VIRTUAL

11th EUROPEAN ZEBRAFISH MEETING 2020

PROGRAM & ABSTRACT BOOK

October 26 - 27, 2020

www.zebrafish2020.org

Content

Scientific Program Committee

Petr Bartunek, Institute of Molecular Genetics, Prague, Czech Republic
Michael Brand, Center for Regenerative Therapies Dresden TU, Dresden, Germany
Elisabeth Busch-Nentwich, University of Cambridge, UK
David Langenau, Massachusetts General Hospital, Harvard Medical School, Boston, USA
Chris Lawrence, Boston Children's Hospital, Boston, USA
Graham Lieschke, Australian Regenerative Medicine Institute, Melbourne, Australia
Christian Mosimann, University of Denver, USA
Trista North, Harvard Medical School, Boston, USA
Liz Patton, MRC Human Genetics Unit, University of Edinburgh, UK
Ewa Snaar-Jagalska, Leiden University, Leiden, Netherlands
Tatjana Sauka-Spengler, Oxford University, Oxford, UK
Stefan Schulte-Merker, Münster University, Münster, Germany
Didier Stainier, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
Leonard Zon, Harvard University, Boston Children's Hospital, HHMI, Boston, USA
Randy Peterson, University of Utah, USA
Francisco Pelegri, MAdison University, USA
Bettina Schmid, LMU Munich, Germany
Massimo Santoro, University of Padua, Italy
Herwig Baier, MPI Munich, Germany

Local Organising Committee

Petr Bartunek, Institute of Molecular Genetics, Prague, Czech Republic
Jana Dvorakova, C-IN, Professional Congress Organiser, Czech Republic
Jana Oltova, Institute of Molecular Genetics, Prague, Czech Republic
Sarka Simova, Institute of Molecular Genetics, Prague, Czech Republic

IMG

European Zebrafish Society

C-IN

Program at Glance

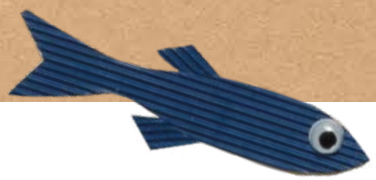
Monday, October 26th

	Virtual Room A	Virtual Room B
08:00		
	Welcome Address	
09:00	Epigenetics, Gene Regulation and Genomic Resources	
	Coffee Break + Virtual Exhibition	
10:00	Morphogenesis and Organogenesis I	
		Diamond Sponsor / The Tecniplast InterZebTEC: a Novel Technical Approach to the Environmental Microbiological Monitoring of Zebrafish
11:00	Coffee Break + Virtual Exhibition	Coffee Break + Virtual Exhibition
	Germ Line, Early Development and Patterning I	Neurobiology I
12:00		
	Lunch Break + Sponsor Pitches	
13:00		
	Keynote Session - Prof. Dr. Nikolaus Rajewsky	
14:00		
	Emerging Technologies	
15:00		
	Community Session	
16:00		
	Coffee Break + Virtual Exhibition	
17:00	Disease Models I	Husbandry and Aquaculture
18:00	Coffee Break + Virtual Exhibition	Coffee Break + Virtual Exhibition
	Regeneration I	Cancer
19:00		

Tuesday, October 27th

	Virtual Room A	Virtual Room B
08^{00}		
	Germ Line, Early Development and Patterning II	Immunity and Infection
09^{00}	Coffee Break + Virtual Exhibition	Coffee Break + Virtual Exhibition
	Regeneration II	Cell Signalling and Metabolism
10^{00}	Coffee Break + Virtual Exhibition	Coffee Break + Virtual Exhibition
11^{00}	Neurobiology II	Chemical Biology and Drug Discovery
12^{00}	Lunch Break + Sponsor Pitches	Lunch Break + Sponsor Pitches
13^{00}	Morphogenesis and Organogenesis II	Circuits and Behavior
14^{00}	Coffee Break + Virtual Exhibition	Coffee Break + Virtual Exhibition
	Disease Models II	Gold Sponsor - Aquaneering / Aquatic Facility Design:
15^{00}		
		Gold Sponsor - Plexx / Iwaki Aquatic Workshop on
	Coffee Break + Virtual Exhibition	Coffee Break + Virtual Exhibition
16^{00}	Stem Cells	Toxicology
17^{00}		
	Keynote Session - Prof. Lalita Ramakrishnan	
18^{00}	Closing Remarks	
19^{00}		

Detailed Program



Monday October 26, 2020

Welcome Address

08:30–08:50 **VIRTUAL ROOM A**

Petr Bartunek (Czech Republic)

Scientific Session: Epigenetics, Gene Regulation and Genomic Resources

08:50–09:55 **VIRTUAL ROOM A**

Chairs: Tatjana Sauka-Spengler (United Kingdom)
Elisabeth Busch-Nentwich (United Kingdom)

08:50 **Cis-regulatory similarities in the zebrafish and human pancreas uncover potential disease-related enhancers**
Jose Bessa (Portugal)

09:05 **Single-cell-resolved dynamics of chromatin architecture delineate cell and regulatory states in wildtype and cloche/npas4l mutant zebrafish embryos**
Scott Lacadie (Germany)

09:17 **The complexity within you: Single-cell analysis revealed hidden heterogeneity in the thyroid gland**
Pierre Gillotay (Belgium)

09:29 **A novel transgenic zebrafish line to study epigenetics during disease and development**
Aniket Gore (USA)

Scientific Session: Morphogenesis and Organogenesis I

10:15–11:20 **VIRTUAL ROOM A**

Chair: Didier Stainier (Germany)

10:15 **Planar Cell Polarity signaling couples nuclear mechanics with changes in muscle differentiation**
Anne M. Merks (Germany)

10:30 **Laminins regulate cardiac growth through restricting second heart field addition**
Emily Noël (United Kingdom)

10:42 **Loss of Smad4 steers embryonic development towards a BMP zero morphotype**
Luca Guglielmi (United Kingdom)

10:54 **The dead box helicase Ddx21 controls cell cycle progression and cell number expansion during embryonic lymphangiogenesis**
Kaska Koltowska (Sweden)

Sponsor Session: Diamond Sponsor / The Tecniplast InterZebTEC: a Novel Technical Approach to the Environmental Microbiological Monitoring of Zebrafish

10:50–11:20 **VIRTUAL ROOM B**

Tecniplast InterZebTEC system: the first attempt to simplify and standardize the methodologies of microbiological investigation in aquatic species by offering the possibility of introducing a simple health and hygiene monitoring routine with a standardized sample collection methodology.
Moderator: Marco Brocca (Italy)

The Tecniplast InterZebTEC: a Novel Technical Approach to the Environmental Microbiological Monitoring of Zebrafish.
Speaker: Gianpaolo Milite (Italy)

Scientific Session: Germ Line, Early Development and Patterning I

11:40–12:45 **VIRTUAL ROOM A**
Chair: Ferenc Mueller (United Kingdom)

11:40 **A Newly Identified Cilium Mechanically Controls Meiotic Chromosomal Pairing and Germ Cell Morphogenesis in Zebrafish And Mouse**
Yaniv Elkouby (Israel)

11:55 **Rbpms2 function in plasticity and sex-specific differentiation of germ cells**
Florence Marlow (USA)

12:07 **Real-time monitoring of Fgf8 propagation during Zebrafish gastrulation and validation of its role as a morphogen**
Rohit Krishnan Harish (Germany)

12:19 **Nodal signalling defines a competency window for the separation of the endodermal from mesodermal lineage through stochastic switching in cell fate**
Andrew Economou (United Kingdom)

Scientific Session: Neurobiology I

11:40–12:45 **VIRTUAL ROOM B**

Chair: Bettina Schmid (Germany)

11:40 **Shining a light on the enteric nervous system**
Gilles Vanwalleghem (Australia)

11:55 **Gigaxonin-E3 ligase controls the initiation of Sonic Hedgehog signaling to sustain motor neuron specification and motility in zebrafish**
Pascale Bomont (France)

12:07 **Purinergic signaling selectively modulates maintenance but not repair neurogenesis in the zebrafish olfactory epithelium**
Mehmet Can Demirler (Turkey)

12:19 **Regulation of axonal growth and synaptogenesis by the cytoplasmic pool of core spliceosomal protein SNRNP70**
Nikolas Nikolaou (United Kingdom)

Keynote Session: Keynote Session – Prof. Dr. Nikolaus Rajewsky

13:30–14:30 **VIRTUAL ROOM A**

Chair: Philipp Junker (Germany)

Single-Cell Approaches in Medicine
Nikolaus Rajewsky (Germany)

Scientific Session: Emerging Technologies

14:30–15:35 **VIRTUAL ROOM A**

Chair: Petr Bartunek (Czech Republic)

14:30 **The Flamingo project makes custom, high-end microscopy more accessible**
Michael Weber (USA)

14:45 **Harmonic generation in-vivo imaging of the zebrafish cardiovascular system**
Guy Malkinson (France)

14:57 **Single-cell phenotypic analysis and dual lineage tracing in real-time with a zebrafish genetic mosaic system**
Bing Xu (USA)

15:09 **Cre-controlled CRISPR (3C) provides an easy method for conditional gene inactivation in zebrafish**
Stefan Hans (Germany)

Scientific Session: Community Session

15:45–16:40 **VIRTUAL ROOM A**
Stefan Schulte-Merker (Germany)
Elizabeth Patton (United Kingdom)
Carole Wilson (United Kingdom)
Kevin Thiessen (Germany)
Periklis Pantazis (United Kingdom)
Apolline Goudmaeker (Belgium)
Tatjana Piotrowski (USA)

Scientific Session: Disease Models I

17:00–18:05 **VIRTUAL ROOM A**
Chair: Ewa Snaar-Jagalska

17:00 **Functional evaluation of catastrophic epilepsy genes: The Epilepsy Zebrafish Project (EZP)**
Scott Baraban (USA)

17:15 **Genetic variants associated to type 2 diabetes modulate endocrine enhancers in vivo**
Ana Eufrásio (Portugal)

17:27 **Abnormal spine development and highly mineralized inclusions in vertebrae in a zebrafish model of CHARGE syndrome**
Maximilian Breuer (Canada)

17:39 **Istaroxime treatment ameliorates calcium dysregulation in a zebrafish model for Phospholamban R14del cardiomyopathy**
Sarah Kamel (Netherlands)

Scientific Session: Husbandry and Aquaculture

17:00–18:05 **VIRTUAL ROOM B**
Chair: Christian Lawrence (USA)

17:00 **Anesthesia does not equal analgesia: pain management in zebrafish**
Karin Finger-Baier (Germany)

17:15 **Physiological effects of common disinfectants – guidelines for zebrafish egg disinfection**
Geoffrey Aliti (Sweden)

17:27 **Sustainable microalgae products to improve zebrafish reproduction and larvae development**
Patricia Diogo (Portugal)

17:39 **Contemporary real-time PCR-based prevalence of infectious agents in laboratory zebrafish colonies (2015–2019)**
Marcus Crim (USA)

Scientific Session: Regeneration I

18:25–19:30 **VIRTUAL ROOM A**
Chair: Michael Brand (Germany)

18:25 **Mapping the cellular dynamics of liver regeneration using targeted photoactivatable cell ablation**
Elke Ober (Denmark)

18:40 **Single-cell analysis of the regenerative niche in the zebrafish heart**
Bo Hu (Germany)

18:52 **Determining the gene regulatory network for hair cell regeneration in the zebrafish adult inner ear at single cell resolution**
Shawn Burgess (USA)

19:04 **Single-cell transcriptomics reveals several populations of cardiac-resident leukocytes in the regenerating zebrafish heart**
João Cardeira-da-Silva (Germany)

Scientific Session: Cancer

18:25–19:30 **VIRTUAL ROOM B**

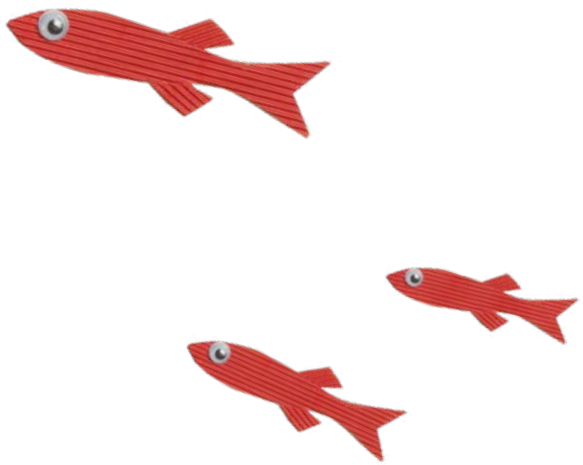
Chairs: Leonard Zon (USA)
David Langenau (USA)

18:25 **The Investigation of the Role of VHL-HIF Signaling in DNA Repair and Apoptosis in Zebrafish**
Hyejeong Rosemary Kim (United Kingdom)

18:40 **The role of P97 segregase in the repair of DNA-protein crosslink in vivo using CRISPR/Cas9 gene editing in zebrafish**
Cecile Otten (Croatia)

18:52 **Using zebrafish to unravel the role of succinate dehydrogenase in tumour development**
Margo Dona (Netherlands)

19:04 **HDAC8: a promising therapeutic target for acute myeloid leukemia**
Marco Spreafico (Italy)



TUESDAY OCTOBER 27, 2020

Scientific Session: Germ Line, Early Development and Patterning II

08:30–09:35 **VIRTUAL ROOM A**

Chair: Yaniv Elkouby (Israel)

08:30 **Identification of Maternal-Effect Genes in Zebrafish by a Maternal Crispant Screen**
Cara Moravec (USA)

08:45 **Circadian roles in reproduction**
Han Wang (China)

08:57 **Autophagy inhibition perturbed definitive hematopoiesis leading to aberrant myeloproliferation in Zebrafish models**
Kazi Md Mahmudul Hasan (Hong Kong)

09:09 **Zebrafish pigment pattern formation – a problem solved or one ripe for discovery?**
Robert Kelsh (United Kingdom)

Scientific Session: Immunity and Infection

08:30–09:35 **VIRTUAL ROOM B**

Chair: Graham Lieschke (Australia)

08:30 **New insights into lymphoid organization in fish: a lymphoid continuity between mucosal tissues and the discovery of a new lymphoid tissue identify the branchial cavity as a lymphoid nexus**
Julien Resseguier (Norway)

08:45 **Reprogramming macrophages and neutrophils by infection and through loading with cargoes via protocells**
Paco López-Cuevas (United Kingdom)

08:57 **Development of gamma/delta T-cells is regulated by cell localization and environmental signals in the thymus**
Baubak Bajoghli (Germany)

09:09 **An evolutionary conserved program of TGFβ signaling-mediated microglia development in zebrafish**
Ferrero Giuliano (Belgium)

Scientific Session: Regeneration II

09:55–11:00 **VIRTUAL ROOM A**

Chair: Christoph Englert (Germany)

09:55 **Make do and make new: how zebrafish rapidly regenerates CNS injury**
Jan Kaslin (Australia)

10:10 **Osteoblast cell migration during zebrafish fin regeneration**
Ivonne Sehring (Germany)

10:22 **Interleukin-11 signaling limits scar formation by antagonizing endothelial-to-myofibroblast transdifferentiation during zebrafish heart regeneration**
Srinivas Allanki (Germany)

10:34 **Investigating the interaction between cardiac subpopulations during adult cardiac regeneration**
Phong Nguyen (Netherlands)

Scientific Session: Cell Signalling and Metabolism

09:55–11:00 **VIRTUAL ROOM B**

Chair: Massimo Santoro (Italy)

09:55 **Yap regulates hematopoietic stem cell formation in response to the biomechanical forces of blood flow**
Wade Sugden (USA)

10:10 **Left side story: Cachd1, Wnt signalling and the habenulae**
Gareth Powell (United Kingdom)

10:22 **Multiple clocks regulate amino acid levels in zebrafish cells**
Rima Siauciunaite (Germany)

10:34 **The increased concentration of 4-Hydroxynonenal in aldh3a1 zebrafish mutants disrupts pancreas development, leading to hyperglycaemia and retina hyaloid vasculature alteration**
Bowen Lou (Germany)

Scientific Session: Neurobiology II

11:20–12:25 **VIRTUAL ROOM A**

Chair: Corinne Houart (United Kingdom)

11:20 **Molecular and functional characterizations of Gfi1ab role in the establishment of specific retinotectal connections**
Shahad Albadri (France)

11:35 **The olfactory epithelia: A novel neural immune tissue**
Kathleen Whitlock (Chile)

11:47 **Synaptic silencing of fast muscle is compensated by rewired innervation of slow muscle**
Fumihito Ono (Japan)

Scientific Session: Chemical Biology and Drug Discovery

11:20–12:25 **VIRTUAL ROOM B**

Chair: Elizabeth Patton (United Kingdom)

11:20 **Identification of bioactive compounds from fungi using zebrafish embryogenesis as read-out**
Jelmer Hoeksma (Netherlands)

11:35 **TRPswitch – a step function chemo-optogenetic ligand for the vertebrate TRPA1 channel**
Pui Ying Lam (USA)

11:47 **The potential of Tetraselmis sp. CTP4 as a source for bone anabolic compounds in zebrafish**
Alessio Carletti (Portugal)

11:59 **Establishing a locomotive zebrafish larvae bioassay using GABAA receptor modulators**
Anke Wilhelm (South Africa)

Scientific Session: Morphogenesis and Organogenesis II

13:10–14:15 **VIRTUAL ROOM A**

Chair: Robin Kimmel (Austria)

13:10 **Calcium Signaling during Primary Angiogenic Sprouting in Zebrafish**
Daniela Panáková (Germany)

13:25 **Adaptive cell invasion of skin-derived ionocytes into hair cell-containing mechanosensory organs**
Daniela Münch (USA)

13:37 **Tissue-specific compensatory mechanisms for maintaining body size in polyploid Zebrafish**
Christopher Small (Canada)

13:49 **Twisting of the heart tube during cardiac looping is a tbx5-dependent and tissue-intrinsic process**
Federico Tessadori (Netherlands)

Scientific Session: Circuits and Behavior

13:10–14:15 **VIRTUAL ROOM B**
Chair: Herwig Baier (Germany)

13:10 **Future state prediction error improves active avoidance behavior by adult zebrafish in virtual reality**
Makio Torigoe (Japan)

13:25 **Functional Imaging and Optogenetic Analysis of Cells Derived from Three Germ Layers in the Larval Zebrafish Gut**
Kohei Hatta (Japan)

13:37 **The left-right directional information in zebrafish is processed through the dorsal lateral habenula-interpeduncular nucleus pathway for decision making**
Bor-Wei Cherng (Japan)

13:49 **Exploring the role of the P2y12 receptor in the zebrafish brain**
Sarra Zenagui (Sweden)

Scientific Session: Disease Models II

14:35–15:40 **VIRTUAL ROOM A**
Chair: Stefan Schulte-Merker (Germany)

14:35 **Adult pdx1-deficient zebrafish display diabetes-induced vasculopathy in the retina**
Jens Kroll (Germany)

14:50 **The nuclear gene rpl18 regulates erythroid maturation via JAK2-STAT3 signaling in zebrafish model of Diamond-Blackfan anemia**
Wei Qin (China)

15:02 **Zebrafish calreticulin loss of function model as a potential therapeutic target for human CALR mutated myeloproliferative neoplasms**
Kazi Md Mahmudul Hasan (Hong Kong)

15:14 **Molecular mechanisms of neural stem cell plasticity and regenerative neurogenesis in Alzheimer's disease model of zebrafish brain: from zebrafish to humans**
Caghan Kizil (Germany)

Sponsor Session: Gold Sponsor – Aquaneering / Aquatic Facility Design: Considerations for Aquatic Facility Planning

14:35–14:50 **VIRTUAL ROOM B**

Planning a new Zebrafish Facility? There's a lot to consider. Aquaneering previews our presentation on Aquatic Facility Design, which we make available to individuals and groups wanting to be informed when making this important step.

Aquaneering is an internationally recognized leader of aquatic housing for zebrafish, Xenopus frogs, and other aquatic species used in medical research, as well as the manufacturer of the largest zebrafish systems in the world. Aquaneering offers unmatched knowledge of highly advanced filtration technologies pioneered within the aquaculture industry, notably our no-maintenance filters that assure undetectable levels of ammonia and nitrites.
Moderator: Bill Kilgore (USA)
Speaker: Bobbi Baur (USA)

Sponsor Session: Gold Sponsor – Plexx / Iwaki Aquatic Workshop on the New LAbREED™ Zebrafish Housing System

15:25–15:40 **VIRTUAL ROOM B**

In this workshop we will be showing you the new Iwaki Aquatic LAbREED™ Zebrafish Housing System featuring new tank designs of 2L, 4.5L, and 9L. The tanks have been designed for improved solids removal while keeping tank assembly simple with just three parts. Our redundant speed-controlled pumps provide constant flow to your zebrafish over time as filters accumulate debris, a first in the industry!

Our life support controller is the most capable and flexible controller on the marketplace and when connected to our new cloud-based software program (Fluent™), you will be able to trend and record water quality over time, set alerts, change parameters, escalate alarms, and so much more. Please join our workshop

to learn about these exciting new features on the LAbREED™ Zebrafish Housing System.

Moderator: Erwin Smulders (Netherlands)

Speaker: Eric Moore

Scientific Session: Stem Cells

16:00–17:05 **VIRTUAL ROOM A**

Chair: Trista North (USA)

16:00 **A csf1rb mutation uncouples two waves of microglia development in zebrafish**
Valerie Wittamer (Belgium)

16:15 **Unveiling the heterogeneity of vertebrate adult Neural Stem Cells**
David Morizet (France)

16:27 **Transposable elements induce a RIG-I-like receptor-mediated inflammation to regulate HSPC emergence**
Stylianos Lefkopoulos (Germany)

16:39 **Redox biology of neural stem cell: understanding the role of antioxidants during retinogenesis**
Shahad Albadri (France)

Scientific Session: Toxicology

16:00–17:05 **VIRTUAL ROOM B**

Chair: Randall Peterson (USA)

16:00 **New insights into the osteotoxicity of benzo[α]pyrene in zebrafish**
Marco Tarasco (Portugal)

16:15 **Automated feature recognition in zebrafish embryos for chemical hazard characterisation**
Stefan Scholz (Germany)

16:27 **Toxicity assessment and behavioral effects of parabens in zebrafish early-life stages**
Carmine Merola (Italy)

16:39 **Zebrafish as a model for investigating noise-Induced physiological stress and hearing loss**
Raquel Vasconcelos (Macao)

Keynote Session: Keynote Session – Prof. Lalita Ramakrishnan

17:20–18:20 **VIRTUAL ROOM A**

Chair: David Langenau (USA)

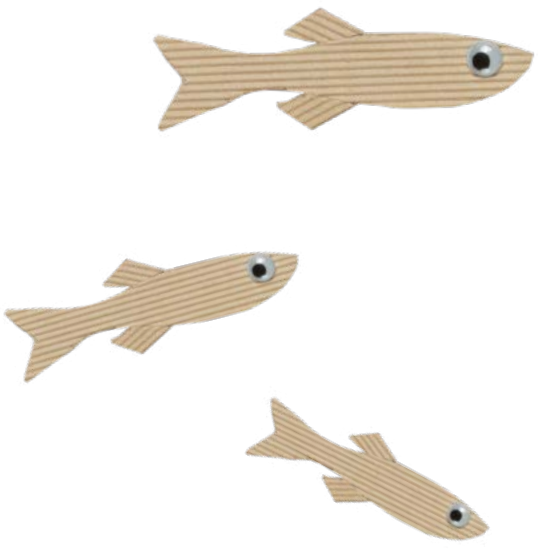
Life-saving therapies for Tuberculosis from the zebrafish

Lalita Ramakrishnan (United Kingdom)

Closing Remarks

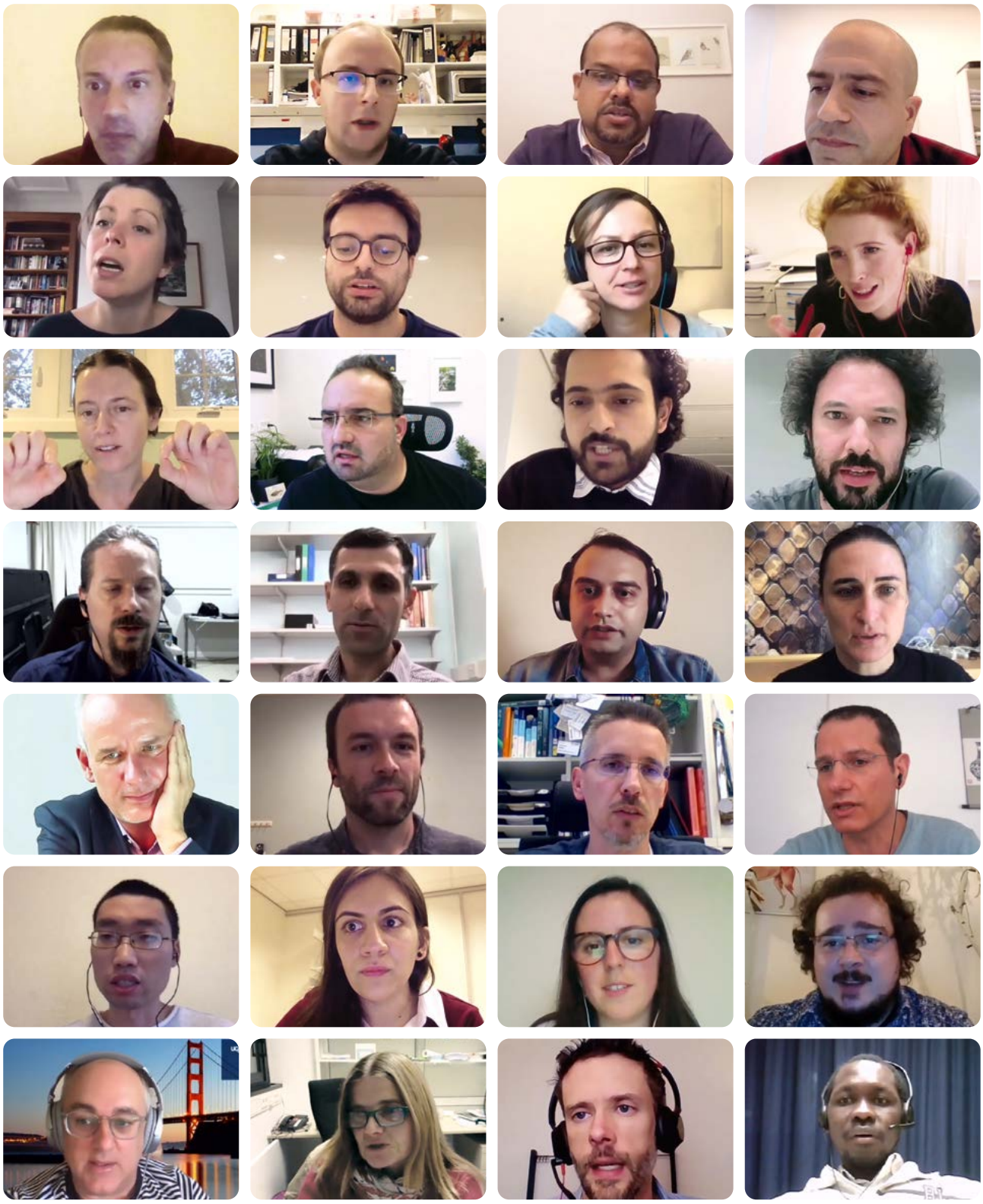
18:20–18:40 **VIRTUAL ROOM A**

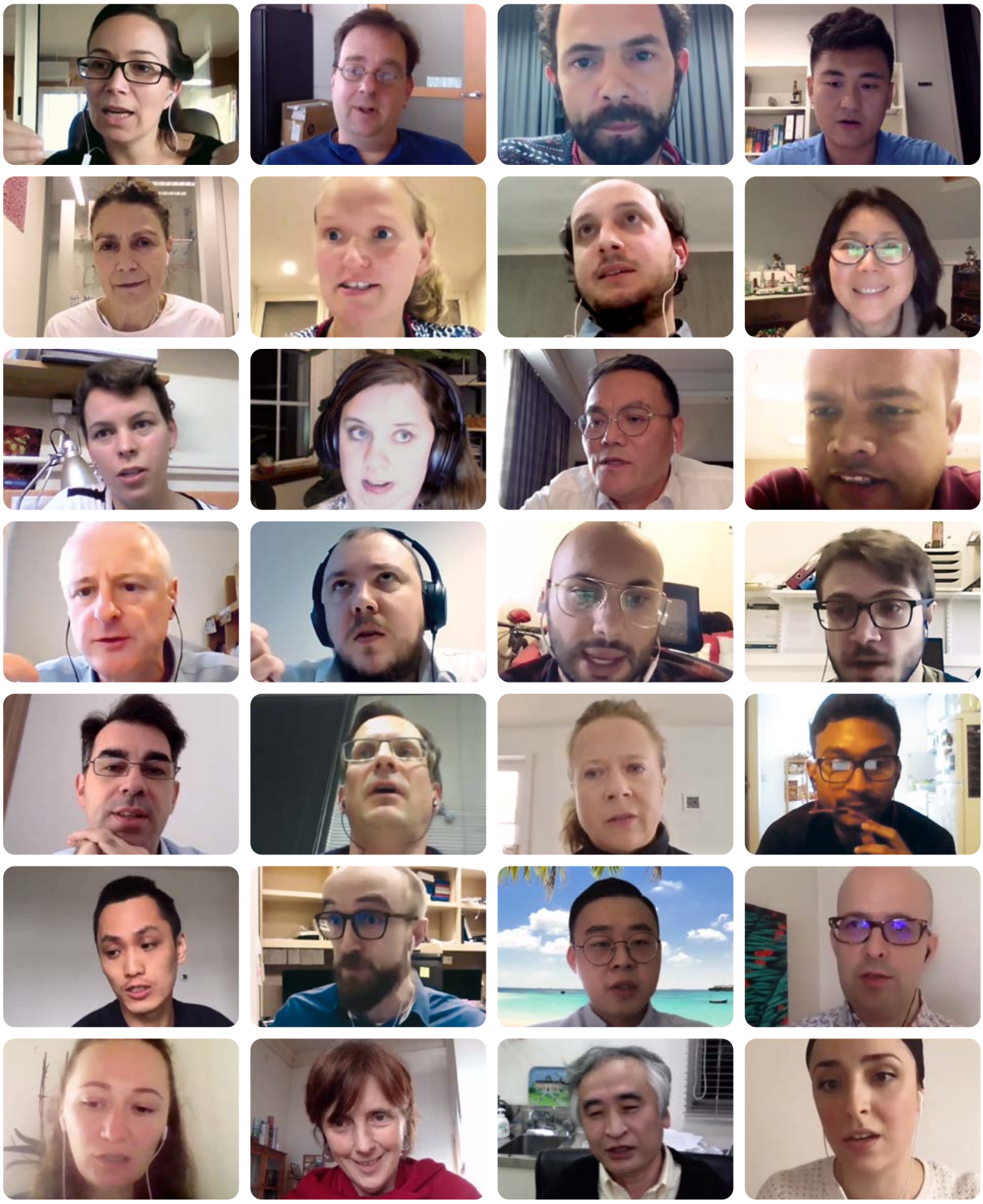
Petr Bartunek (Czech Republic)
Stefan Schulte-Merker (Germany)
Lilianna Solnica-Krezel (USA)

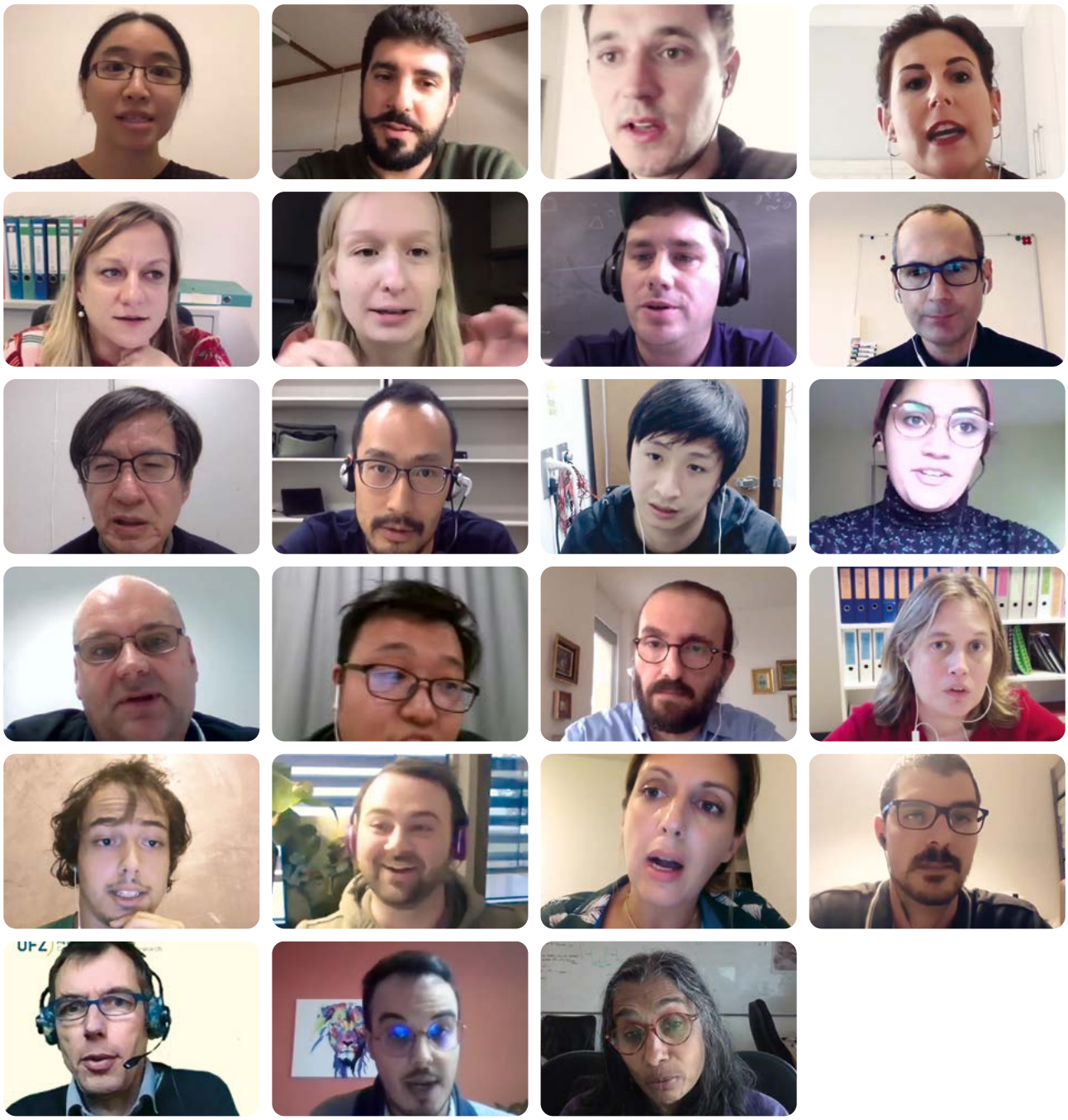


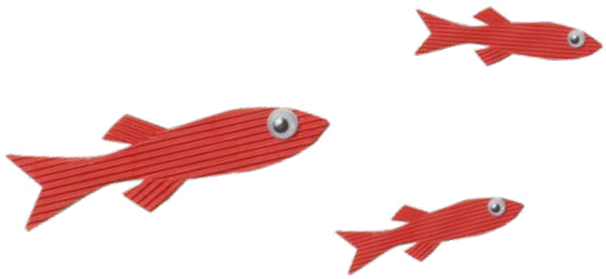
Zebrafish 2020 Chairs

Zebrafish 2020 Speakers









List of Oral Abstracts

Epigenetics, Gene Regulation and Genomic Resources

Cis-regulatory similarities in the zebrafish and human pancreas uncover potential disease-related enhancers

SESSION: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Renata Bordeira-Carriço[1], Joana Teixeira[1], Marta Duque[1], Mafalda Galhardo[1], Diogo Ribeiro[1], Rafael Dominguez-Acemel[2], Panos Firbas[2], Juan Tena[2], Ana Eufrasio[1], Joana Marques[1], Fabio Ferreira[1], Telmo Freitas[1], Fatima Carneiro[3], Jose Luis Goméz-Skarmeta[2], Jose Bessa[1]

[1] *Instituto de Investigação e Inovação em Saúde i3S- Universidade do Porto, Vertebrate development and regeneration, Porto, Portugal*
[2] *Centro Andaluz de Biología del Desarrollo CABD- Universidad Pablo de Olavide, Gene Regulation and Morphogenesis, Seville, Spain*
[3] *Faculdade de Medicina da Universidade do Porto FMUP, Departamento de Patologia, Porto, Portugal*

ABSTRACT TEXT

Pancreatic cancer and diabetes are pancreas diseases with a dramatic societal burden, whose genetic causes are still largely unknown. Alterations in non-coding cis-regulatory elements (CREs) of DNA, can contribute to human diseases by affecting gene expression. However, functional testing of CREs *in vivo* is not fully explored. We analysed histone modifications, transcription, chromatin accessibility and interactions to identify zebrafish pancreas CREs and their human functional equivalents, uncovering disease-associated sequences across species. We found a human pancreatic enhancer whose deletion impairs the tumour-suppressor gene *ARID1A* expression, having thus a potential tumour-suppressor role. Additionally, human genomic deletions in the landscape of *PTF1A* have been associated to pancreatic agenesis. We identified a zebrafish *ptf1a* distal enhancer whose deletion generates pancreatic agenesis, demonstrating the causality of this condition in humans. Our results further demonstrate that this phenotype is a consequence of loss of pancreas progenitor cells. This work demonstrates the interspecies functional equivalency of cis-regulatory elements, establishing new bridges between two phylogenetically distant species, providing an invaluable contribution for the prediction of new disease-causative enhancers and the study of their role in human disease.

Single-cell-resolved dynamics of chromatin architecture delineate cell and regulatory states in wildtype and cloche / npas4l mutant zebrafish embryos

SESSION: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Alison McGarvey[1], Wolgang Kopp[1], Dubravka Vučićević[1], Rieke Kempfer[1], Kenny Mattonet[2], Antje Hirsekorn[1], Ilija Bilić[1], Alexandra Trinks[3], Anne Merks[4], Daniela Panáková[4], Ana Pombo[1], Altuna Akalin[1], Jan Philipp Junker[1], Didier Stainier[2], David Garfield[5], Uwe Ohler[1], Scott Lacadie[1]

[1] *Max Delbrück Center for Molecular Medicine, Berlin Institute of Medical Systems Biology, Berlin, Germany*
[2] *Max Planck Institute for Heart and Lung Research, Department of Developmental Genetics, Bad Nauheim, Germany*
[3] *Humboldt Universität Berlin, IRI Life Sciences, Berlin, Germany*
[4] *Max Delbrück Center for Molecular Medicine, Cardiovascular & Metabolic Diseases, Berlin, Germany*
[5] *Bayer Pharma AG, Bayer Pharma AG, Berlin, Germany*

ABSTRACT TEXT

DNA accessibility of cis regulatory elements (CREs) reflects activity that drives cell differentiation during development. The underlying CRE dynamics controlling developmental gene expression remain largely unknown. We have characterized the genome-wide chromatin architecture of the whole 24 hpf stage zebrafish embryo, at bulk and single cell resolution, to generate a resource of cell type-specific candidate CREs. We generated accessibility profiles for ~23,000 single nuclei using sci-ATAC-seq and developed a tool, named ScregSeg, which utilizes Hidden Markov Models (HMMs) to address key challenges in analyzing single-cell accessibility profiles. We show that diverse cell types can be grouped in silico by their accessibility profiles and have classified complex patterns of CRE dynamics. Sequence analysis considering CRE dynamics allowed us to infer cell type-specific DNA binders. Integrating histone modification-based classifications with sci-ATAC-seq and bulk in situ Hi-C, we show clear relationships between promoter chromatin states, constitutive accessibility, and 3D insulation, as well as between co-accessibility and 3D interactions, thereby highlighting regulatory principles active during zebrafish

development. Lastly, we apply sci-ATAC-seq to npas4l mutant embryos, which lack blood and endothelial cells, and observe unexpected changes in muscle, epidermal, and caudal precursor cell numbers. Furthermore, we detect candidate cell type-specific npas4l CREs, suggesting an intricate network controlling its expression. Our work constitutes a valuable resource for future studies in developmental, molecular, and computational biology.

The complexity within you: Single-cell analysis revealed hidden heterogeneity in the thyroid gland

SESSION: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Pierre Gillotay[1], Meghna Shankar[1], Elif Eski[1], Susanne Reinhardt[2], Annekathrin Kränkel[2], Juliane Bläsche[2], Andreas Petzold[2], Gokul Kesavan[2], Christian Lange[2], Michael Brand[2], Vincent Detours[1], Sabine Costagliola[1], Sumeet Pal Singh[1]

[1] *Université Libre de Bruxelles, Institut de recherche interdisciplinaire en biologie humaine et moléculaire, Brussels, Belgium*

[2] *TU Dresden, Center for Molecular and Cellular Bioengineering, Dresden, Germany*

ABSTRACT TEXT

The thyroid is an endoderm-derived endocrine gland that acts as a master regulator of many biological processes including basal metabolism, foetal growth and brain development. Thyroid function is mediated by the thyroid hormones produced in the thyroid follicles, which are spherical structures composed of only one cell type: the thyroid follicular cells (TFC). So far, TFC have been considered as a homogenous population and little is known about the differences that might exist between TFC. To explore the transcriptional heterogeneity within the TFC populations, we perform high-throughput single-cell RNA-sequencing of 3 and 8 mpf zebrafish thyroid. Transcriptome of 6200 cells was profiled to generate an atlas of the thyroid gland and its surrounding tissue. Profiling of the TFC revealed two sub-populations with distinct transcriptomes. Both populations were expressing same level of thyroglobulin but were differentially expressing genes such as cathepsin B (ctsba) and iyd (both are actors of the thyroid hormone metabolism). Most importantly, both populations displayed differential expression of *pax2a* (transcription factor driving the thyroid progenitors specification). This differential expression was inversely correlated with that of cstba and iyd. To validate this heterogeneity; we first generated a *pax2a* knock-in line (*pax2a*^{mKO2}) using CRISPR-Cas9. Analysis of the thyroid gland of adult *pax2a*^{mKO2} zebrafish revealed the presence of TFC with low expression of *pax2a*. Interestingly, these *pax2a*-low TFC were spread across the thyroid gland. To understand the differences between both populations we combined our *pax2a*^{mKO2} line with a TG(*tg*:EGFP-nls) to isolate thyroid cells based on GFP and mKO2 expression for transcriptome profiling. Taken together, our data present the first single-cell atlas of a vertebrate thyroid and highlight the transcriptional complexity of its cell populations paving the way for further characterisation of the cellular heterogeneity of the TFC.

A novel transgenic zebrafish line to study epigenetics during disease and development

SESSION: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Aniket Gore[1], Kiyohito Taimatsu[1], Miranda Marvel[1], Daniel Castranova[1], Avery Swearer[1], Olivia Carpinello[1], Keith Barnes[1], Andrew Davis[1], Brant Weinstein[1]

[1] *Eunice Kennedy Shriver National Institute of Child Health and Human Development- NIH, Division of Developmental Biology, Bethesda, USA*

ABSTRACT TEXT

Large scale human epigenome sequencing projects and additional studies from several model organisms have revealed that tissue-specific epigenetic marks are correlated with cellular identities and cell type-specific gene expression. Currently, few tools are available to visualize the dynamic nature of epigenetic gene regulation in live vertebrate embryos at the cellular level. We have developed a novel *Tg(dazlCGI-ef1a:gfpd2)* "EpiTag" transgenic line that reliably reports dynamic tissue-specific epigenetic changes during development, regeneration, and disease. The transgenic construct used in this line contains a CpG island from the *dazl* gene, a germ cell-specific gene targeted for silencing in all somatic cells. The *dazl* CpG island is cloned next to a ubiquitously expressing *ef1a* promoter driving expression of destabilized GFP (GFPd2), allowing for dynamic visualization of rapid changes in GFP reporter expression in living animals. The EpiTag line begins expressing GFP at around 6 hpf. GFP fluorescence peaks at around 24 hpf, begins to fade by 48 hpf and is almost undetectable by 5 dpf. GFP expression after 24 hpf can be reactivated by treatments that interfere with epigenetic silencing, validating

the line as an effective "epigenetic reporter." The epigenetic reporter is silenced in all adult tissues except for testis and ovary, where GFP is reactivated in developing spermatogonia and oocytes, respectively. We are currently using this line to carry out an F3 ENU mutagenesis screen to identify recessive tissue-specific mutants in epigenetic silencing. This highly successful screen has already identified mutants defective in epigenetic silencing in a variety of different tissues. We have also shown that EpiTag GFP expression is reactivated during regeneration and in selected tumors. Our findings highlight the strength and versatility of the EpiTag reporter for studying dynamic epigenetic changes during development, regeneration, and disease.

Morphogenesis and Organogenesis I

Planar Cell Polarity signaling couples nuclear mechanics with changes in muscle differentiation

SESSION: MORPHOGENESIS AND ORGANOGENESIS I

Anne M. Merks[1,2], *Marie Dreher*[1], *Alexander M. Meyer*[1], *Daniela Panáková*[1,2]

[1] *Max Delbrück Center for Molecular Medicine, Cardiovascular and Metabolic Disease, Berlin, Germany*
[2] *DZHK German Center for Cardiovascular Research, partner site Berlin, Berlin, Germany*

ABSTRACT TEXT

The nucleus can act as a mechanosensor to directly modulate gene expression. Here, we identify a role for Wnt non-canonical Planar Cell Polarity (PCP) signaling in regulating mechanosensing properties of the nuclei in heart muscle cells during early cardiac development. We have discovered that nuclei of ventricular cardiomyocytes undergo remarkable morphological changes during heart chamber remodeling in a tightly temporally-controlled manner. These changes in nuclear morphology depend on contractile actomyosin. PCP signaling regulates nuclear shape changes as PCP-deficient hearts display abnormalities in nuclear morphology. PCP signaling affects the actomyosin contractility by targeting not only the cytoplasmic, but also perinuclear and nuclear actomyosin. These PCP-dependent events occurring in the linear heart tube stage are linked to transcriptional changes in gene programs regulating muscle differentiation and are ultimately manifested as defects in sarcomerogenesis long after the heart chambers are formed. Thus, PCP signaling affects the mechanosensing properties of the nucleus to alter downstream mechanotransduction signaling and with that associated changes in gene expression.

Laminins regulate cardiac growth through restricting second heart field addition

SESSION: MORPHOGENESIS AND ORGANOGENESIS I

Christopher Derrick[1], *Farah Hussein*[1], *Eric Pollitt*[1], *Emily Noël*[1]

[1] *University of Sheffield, Biomedical Science, Sheffield, United Kingdom*

ABSTRACT TEXT

Congenital heart defects occur in around 1 % of live births, and are structural malformations of the heart due to improper cardiac development. Heart looping morphogenesis is a critical stage in early vertebrate heart development when the heart transitions from a linear tube to an asymmetrically looped organ. Concomitantly, cells migrate into both cardiac poles from the Second Heart Field (SHF), and this morphogenesis and growth are intimately linked in heart development. The heart tube comprises an outer layer of contractile myocardium surrounding the endocardium, separated by the cardiac extracellular matrix (ECM), and we are interested in how cardiac ECM supports heart morphogenesis.

Laminins are ECM components consisting of diverse heterotrimeric isoforms made up of combinations of α, β, and γ chains. Despite being a core component of basement membranes, no role for laminin has been identified in early vertebrate heart morphogenesis. We identified dynamic and tissue-specific expression of laminin subunit genes in the developing zebrafish heart, supporting a role for one or more laminin isoforms in heart morphogenesis. Analysis of *lamc1* mutants reveals abnormal looping morphology at 2dpf and increased heart size by 3dpf. Distinctly, loss of *lamb1a* also results in increased heart size but does not impact early heart looping, highlighting that distinct laminin isoforms perform specific roles in cardiac development. *lamb1a* mutants have an increase in newly-added SHF cells to the atrium at 2dpf, demonstrating that *lamb1a* limits SHF addition to the venous pole. Finally, knockdown of *tnnt2a* in *lamb1a* mutants rescues heart size at 3dpf, suggesting interactions between cell migration and biomechanics are regulated by *lamb1a* during heart development. Together, this represents the first description of multiple roles for laminins in early vertebrate heart morphogenesis, reinforcing the importance of specialised ECM composition in distinct aspects of cardiac development.

Loss of Smad4 steers embryonic development towards a BMP zero morphotype

SESSION: MORPHOGENESIS AND ORGANOGENESIS I

Luca Guglielmi[1], *Claire Heliot*[1], *Sunil Kumar*[2], *Ilaria Gori*[1], *Christopher Barrington*[3], *Yuriv Alexandrov*[2], *Paul French*[2], *James McGinty*[2], *Caroline Hill*[1]

[1] *The Francis Crick Institute, Developmental Signalling, London, United Kingdom*
[2] *Imperial College London, Department of Phisics, London, United Kingdom*
[3] *The Francis Crick Institute, Bioniformatics and Biostatistics, London, United Kingdom*

ABSTRACT TEXT

In vertebrates, the ligands of the TGFβ family are fundamental for embryonic development and their de-regulated signalling underlies a number of human diseases, the most prominent being cancer. Smad4 is typically considered a core component of the TGFβ family signalling pathways and essential for the activity of both the BMP and the TGFβ/Activin/Nodal branches. Specifically, Smad4 interacts with activated R-SMADs forming complexes that accumulate in the nucleus to regulate the transcription of different target genes. The requirement for Smad4 for all Nodal and BMP signalling has always been assumed based on the classical description of the pathway. However, this assumption has never been extensively tested, especially *in vivo*. In order to thoroughly characterize its role, we deleted *smad4a* in zebrafish embryos using CRISPR/Cas9 and interrogated the consequences of its deletion on BMP and Nodal signalling activity. Intriguingly, we find that while loss of Smad4a abolishes expression of BMP target genes, the expression of Nodal target genes is robustly maintained. Surprisingly, we find that this results from Smad4-independent Nodal signalling. In line with these findings, MZ*smad4a* mutant embryos express endoderm and mesoderm markers, while ventral epidermal genes are suppressed in favour of dorsal neural tissue and dorsal–ventral patterning is disrupted. Finally, we ask how the Smad4 mutant morphology relates to embryos lacking BMP or Nodal signalling. To this end we have developed an optical projection tomography (OPT) pipeline enabling the extraction and quantitation of morphological feature in 24 hpf fluorescently-labelled embryos. By analyzing both embryo shape and the expression of specific markers we are exploring the relationship between levels of BMP and Nodal signalling and embryo morphogenesis. Notably, we show that MZ*smad4a* mutants are morphologically indistinguishable from embryos in which BMP signalling have been pharmacologically or genetically perturbed.

The dead box helicase Ddx21 controls cell cycle progression and cell number expansion during embryonic lymphangiogenesis

SESSION: MORPHOGENESIS AND ORGANOGENESIS I

Kaska Koltowska[1,2], Kazuhide Okuda[3], Jun Chen[2], Maria Rondon Galeano[2,3], Hannah Arnold[1], Gregory Baillie[2], Ignat Leshchiner[4], Wolfram Goessling[5], Kelly Smith[6], Cas Simons[7], Stefan Schulte-Merker[8], Ben Hogan[2,9]

1 *Uppsala University, Department for Immunology- Genetics and Pathology, Uppsala, Sweden*
2 *The University of Queensland, Institute for Molecular Bioscience, Brisbane, Australia*
3 *University of Melbourne, Department of Anatomy and Neuroscience and Sir Peter MacCallum Department of Oncology, Melbourne, Australia*
4 *Brigham and Women's Hospital, Harvard Stem Cell Institute, Boston, USA*
5 *Brigham and Women's Hospital, Harvard Stem Cell Institute-, Boston, USA*
6 *University of Melbourne, Department of Physiology, Melbourne, Australia*
7 *Murdoch Children's Research Institute, Murdoch Children's Research Institute, Melbourne, Australia*
8 *WWU Münster, Institute of Cardiovascular Organogenesis and Regeneration, Münster, Germany*
9 *University of Melbourne, Department of Anatomy and Neuroscience and Sir Peter MacCallum Department of Oncology-, Melbourne, Australia*

ABSTRACT TEXT

The regulation of tissue growth is a complex and multifactorial process dependent on tissue-specific and general "housekeeping" cellular machinery. It is becoming increasingly clear that presumed housekeeping pathways can have selective and tissue specific roles in development and disease. Here, we used an unbiased forward genetic screen to uncover a role for the RNA helicase Ddx21 in lymphangiogenesis. Ddx21 regulates Pol1 mediated rRNA transcription and ribosomal biogenesis. A zebrafish *ddx21* mutant displayed a loss of developmental lymphangiogenesis, while forming a relatively normal blood vasculature. Another mutant isolated in a forward genetic screen identified *topoisomerase 3a* (*top3a),* which also showed a loss of lymphatic vessels but normal blood vessels and fails to express normal levels of Ddx21. *ddx21* expression is enriched in specific tissues, including endothelium and blastocyst cell transplantation experiments revealed a cell autonomous role within lymphatic endothelial cells. Ddx21 mutants genetically interact with the Vegfr3 pathway and Ddx21 is essential for pathological proliferation of vasculature driven by ectopic Vegfc expression. Mechanistically, zebrafish and human endothelial cells show arrest of the cell cycle at G1 phase in the absence of Ddx21. p53 phosphorylation and p21 levels are robustly upregulated upon Ddx21 loss and inhibition of p53 rescues lymphatic phenotypes in *ddx21* and *top3a* mutants. This work suggests a pathway acting downstream of Vegfc-Vegfr3 signalling promoting endothelial cell proliferation and growth lymphatic vessels growth. Lymphatics appear to be more sensitive to loss of this pathway *in vivo* than blood vasculature. This may open up opportunities to target Ddx21 and cell cycle progression in lymphatics in pathological contexts of excessive lymphatic growth.

Germ Line, Early Development and Patterning I

A Newly Identified Cilium Mechanically Controls Meiotic Chromosomal Pairing and Germ Cell Morphogenesis in Zebrafish and Mouse

SESSION: GERM LINE, EARLY DEVELOPMENT AND PATTERNING I

Avishag Mytlis[1], Vineet Kumar[1], Karine Levy[1], Rachael Deis[1], Farouq Nather[1], Hagai Eitan[1], Amal Shawahny[1], Yaniv Elkouby[1]

[1] *The Hebrew University of Jerusalem, Developmental Biology and Cancer Research, Jerusalem, Israel*

ABSTRACT TEXT

In meiosis, chromosomal pairing requires cytoplasmic mechanical forces. In pairing, telomeres associate with centrosome-based perinuclear microtubules (MTs) via Sun/KASH proteins on the nuclear envelope (NE). MTs facilitate telomere rotation on the NE, shuffling chromosomes for homology searches. Telomeres are then pulled towards the centrosome and cluster on the NE, looping their chromosomes to the other side. This configuration stabilizes pairing and is called the zygotene chromosomal bouquet. How bouquet forces are generated and regulated is poorly understood. Here, we identify an oocyte cilium that essentially connects to the bouquet machinery. We detected tubulin cables that extend from oocyte centrosomes, which we confirmed as cilia by TEM analysis as well as molecular markers, such as tubulin acetylation and glutamylation and Arl13b. Zygotene oocytes develop in a compact cellular organization called the germline cyst. 3-dimensional rendering of SBF-SEM and confocal data, show that cilia tangle between oocytes like scaffolds throughout the cyst. The cilia form specifically during bouquet stages, and live imaging show cilia movements that coordinate with chromosomal rotations. Thus, a cytoskeletal cable system extends from the cilium through the centrosome and MTs to NE-associated telomeres, as the machinery for chromosomal pairing and as a physical framework for the cyst. Analysis of the ciliary mutants *cep290, kif7,* and their combination, demonstrates that the zygotene cilium is essential for bouquet formation and germline cysts integrity. Ciliary defects consequently lead to adult ovarian dysgenesis and sterility. Complete cilia loss induces extensive apoptosis of deficient oocytes. We further show that the zygotene cilium is conserved in male meiosis, as well as in mouse oocytes. Our work uncovers the zygotene cilium as a newly identified player in meiosis that mechanically regulates chromosomal pairing and physically reinforces germ cell morphogenesis.

Rbpms2 function in plasticity and sex-specific differentiation of germ cells

SESSION: GERM LINE, EARLY DEVELOPMENT AND PATTERNING I

Shannon Romano[1], Yulong Liu[2], Bruce Draper[2], Florence Marlow[1]

[1] *Icahn School of Medicine at Mount Sinai, Department of Cell- Developmental- and Regenerative Biology, New York, USA*

[2] *University of California Davis, Department of Molecular and Cellular Biology, Davis, USA*

ABSTRACT TEXT

Sex specific differentiation of germ cells (GCs) is essential to fertility in sexually reproducing species. In zebrafish this process begins with formation of a bipotential, ovary-like gonad that initially develops oocyte-like cells (OLCs). Maintenance of OLCs is required for female gonad development, but how sex-specific programming of GCs is regulated is unknown. Rbpms2 is a conserved Rbp and is essential for female fertility. *rbpms2* mutants form a bipotential gonad, but eventually differentiate as males. This suggests that GCs lacking Rbpms2 adopt male fate due to a defect in female-specific differentiation. To identify Rbpms2 targets involved in sex-specific differentiation, we generated a transgenic line expressing tagged Rbpms2 in oocytes and performed RNA-immunoprecipitation and RNA-sequencing to identify Rbpms2-bound RNAs. Surprisingly, twenty percent of Rbpms2 bound RNAs in oocytes have known roles in spermatogenesis. This observation and the all-male phenotypes of *rbpms2* mutants suggests that Rbpms2 is a translational repressor of male RNAs. Accordingly, inactivation of Rbpms2 or its absence, would cause GCs to switch from female identity to male fate. Consistent with this model, genetic and epistasis analysis place *rbpms2* upstream of *dmrt1*, a key regulator of male differentiation. However, unlike mutants lacking *rbpms2* or *dmrt1* alone, *rbpms2; dmrt1* double mutants are sterile, thus Rbpms2 may promote translation of factors required for

female fates. Accordingly, we found targets that phenocopy Rbpms2 loss-of-function and link acquisition of female fate to regulation of nutrient sensing pathways. Our findings indicate that undifferentiated GCs in the female germline are endowed with RNAs to drive both male and female differentiation programs, thus accounting for their plasticity. In this model, a binary fate decision, acquisition of male or female GC fate is governed by Rbpms2, which acts as a program switch to repress male fate and promote female identity.

Real-time monitoring of Fgf8 propagation during Zebrafish gastrulation and validation of its role as a morphogen

SESSION: GERM LINE, EARLY DEVELOPMENT AND PATTERNING I

Rohit Krishnan Harish[1], *Michael Brand*[2]

[1] *Center for Regenerative Therapies Dresden CRTD- Technische Universität Dresden TUD, Ph.D. Student, Dresden, Germany*
[2] *Center for Regenerative Therapies Dresden- Technische Universität Dresden, Professor- Director, Dresden, Germany*

ABSTRACT TEXT

The induction of distinct cell types from a field of naïve cells is a fundamental challenge faced by all multicellular organisms during development. One mechanism to achieve this is to utilize signaling molecules known as morphogens. These molecules are secreted from a localized source, distribute through the target tissue in a graded manner and induce distinct cellular responses depending on different concentration thresholds. The Fibroblast growth factor 8 (Fgf8) is one such molecule which performs inductive roles in mesoderm formation, neural patterning and organogenesis in vertebrates. During Zebrafish gastrulation, Fgf8 transcripts are detected at the embryonic margin and its target genes are expressed at increasingly broader domains away from the source. A previous study relied on mRNA micro-injection to show that tagged-Fgf8 produced from an artificial source in the embryo is capable of forming a protein gradient. However, a complete study detailing the morphogenic activity of endogenous Fgf8 has been lacking. In our study, we use an Fgf8-EGFP knock-in fish line, generated using CRISPR/Cas9, to monitor endogenous Fgf8 propagation in the developing neural plate during gastrulation. By combining sensitive imaging techniques with single-molecule Fluorescence Correlation Spectroscopy, we demonstrate that Fgf8 produced at the embryonic margin, moves by free diffusion through the extracellular spaces and forms a graded distribution towards the animal pole. Overlaying this Fgf8 gradient with expression profiles of its downstream targets has enabled us to determine the input-output ratio of Fgf8 mediated patterning. Furthermore, through micro-injection of membrane-tethered versions of Fgf8, we show that its diffusion from an ectopic source is indeed necessary for patterning the surrounding tissue. Taken together, our work establishes that Fgf8 functions as a morphogen during Zebrafish gastrulation, with its diffusion being critical in achieving this function.

Nodal signalling defines a competency window for the separation of the endodermal from mesodermal lineage through stochastic switching in cell fate

SESSION: GERM LINE, EARLY DEVELOPMENT AND PATTERNING I

Andrew Economou[1], *Luca Guglielmi*[1], *Philip East*[2], *Ameila Edwards*[3], *Caroline Hill*[1]

[1] *The Francis Crick Institute, Developmental Signalling Lab, London, United Kingdom*
[2] *The Francis Crick Institute, Bioinformatics and Biostatistics, London, United Kingdom*
[3] *The Francis Crick Institute, Advanced Sequencing, London, United Kingdom*

ABSTRACT TEXT

One of the most fundamental events in early development is the separation of the three germ layers: ectoderm, mesoderm and endoderm. In zebrafish, this process is regulated by the TGF-β related signalling ligand Nodal. We have previously demonstrated that Nodal signalling initiates a cascade whereby as well as promoting endoderm induction, Nodal is also required to suppress inhibitory Fgf signals, which themselves contribute to mesoderm induction. However, not all cells within the margin which experience high Nodal and low Fgf signalling are induced to an endodermal fate.

By combining timed drug treatments, with quantitative imaging and single cell transcriptomics, we show that during early epiboly sustained Nodal signalling in the ventrolateral margin is required to establish a bipotential progenitor state where cells initially fated

to become mesoderm can switch to an endodermal fate in a seemingly stochastic manner. While Nodal signalling is required for this cell fate switch, Fgf signalling modulates the likelihood of switching, with switching more likely if Fgf signalling is reduced. Unlike endodermal progenitors, which do not require sustained signalling inputs once induced, the mesodermal progenitor state requires sustained Nodal and Fgf signalling until gastrulation, at which point cells commit to the mesodermal lineage and diverge both molecularly and behaviourally from the endoderm.

Therefore, we show that the separation of the germ layers is not simply a readout of relative signalling profiles at the margin of the embryo in a deterministic manner. Rather, we propose a more stochastic process, where Nodal signalling effectively establishes a window of competency during which cells can switch from a mesodermal fate to an endodermal fate, with the likelihood of switching and the length of the window determining the size of the endodermal population.

Neurobiology I

Shining a light on the enteric nervous system

SESSION: NEUROBIOLOGY I

Gilles Vanwalleghem[1], *Rajlakshmi Sawale*[1], *Riley Heather*[1], *Ethan Scott*[1]

[1] *The University of Queensland, Queensland Brain Institute, Brisbane, Australia*

ABSTRACT TEXT

The gut-brain axis is a physiological communication network between the microbiome, enteric and central nervous system. The microbiome interacts directly with the enteric nervous system (ENS), a complex network that drives and regulates multiple gut functions such as motility, homeostasis, and nutrient uptake. The ENS interacts with the central nervous system (CNS) in multiple ways, through endocrine and immune signalling, as well as through direct neuronal signalling via the vagus nerve.

The gut microbiome can affect behaviour and cognition, and patients with psychiatric (anxiety, depression) or neurological disorders (autism spectrum disorders, Parkinson's disease) often show gastrointestinal comorbidities.

Our goal is to understand the bidirectional communication between the gut microbiome and the nervous system, and how dysregulation of this communication can affect behaviour. As a transparent vertebrate animal, and with powerful light-based tools to monitor and manipulate neurons, the larval zebrafish is a powerful model in neuroscience. Our first step is to characterize the functional development of the ENS in larval zebrafish and investigate the influence of the gut microbiome on its development. We are using light sheet microscopy to image the activity of the ENS neurons in 3 to 7 days post fertilization (dpf) larvae using a nuclear-targeted genetically encoded calcium indicator (GECI), GCaMP6s in wild-type and germ-free zebrafish larvae. We observed a transition period where the spontaneous activity increases while the network self organizes similar to what was observed in the optic tectum of larval zebrafish. In order to understand how the network of neurons in the ENS is anatomically organized, we are also carrying out serial block face scanning electron microscopy analysis of 5dpf larval zebrafish. This study will lay the foundations for future studies of the gut-brain axis in disease models.

Gigaxonin-E3 ligase controls the initiation of Sonic Hedgehog signaling to sustain motor neuron specification and motility in zebrafish

SESSION: NEUROBIOLOGY I

Pascale Bomont[1], *Karolina Mysiak*[1], *Yoan Arribat*[2], *Léa Lescouzères*[1], *Alexia Boizot*[3], *Maxime Ruiz*[4], *Mireille Rossel*[5]

[1] *INSERM U1051, Neurosciences, Montpellier, France*
[2] *UNIL-CHUV, Sciences Biomédicales, Lausanne, Switzerland*
[3] *UNIL-CHUV, Biologie médicale, Lausanne, Switzerland*
[4] *Stein Eye Institute, Health, Los Angeles, USA*
[5] *MMDN, Neurosciences, Montpellier, France*

ABSTRACT TEXT

The Hedgehog family of morphogens represents an evolutionarily conserved pathway essential for embryonic development, tissue homeostasis, and tumorigenesis. In vertebrates, Sonic Hedgehog (Shh) assigns neuronal and muscle fate, acting in a graded manner to pattern the dorso-ventral axis of the neural tube and the muscles. The last decade has shown that ubiquitination serves as a pivotal mechanism in regulating Shh activity but the identity of the E3 ligase(s) that control(s) the initiation of signaling is unknown.

With a long-lasting interest in a severe neurodegenerative disease called giant axonal neuropathy (GAN), we show here that the gigaxonin-E3 ligase governs Shh induction, by controlling the degradation of the Shh-bound Patched receptor. Using both morpholino oligonucleotides and CRISPR technology, we demonstrate that, similarly to Shh inhibition, repression of gigaxonin in zebrafish impairs motor neuron specification and somitogenesis, and abolishes neuromuscular junction formation and locomotion. Moreover, we evidence that Shh signaling is impaired in gigaxonin null zebrafish and is corrected by both pharmacological activation of the Shh pathway and human gigaxonin. The gigaxonin-dependent inhibition of Shh activation is also seen in different biological systems, in primary fibroblasts from GAN patients and in a Shh activity reporter line depleted in gigaxonin.

Altogether, our findings establish gigaxonin as a key E3 ligase that positively controls the initiation of Shh transduction to sustain motor neuron specification and motility in zebrafish. Also, this work presents the first phenotypcial animal model for GAN, and provides the first hints for the hypothesis that the human GAN pathology has a developmental origin.

Purinergic signaling selectively modulates maintenance but not repair neurogenesis in the zebrafish olfactory epithelium

SESSION: NEUROBIOLOGY I

Mehmet Can Demirler[1], *Uğurcan Sakızlı*[1,2], *Burak Bali*[1,3], *Yiğit Kocagöz*[1], *Sema Elif Eski*[1,4], *Arda Ergönen*[1], *Aysu Şevval Alkiraz*[1], *Xalid Bayramli*[1], *Thomas Hassenklöver*[5], *Ivan Manzini*[5], *Stefan H. Fuss*[1]

[1] *Boğaziçi University, Department of Molecular Biology and Genetics, Istanbul, Turkey*
[2] *Kobe University, Faculty of Medicine, Kobe, Japan*
[3] *University of Göttingen Medical Center, Institute of Auditory Neuroscience and InnerEarLab, Göttingen, Germany*
[4] *Université de Libre Bruxelles, Institut de Recherche Interdisciplinaire en Biologie Humaine et moléculaire, Brussels, Belgium*
[5] *Justus Liebig University Giessen, Department of Animal Physiology and Molecular Biomedicine, Giessen, Germany*

ABSTRACT TEXT

The peripheral olfactory epithelium (OE), different from other neuronal structures, has the unique capacity to continuously generate new olfactory sensory neurons (OSNs) and to regenerate efficiently after acute injury. OSN neurogenesis must be tightly controlled, as both over- and underproduction of nerve cells would be detrimental to olfactory function. However, the underlying signals that regulate stem/progenitor cell activity are only poorly understood. Here we examine the contribution of purinergic signaling to OSN neurogenesis from basal progenitors in zebrafish, using Ca^{2+}-imaging of purinergic responses, molecular identification of purine-sensitive cell types, and cell proliferation assays after purine stimulation.

To identify non-neuronal cells that are implicated in OSN neurogenesis, we studied physiological responses to a series of purine compounds by measuring Ca^{2+}-signals in acute tissue slices through the OE. We observed ATP-responding cells throughout the basal and intermediate layers of the OE, which could be partially blocked by the P2Y receptor antagonist Suramin.

To further characterize these cells, we performed morphometric comparisons of purine-responsive cells and cell populations expressing cell type-specific molecular markers. Most ATP-sensitive cells correlated with cells expressing the stem cell marker Sox2, which could be further subdivided into a Cytokeratin II-positive glia and a basal Krt5-positive progenitor cell population.

To test the biological relevance of purinergic signaling in the OE directly, we stimulated adult fish by intraperitoneal injection of ATP and observed an increase in neurogenic cell proliferation in the OE, which was revertible by Suramin treatment. The results suggest that purine release in the OE from damaged or dying OSNs stimulates OSN neurogenesis from purine-sensitive progenitor cells to ensure a constant number of OSNs over time.

Work on this study was supported by TÜBİTAK grant 113T038 and BMBF grant 1364480.

Regulation of axonal growth and synaptogenesis by the cytoplasmic pool of core spliceosomal protein SNRNP70

SESSION: NEUROBIOLOGY I

Nikolas Nikolaou[1], *Fursham Hamid*[2], *Patricia Gordon*[2], *Richard Taylor*[2], *Corinne Houart*[2]

[1] *University of Bath, Biology and Biochemistry, Bath, United Kingdom*
[2] *King's College London, Centre for Developmental Neurobiology, London, United Kingdom*

ABSTRACT TEXT

Neural circuit function and ultimately animal behavior depend on the precise formation of synaptic connections in the brain. RNA processing plays a major role in circuit development and is carried out by an army of RNA-binding proteins. Mutations in genes encoding such factors are often associated with neurological conditions, demonstrating the important roles RNA-binding proteins have for nervous system function. Spliceosomal proteins is a major class of RNA-binding proteins and they drive constant and alternative splicing of pre-mRNAs, processes known to take place in the nucleus of all eukaryotic cells. Recent evidence indicated the presence of spliceosomal proteins in neurites; however, their cytoplasmic roles are not yet understood. We found SNRNP70, a core component of the U1 snRNP particle, to be localized in cytoplasmic puncta closely associated with RNA granules inside axons. We thus hypothesized that the cytoplasmic pool of SNRNP70 regulates neuronal connectivity. We first generated a zygotic null *snrnp70* mutant and found that homozygous null embryos show widespread cell death in the brain likely due to the important roles of nuclear SNRNP70 as part of the spliceosome. We thus focused our examinations in the largely unaffected spinal cord and identified motor axonal growth and branching phenotypes as well as neuromuscular synaptogenesis defects. Through cell transplantation experiments we found that SNRNP70 is required cell-autonomously in motor neurons for the clustering of acetyl-choline receptors at the neuromuscular junction. To our surprise these neuromuscular defects can be restored by transgenic expression of a SNRNP70 variant whose localization is restricted to the cytoplasm, demonstrating that the axonal pool of SNRNP70 is an important regulator of neuromuscular connectivity. Finally, we show that cytoplasmic SNRNP70 is functionally required for the processing of a restricted set of neuronal transcripts during development.

Emerging Technologies

The Flamingo project makes custom, high-end microscopy more accessible

SESSION: EMERGING TECHNOLOGIES

Michael Weber[1], *Jan Huisken*[1], *Joe Li*[1], *Todd Bakken*[1], *Kurt Weiss*[1]

[1] *Morgridge Institute for Research, Medical Engineering, Madison, USA*

ABSTRACT TEXT

Whenever a new microscopy technology gets presented, researches want to evaluate and use it for their own imaging ideas: You can collaborate with the inventor, try to build your own copy or wait until it becomes available commercially or in your core facility. None of these solutions might work in your particular situation or for your delicate sample.

We developed Flamingo, a modular and shareable light sheet microscope that travels to where you need it. The primary goal of the design process was to come up with a microscope that packs all the optical performance and most of the features of our existing, stationary microscopes in a compact and portable framework. The Flamingo supports different light sheet configurations and has a high level of modularity. We collaborated with many developmental biologists in several institutions who wanted to try light sheet microscopy, and we have used Flamingos for workshops and virtual talks. The microscopes performed well with a variety of biological samples, including zebrafish, worms and plants, even in long time-lapses. The results are on par with images recorded on our well-proven but complex and stationary microscopes. Best of all, the entire setup fits in two large suitcases and can be moved from lab to lab, packed in the trunk of a car, and shipped over long distances. Setting it up only takes about an hour and our software has unique remote-control options to supervise the microscope from afar.

With Flamingo, we revolutionize how and where high-powered research microscopes are setup and used. We aim to democratize light microscopy by bringing it to campuses and labs that may not be able to afford a commercial system. Our project has transformative benefits for biologists, as it allows them to do studies on living organisms close to where they reside. We see large potential in expanding our concept to areas beyond light sheet microscopy, and we would be happy to get new partners on board to push this idea forward.

Harmonic generation in-vivo imaging of the zebrafish cardiovascular system

SESSION: EMERGING TECHNOLOGIES

Guy Malkinson[1,2], *Pierre Mahou*[2], *Elodie Chaudan*[3], *Thierry Gacoin*[3], *Ali Sonay*[4], *Periklis Pantazis*[4], *Emmanuel Beaurepaire*[2], *Willy Supatto*[2]

[1] *College de France- Inserm, Center for Interdisciplinary Research in Biology CIRB, Paris, France*
[2] *Ecole Polytechnique- CNRS, Laboratory for Optics and Biosciences, Palaiseau, France*
[3] *Ecole Polytechnique- CNRS, Laboratory of Condensed Matter Physics, Palaiseau, France*
[4] *Eidgenössische Technische Hochschule ETH Zurich, Department of Biosystems Science and Engineering, Basel, Switzerland*

ABSTRACT TEXT

Zebrafish is a well-established model for studying the cardiovascular system. The transparency of the embryo makes it accessible for imaging, enabling high-resolution studies of developmental processes in the living embryo. Multiphoton (MP) microscopy (MPM) is adapted for in-vivo studies as it enables deep-tissue imaging and low photo-damage levels. MPM also generates, along the excitation axis, other "label-free" contrast mechanisms within the biological tissue, namely Second Harmonic Generation (SHG) and Third Harmonic Generation (THG). SHG and THG do not rely on fluorescent probes but can be simultaneously collected with them, yielding multi-modal detection (Evanko, Nat Meth, 2010). Here we report on our recent achievements in harnessing the advantages of label-free imaging in zebrafish to study its cardiovascular system in-vivo. (1) Using the orthogonal configuration of a MP light-sheet microscope (Truong et al., Nat Meth,2011; Mahou et al., Nat Meth, 2014), we achieved multi-modal imaging of embryos expressing fluorescent proteins in their blood vessels, injected with special nanoprobes (NP) that generate SHG also along the orthogonal axis. We successfully performed single-particle

microangiography by imaging the NPs in the blood flow at rates of up to 180 frames per second, and tracking their trajectories (Malkinson et al., ACS Photonics, 2020). (2) In a separate set of experiments, we used classical point-scanning microscopy and successfully collected SHG and THG signals from entire cardioplegic hearts in intact embryos, and further characterized and quantified cardiac structures in both wild type and mutant embryos. We demonstrate that harmonics imaging is an efficient and straightforward technique to study the zebrafish heart.Together these advances open up new opportunities to perform "label-free" multi-modal in-vivo imaging in zebrafish and thus to expand the understanding of biological physiological processes as they occur during development.

Single-cell phenotypic analysis and dual lineage tracing in real-time with a zebrafish genetic mosaic system

SESSION: EMERGING TECHNOLOGIES

Bing Xu[1], *Sarah Kucenas*[2], *Hui Zong*[1]

[1] *University of Virginia, Microbiology- Immunology and Cancer biology, Charlottesville, USA*
[2] *University of Virginia, Biology, Charlottesville, USA*

ABSTRACT TEXT

Gene knockout with high spatial resolution, achieved by conditional knockout (CKO) in mice, is critical for studying gene functions in vivo. As a model organism, Zebrafish provides unique advantage with its transparent body, enabling real-time studies of development and disease progression. However, it's challenging to establish CKO in zebrafish due to technical difficulties of making floxed alleles. Even if successful, tissue-level CKO is still not optimal for spatial resolution, especially to distinguish cell autonomous from non-cell autonomous gene functions. Here, we present a novel genetic mosaic system, termed zMADM (zebrafish Mosaic Analysis with Double Markers), adopted from the mouse MADM system. Via Cre/loxP mediated *inter*-chromosomal mitotic recombination, MADM generates sporadic, GFP^+ mutant cells along with their RFP^+ sibling WT cells. Key features of MADM include: first, the color-genotype matching is 100 %; second, sparse, well-labeled cells allow for single-cell resolution analysis that distinguishes cell autonomous from non-cell autonomous gene functions; and third, RFP^+ WT cells serve as the perfect internal control for GFP^+ mutant sibling cells to reveal even the subtlest phenotypes. Due to these appealing features, mouse MADM was broadly adopted in many fields and resulted in high impact findings, including neurosciences, developmental biology, and cancer biology, etc. To create zMADM, we knocked the MADM cassettes into the pre-selected genomic region with CRISPR/Cas9. The successful establishment of zMADM was then confirmed by injecting Cre mRNA or plasmids into the eggs of zMADM zebrafish, achieving a labeling efficiency of ~0.5 %, sparse enough for single-cell analysis. Finally, we used live imaging to witness the birth of two sibling cells and their subsequent development in zMADM, demonstrating its application for dual lineage tracing. Once broadly distributed, we anticipate that zMADM should help unleash the full power of zebrafish genetics.

Cre-controlled CRISPR (3C) provides an easy method for conditional gene inactivation in zebrafish

SESSION: EMERGING TECHNOLOGIES

Stefan Hans[1], *Daniela Zöller*[1], *Anja Machate*[1], *Michaela Geffarth*[1], *Michael Brand*[1]

[1] *Technische Universität Dresden, CRTD - Center for Regenerative Therapies Dresden, Dresden, Germany*

ABSTRACT TEXT

Gene inactivation is a powerful tool to determine gene function. Conventional germline inactivation of a gene however often results, due to its consequences for all cells, in deleterious effects, or even embryonic lethality, which impedes the analysis of gene function at later stages. To solve this issue, conditional gene inactivation strategies have been developed. The most commonly used technique employs the Cre/loxP system in which $CreER^{T2}$ variants provide additional temporal control. Cre recombinase promotes strand exchanges between two loxP target sites and depending on their orientation, recombination results either in the excision or inversion of the intervening DNA sequence. Thus, conditional gene inactivation can be achieved in a Cre-dependent manner if a gene or critical exon is flanked by loxP sites. However, although CRISPR/Cas9 technology has greatly improved genome editing, targeting loci with two loxP sites is time and labor consuming.

Here, we propose Cre-controlled CRISPR ('3C') as an easy and straightforward system that allows conditional gene inactivation in a Cre-dependent manner. 3C requires only the generation a stable transgenic line which harbors a Cre effector construct expressing Cas9 after a successful recombination event. In addition, the same transgenic construct is used to express one or more gRNAs under a ubiquitous U6 promoter. As a proof-of-principle, we used a well-established target site in *tyrosinase*, the gene required for converting tyrosine into the pigment melanin. We demonstrate the functionality of 3C using Cre mRNA injections and various Cre/CreER^{T2} driver lines, resulting in loss of pigmentation in a Cre-dependent manner. Moreover, we will present results from our genome editing sequence analysis confirming high level mutagenesis achieved in recombined cells. Our data show that the '3C' conditional gene inactivation system is simple and fast, and allows for conditional manipulation of multiple genes simultaneously.

Disease Models I

Functional evaluation of catastrophic epilepsy genes: The Epilepsy Zebrafish Project (EZP)

SESSION: DISEASE MODELS I

Colleen Carpenter[1], Aliesha Griffin[1], Jing Liu[1], Rosalia Paterno[1], Kyla Hamling[1], Maia Moog[1], Matthew Dinday[1], Brian Grone[1], Francisco Figueroa[1], Mana Anvar[1], Chinwendu Ononuju[1], Scott Baraban[1]

[1] *University of California- San Francisco UCSF, Neurological Surgery, San Francisco, USA*

ABSTRACT TEXT

Catastrophic childhood epilepsies lead to medically intractable early-life seizures accompanied by debilitating neurodevelopmental, cognitive and behavioral problems. Patient exome sequencing identified ~70 *de novo* single-gene mutations in this rare disease population. Unfortunately, our understanding and treatment of these epilepsies is limited. Zebrafish (*Danio rerio*) are an attractive system for modeling epilepsy, and previously used by our laboratory to study Dravet Syndrome (Baraban et al. Nat. Comm. 2013; Griffin et al. Brain 2017; Griffin et al. Brain Comm. 2019). Here, we present the first large-scale study to define functional consequences of monogenic epilepsy gene mutations using zebrafish. Regions of similarity between zebrafish and human were identified using BLAST and gene expression levels assessed between 0 and 7 days post-fertilization (dpf). Loss-of-function zebrafish lines were generated for 40 genes using clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 gene editing and raised to F3 or greater. Larvae from all 40 lines were screened using electrophysiological, survival, behavioral, and morphological assays (3–14 dpf) with *post hoc* genotyping. Spontaneous ictal-like seizure activity, characterized as long-duration, large-amplitude, multi-spike events were confirmed in 8 lines; *arxa*, *eef1a2*, *gabrb3*, *pnpo*, *scn1lab*, *strada, grin1b* and *stxbp1b*. Early fatality was noted in *aldh7a1*, *depdc5*, *sik1* and *scn1lab* mutant lines. Zebrafish mutants for 3 of these epileptic lines were also noted to have abnormal activity in a series of behavioral assays. One mutant line, *arxa*, was shown to have reduced numbers of *Dlx-GFP* expressing interneurons assessed using volumetric light sheet imaging microscopy. Overall, our work shows that zebrafish can be used to rapidly and efficiently model rare genetic epilepsies. We envision these zebrafish lines as a starting point for further functional analysis and/or identification of new therapies.

Genetic variants associated to type 2 diabetes modulate endocrine enhancers in vivo

SESSION: DISEASE MODELS I

Ana Eufrásio[1], *Chiara Perrod*[1], *Fábio J. Ferreira*[1], *Marta Duque*[1], *Mafalda Galhardo*[1], *José Bessa*[1]

[1] *i3s - Instituto de Investigação e Inovação em Saúde, Instituto de Biologia Molecular e Celular, Porto, Portugal*

ABSTRACT TEXT

Type 2 diabetes (T2D) is a complex disease partially characterized by endocrine pancreatic dysfunction. Several genome-wide association studies have shown an association between single nucleotide polymorphisms (SNPs) and T2D, being the vast majority of these variants located in putative endocrine pancreatic enhancers. This suggests that these SNPs may modulate the enhancer activity and, consequently, gene expression. We have performed *in vivo* mosaic transgenesis assays in zebrafish to test the enhancer activity of sequences overlapping with T2D associated *loci*. We found that six out of ten tested sequences are endocrine pancreatic enhancers. The risk variant of two sequences decreased enhancer activity, while in another two incremented it. One of the latter (rs13266634) is located in a *SLC30A8* exon, encoding a tryptophan-to-arginine substitution described as the cause of the SLC30A8 impairment, being this the canonical explanation for T2D risk association. However, other T2D associated SNPs that truncate SLC30A8, confer protection against T2D, contradicting this explanation. Here, we clarify this incongruence by showing that rs13266634 boosts the activity of an overlapping enhancer (seq132) suggesting a *SLC30A8* gain-of-function as the cause for the increased risk for T2D. Further exploring seq132, we found a single nucleotide variation in a putative binding site for PDX1, an important endocrine pancreatic transcription factor. Additionally, we observed a chromatin interaction between seq132 and *Slc30a8* promoter gene using murine cells. Targeting the seq132 using CRISPR, we showed that seq132 enhancer belongs to the regulatory landscape of *Slc30a8*. Overall, this work uses an *in vivo* system to validate endocrine pancreatic enhancers that overlap with T2D associated SNPs, showing several cases where nucleotide variations may result in complex enhancer modulations, including a poorly understood case where a coding SNP changes the activity of an enhancer.

Abnormal spine development and highly mineralized inclusions in vertebrae in a zebrafish model of CHARGE syndrome

SESSION: DISEASE MODELS I

Maximilian Breuer[1], *Maximilian Rummler*[2], *Charlotte Zaouter*[1], *Bettina Willie*[2], *Kessen Patten Shunmoogum*[1]

[1] *INRS – Centre Armand Frappier Santé Biotechnologie, Biology, Laval, Canada*
[2] *Shriners Hospital for Children - Canada, Research, Montreal, Canada*

ABSTRACT TEXT

CHARGE syndrome is caused by mutations in the chromatin remodeller, CHD7. CHARGE patients, among other symptoms, present with features of idiopathic scoliosis in over 60 % of cases, reduced bone mineral density and osteopenia. Effective disease models are sparse, and the underlying mechanisms remain elusive. Here, we detect and quantitatively analyze skeletal abnormalities in adult *chd7*$^{-/-}$ zebrafish and young larvae.

Our study is the first to identify that young *chd7*$^{-/-}$ larvae present with scoliosis and kyphosis already at 9 dpf. Gene expression analysis confirmed the significant reduction of osteoblast markers and a delay in vertebrae mineralization. Using MicroCT analyses, we further characterized structural abnormalities in adult *chd7*$^{-/-}$ fish. All structural entities of the spine, Weberian apparatus, precaudal vertebrae and caudal vertebrae show altered morphology along with highly variable bone mineral density and bone volume. Strikingly, in *chd7*$^{-/-}$ fish we observed highly mineralized inclusions in the vertebral structure. Finally, we detected a specific depletion in the expression of *col2a1a* in vertebral cartilage along with a significantly reduced number of chondrocytes.

Our study is the first to elucidate the mechanisms underlying spinal development in both larvae and adult *chd7*$^{-/-}$ zebrafish resulting in decreased spinal integrity. To investigate the underlying pathways of spinal deformities in CHARGE syndrome, the *chd7*$^{-/-}$ zebrafish will be greatly advantageous.

Istaroxime treatment ameliorates calcium dysregulation in a zebrafish model for Phospholamban R14del cardiomyopathy

SESSION: DISEASE MODELS I

Sarah Kamel[1], Chantal van Opbergen[2], Charlotte Koopman[1,2], Arie Verkerk[3,4], Yeszamin Onderwater[1], Sonja Chocron[1], Clarissa Polidoro Pontalti[2], Marc Vos[2], Teun de Boer[2], Toon van Veen[2], Jeroen Bakkers[1,2]

1 *Hubrecht Institute-Royal Netherlands Academy of Arts and Sciences KNAW- University Medical Center Utrecht, Cardiac Development and Genetics, Utrecht, Netherlands*
2 *University Medical Center Utrecht, Medical Physiology- Division of Heart & Lungs, Utrecht, Netherlands*
3 *University of Amsterdam- Amsterdam University Medical Centers, Medical Biology- Amsterdam Cardiovascular Sciences, Amsterdam, Netherlands*
4 *University of Amsterdam- Amsterdam University Medical Centers, Experimental Cardiology, Amsterdam, Netherlands*

ABSTRACT TEXT

The heterozygous phospholamban (*PLN*) p.Arg14del (R14del) is a Dutch founder mutation identified in patients with dilated or arrhythmogenic cardiomyopathy. Patients suffer a high risk of developing malignant ventricular arrhythmias and end-stage heart failure, leading to high mortality and poor prognosis. Little is known about the physiological processes preceding PLN R14del induced cardiomyopathy, which is characterized by sub-epicardial accumulation of fibrofatty tissue, and a specific drug treatment is currently lacking. Here, we have generated a knock-in PLN R14del zebrafish model to better dissect the early mechanism and the underlying process that leads to remodeling and heart failure. Hearts from adult zebrafish with the R14del mutation display age-related remodeling with sub-epicardial inflammation and fibrosis. Echocardiography revealed contractile pulsus alternans before overt structural changes occurred, which correlated at the cellular level with action potential duration alternans. These functional alterations are preceded by diminished Ca^{2+} transient amplitudes in embryonic hearts. We found that istaroxime treatment ameliorates the *in vivo* Ca^{2+} dysregulation, rescues the cellular APD alternans, while it improves cardiac relaxation. Thus, we present novel insight into the pathophysiology of *PLN* R14del cardiomyopathy and identify istaroxime as a potential novel drug for its treatment.

Husbandry and Aquaculture

Anesthesia does not equal analgesia: pain management in zebrafish

SESSION: HUSBANDRY AND AQUACULTURE

Karin Finger-Baier[1], Lynne Sneddon[2], Simon Tinman[3], Bertrand Collet[4], Angeleen Fleming[5], Paul Schroeder[6], Ana Roque[7]

1 *Max Planck Institute of Neurobiology, Genes-Circuits-Behavior, Martinsried, Germany*
2 *University of Liverpool, Institute of Integrative Biology, Liverpool, United Kingdom*
3 *Bar Ilan University, Faculty of Life Sciences, Ramat Gan, Israel*
4 *Université Paris-Saclay, INRAE Virologie et Immunologie Moléculaires, Jouy-en-Josas, France*
5 *University of Cambridge, Dept. of Physiology- Development and Neuroscience, Cambridge, United Kingdom*
6 *Oxford University, Department of Biomedical Services, Oxford, United Kingdom*
7 *IRTA Institute of Agrifood Research and Technology, IRTA Sant Carles de la Ràpita, Sant Carles de la Ràpita, Spain*

ABSTRACT TEXT

Zebrafish, like all other vertebrates, are deemed sensitive to pain by the European directive 2010/63/EU. In addition, there are numerous scientific publications in support of fishes' ability to feel pain and there is also experimental evidence that pain in zebrafish can be alleviated by analgesics. Still, outside of pain research groups, most zebrafish researchers to this day use general anesthesia as means of pain

management during procedures and little is reported in terms of peri-operative provision of analgesics. The widespread use of anesthesia for pain management needs to be critically discussed and alternative options should be considered.

Effective pain management requires recognizing potentially painful procedures, the capability of assessing pain in zebrafish, profound knowledge of analgesia available for zebrafish and the refinement of peri-operative care.

Hence, FELASA, the Federation of European Laboratory Animal Science Associations, established the "FELASA working group on Pain management in zebrafish". Its aim is to propose good practice guidelines for the management of pain in zebrafish, both during and following potentially painful procedures, thus reducing fish suffering and improving peri-operative care.

We will highlight some of the working group's findings thus far and exchange experiences with the community regarding currently unpublished peri-operative care protocols.

Physiological effects of common disinfectants – guidelines for zebrafish egg disinfection

SESSION: HUSBANDRY AND AQUACULTURE

Geoffrey Aliti[1], *Adrian Ekehorn Gimdal*[1], *Lars Westberg*[1], *Petronella Kettunen*[1]

[1] *University of Gothenburg, Neuroscience and Physiology, Gothenburg, Sweden*

ABSTRACT TEXT

The advantages of using zebrafish (*Danio rerio*) keep emerging since their introduction to the scientific community as model animals for human biological processes. This is due to the fact that the genetic composition of zebrafish is similar to humans and that about 84 % of zebrafish genes are being associated with human diseases. This has led to increased use of zebrafish and establishment of zebrafish facilities all over the world. However, the appreciation of zebrafish as a model animal has come up with challenges, especially zebrafish disease transmission between and within facilities, due to sharing of mutant and transgenic lines. These diseases manifest as clinical and subclinical conditions, e.g. mycobacteria and pseudoloma infections. Therefore, biosecurity and 'egg only' practices have been encouraged for the facilities. To prevent disease spread, shared eggs can be treated by surface disinfection, while survival, development and physiology of the embryos should not be impaired by the treatments. At the same time, treatment conditions must ensure proper disinfection. Therefore, the aim of the study was to test the physiological effects of several common disinfectants on zebrafish eggs and embryos and present guidelines for egg disinfection based on these findings.

We tested different disinfectants of different pH and concentrations, including sodium hypochlorite, povidone-iodine (PVPI), and hydrogen peroxide. Eggs/embryos of different ages were placed in sieves, immersed in disinfectant and then rinsed. The effects of disinfection were tracked over time and included survival, morphological defects and behaviour.

We found that the physiological effects of the disinfectants were dependent on egg/embryo age, pH and concentration. Based on our findings, we will present guidelines for egg disinfection that will allow for proper disinfection of pathogens while promoting survival and normal development of the animals.

Sustainable microalgae products to improve zebrafish reproduction and larvae development

SESSION: HUSBANDRY AND AQUACULTURE

Patricia Diogo[1,2], *Gil Martins*[1,3], *Tamára Santos*[1], *Elsa Cabrita*[1,4], *Victória del Pino*[2], *João Navalho*[2], *João Varela*[1,4], *Paulo J. Gavaia*[1,3]

[1] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *Necton SA, Research and Development Unit, Olhão, Portugal*
[3] *Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal*
[4] *Faculty of Sciences and Technology, University of Algarve, Faro, Portugal*

ABSTRACT TEXT

Industrially produced microalgae are sustainable resources to improve zebrafish reproductive potential and reduce live feed. Investigating the effect of different commercial products for live feed enrichment is essential to improve zebrafish nutrition and reproduction. Our

objective was to investigate the reproductive performance of zebrafish fed Zebrafeed® (Sparos) and once or twice a week with artemia enriched with different commercial products: PhytoBloom® Green Formula (Necton SA), Red Pepper© (Bernaqua) and Selco® (INVE). PhytoBloom is a liquid concentrated of *Nannochloropsis* sp., which is the main microalgae used in aquaculture, due to its high EPA and ARA content and antioxidant properties, relevant for reproduction and gametes quality. *Nannochloropsis* sp. is commonly encapsulated in live feed for zebrafish husbandry to improve nutrition, stress management and fish welfare. The broodstock was established with males (n=20) separated from females (n=20), reproduced weekly. The reproductive performance was evaluated according to the number of eggs, hatching rate, egg lipid content, and gametes quality by sperm motility and plasma membrane integrity. The offspring quality was evaluated according to larvae length and dry weight. Males showed improved sperm total motility and membrane integrity in high DHA treatments (Zebrafeed, Red Pepper twice a week), which could support spermatogenesis, plasma membrane composition and fluidity. Females showed significantly higher egg total lipids content with *Nannochloropsis* sp. once a week, which can improve offspring development. Our work contributes to improve knowledge on zebrafish nutritional requirements for reproduction. Developing microalgae blends with suitable dietary composition for zebrafish adults and larvae will bring further improvements to reproductive performance and zebrafish husbandry.

Work funded by ZEBRABLOOM-ALG-01-0247-FEDER-039896, Foundation for Science and Technology through UIDB/04326/2020 and INTERREG-Portugal-Spain project 0055 ALGARED+ 5E.

Contemporary real-time PCR-based prevalence of infectious agents in laboratory zebrafish colonies (2015–2019)

SESSION: HUSBANDRY AND AQUACULTURE

Marcus Crim[1], *Marcia Hart*[1], *Cynthia Besch-Williford*[1], *Robert Livingston*[1]

[1] *IDEXX BioAnalytics, IDEXX BioAnalytics, Columbia, USA*

ABSTRACT TEXT

Clinical and subclinical spontaneous infections can have undesirable consequences for zebrafish research, including invalid or misinterpreted experimental data, zoonotic infections, or elevated zebrafish morbidity and mortality. Prevalence is an important risk factor for introducing an infectious agent to a zebrafish colony. We report the rate of positive real-time PCR results for 18 infectious agents in pooled fish and environmental samples submitted to a major commercial diagnostic laboratory over a 5-year period, representing 230 institutions globally, including academic and government institutions, pharmaceutical companies, and biotechnology companies. Prevalence for some agents varied widely among institutions. The overall percentage of positive test results varied by sample type, with higher positive rates in pooled fish samples for Zebrafish picornavirus and all common parasites except *Myxidium streisingeri* – believed to have a two-host life cycle with environmental stages (myxospore and actinospore). Conversely, a higher percentage of eight *Mycobacterium* spp., facultative pathogens that participate in system biofilms, tested positive in environmental samples compared to pooled fish samples. The most frequently detected agents in pooled zebrafish were Zebrafish picornavirus (26.5 %), *Pseudoloma neurophilia* (19.9 %), *M. streisingeri* (16.0 %), *M. chelonae* (9.7 %), *Pseudocapillaria tomentosa* (7.6 %), and *M. haemophilum* (2.6 %). The most frequently detected agents in environmental samples were *M. chelonae* (44.8 %), *M. fortuitum* (35.1 %), *M. streisingeri* (23.5 %), *M. gordonae* (21.8 %), *M. abscessus* (18.7 %), and Zebrafish picornavirus (16.7 %). Thus, institutional prevalence can differ from overall prevalence, and some pathogens may be missed by exporting institutions testing only less sensitive sample types. Obtaining health information including sampling methodology from exporting institutions is critical when assessing risk associated with the importation of zebrafish.

Regeneration I

Mapping the cellular dynamics of liver regeneration using targeted photoactivatable cell ablation

SESSION: REGENERATION I

Elke Ober[1], *Elizabeth Ambrosio*[1], *Charlotte Bailey*[1], *Iris Unterweger*[1], *Jes Dreier*[1], *Younes Barooji*[2]

[1] *University of Copenhagen, NNF Center for Stem Cell Biology - DanStem, Copenhagen, Denmark*
[2] *University of Copenhagen, Niels Bohr Institute, Copenhagen, Denmark*

ABSTRACT TEXT

The liver has an intricate tissue architecture, which has the capacity to regenerate following damage from injury or disease. However, the cellular behaviours and interactions that underpin the re-establishment of liver architecture during regeneration remain unclear. Here, we report the development and application of a novel optogenetic hepatocyte ablation tool, LIVERZAP, to examine the cell behavioural dynamics of liver regeneration *in vivo*. Upon activation with near-infrared light, LIVERZAP induces rapid, spatiotemporally controllable ROS-mediated liver injury in zebrafish. This leads to rapid and dynamic remodelling of the biliary ductal network concomitant with dramatic hepatocyte loss. We show that both liver architecture and mass are rapidly restored through de-differentiation of biliary epithelial cells (BECs) and predominantly progenitor-cell driven regeneration. The rapid injury paradigm of LIVERZAP, only 12 min, allows for *live*-imaging of both injury and recovery phases. By light sheet imaging, we show that a temporal wave of cell proliferation, as well as a subset of actively migrating BECs rebuilt the 3D biliary network, challenging the idea that restoration of tissue architecture is predominantly passive and driven by mitotic pressure pushing neighbors into place. We propose that active migration is a critical component of liver regeneration.

Single-cell analysis of the regenerative niche in the zebrafish heart

SESSION: REGENERATION I

Bo Hu[1], *Bastiaan Spanjaard*[1], *Sara Lelek*[2], *Daniela Panakova*[2], *Jan Junker Philipp*[1]

[1] *Max-Delbrück-Center for Molecular Medicine, Berlin Institute for Medical Systems Biology, Berlin, Germany*
[2] *Max-Delbrück-Center for Molecular Medicine, Electrochemical Signaling in Development and Disease, Berlin, Germany*

ABSTRACT TEXT

The zebrafish heart has the remarkable capacity to fully regenerate after a cryoinjury mimicking human myocardial infarction. It is well established that new cardiomyocytes are generated by dedifferentiation and proliferation of remaining cardiomyocytes, and it has been shown that instructive signals for cardiomyocyte regeneration emanate from other cellular sources, such as epicardium and immune cells. However, the cellular composition of the regenerative niche and the molecular interactions between the individual cell types remain largely unclear. Here, we systematically dissect the cell type diversity of the regenerating heart by single-cell RNA-seq of in total >300,000 cells at different time points throughout the regeneration process. We observe a large diversity of novel cell types, in particular among immune cells and fibroblasts, some of which are completely absent in the uninjured heart. Our results demonstrate that fibrosis is mediated by a multitude of cell types, including bona fide fibroblasts but also cell types with other main functions. Interestingly, many of these fibroblast-like cell types express regulatory factors that are implicated in heart regeneration. We systematically assess the origin, location and function of these putative cellular drivers of heart regeneration by combining CRISPR/Cas9 lineage tracing, computational pseudo-time analysis, single-molecule fluorescent in situ hybridization, and computational ligand-receptor analysis. We find that the cell types of the regenerative niche have distinct origins and locations, which, together with their transcriptome, yields valuable information about their function. Specifically, we validate the role of perivascular cells for neovascularization after injury in functional experiments. In summary, this work establishes the foundation for a systematic understanding of cellular drivers of heart regeneration.

Determining the gene regulatory network for hair cell regeneration in the zebrafish adult inner ear at single cell resolution

SESSION: REGENERATION I

Erin Jimenez[1], Wei Song[2], Ivan Ovcharenko[2], Shawn Burgess[1]

[1] *National Human Genome Research Institute, Translational and Functional Genomics Branch, Bethesda, USA*
[2] *National Center for Biotechnology Information, Computational Biology Branch, Bethesda, USA*

ABSTRACT TEXT

Millions of Americans experience a hearing or balance disorder due to permanent hair cell. The hair cells are the mechanosensory cells used in the auditory and vestibular organs of all vertebrates and in the lateral line systems of aquatic vertebrates. In zebrafish and other non-mammalian vertebrates, hair cells turn over during homeostasis and regenerate completely after being destroyed or damaged by acoustic or chemical exposure, while in mammals, destroying or damaging hair cells results in permanent impairments to hearing/balance. Our goal is to identify in vivo the enhancers that are involved in repairing a functional vertebrate inner ear. We developed a transgenic zebrafish to permit conditional and selective ablation of hair cells in the adult zebrafish inner ear using the human diphtheria toxin receptor (hDTR) gene under the control of the *myo6b* promoter. Cell ablation is achieved by injection of diphtheria toxin. We investigated the genome-wide epigenome and transcriptome of single cells from the inner ear at consecutive time-points following hair cell ablation of adult zebrafish. We identified open chromatin locations using single-cell ATAC-seq. Using machine learning on the induced open chromatin revealed transcription factor motif patterns in each cell type as a consequence of regeneration. We detected a pattern of overlapping Sox- and Six-family transcription factors, suggesting a combinatorial program of TF determining cell responses and cell fate. We correlated enhancer activation with trascription (based on scRNA-seq) from the genomic region to link and identify gene regulatory networks. Correlation of RNA expression and chromatin accessibility in single cells revealed the genetic program that occurs in a regenerating inner ear. Correlating cell type, enhancer activation, and transcription factor expression allowed us to begin to understand the combinatorial "code" of TF's that first initiate regeneration and then instruct hair cell differentiation.

Single-cell transcriptomics reveals several populations of cardiac-resident leukocytes in the regenerating zebrafish heart

SESSION: REGENERATION I

João Cardeira-da-Silva[1], Bo Hu[2], Stefan Guenther[3], Michail Yekelchyk[3], Jens Preussner[4], Philipp Junker[2], Didier Stainier[1]

[1] *Max-Planck-Institute for Heart and Lung Research, Department of Developmental Genetics, Bad Nauheim, Germany*
[2] *Max Delbrück Center for Molecular Medicine, Berlin Institute for Medical Systems Biology, Berlin, Germany*
[3] *Max-Planck-Institute for Heart and Lung Research, Department of Cardiac Development and Remodelling, Bad Nauheim, Germany*
[4] *Max-Planck-Institute for Heart and Lung Research, Bioinformatics Core Unit BCU, Bad Nauheim, Germany*

ABSTRACT TEXT

The adult mammalian heart is unable to regenerate following myocardial infarction. Post-ischemic tissue remodeling involves the formation of a permanent scar that impairs cardiac function. In contrast, the zebrafish heart fully regenerates upon ventricle cryoinjury through processes involving the initial formation of a scar, cardiomyocyte cell-cycle reentry, progressive scar resolution, and formation of new functional tissues[1]. The immune response is thought to modulate the regenerative outcome[2]. Yet, given its complexity, the regenerative properties of particular immune populations remain to be identified. Here, we aimed to identify and characterize immune cell (leukocyte) subsets with potential pro-regenerative roles during zebrafish cardiac regeneration.

Single-cell transcriptomic data revealed 6 major leukocyte populations, with defined temporal profiles throughout zebrafish heart regeneration. Together with cyto/histological analyses, we identified macrophage and neutrophil-like subsets present in the uninjured heart, indicative of cardiac-resident leukocytes. One of these populations is composed of antigen-presenting *epdl1*-positive macrophages/dendritic cells under tight differentiation control. These resident leukocytes likely contribute to both early pro-inflammatory and

intermediate-to-late inflammation-resolving macrophage populations, thus limiting the inflammation levels following injury. In addition, resident neutrophil-like cells potentially shift towards *granulin*-positive pro-inflammatory neutrophils at early, but not at intermediate-to-late stages.

This work constitutes a basis to understand the immune regulation of heart regeneration in a regenerative model, with prospective therapeutic value for regeneration-incompetent hearts. Moreover, it sheds light onto cardiac-resident leukocytes as quick responders to cardiac injury and modulators of regeneration.

1. González-Rosa et al. 2017. Regeneration 4:105-123.
2. Lai et al. 2017. eLife 6:e25605.

Cancer

The Investigation of the Role of VHL-HIF Signaling in DNA Repair and Apoptosis in Zebrafish

SESSION: CANCER

Hyejeong Rosemary Kim[1], *Kiran Santhakumar*[2], *Eleanor Markham*[1], *Davide Baldera*[1], *David Greenald*[3], *Helen Bryant*[4], *Sherif El-Khamisy*[5], *Freek van Eeden*[6]

[1] *University of Sheffield, Biomedical Science, Sheffield, United Kingdom*
[2] *SRM Institute of Science and Technology, Department of Genetic Engineering, Kattankulathur, India*
[3] *University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, United Kingdom*
[4] *University of Sheffield, Department of Oncology & Metabolism, Sheffield, United Kingdom*
[5] *University of Sheffield, Department of Molecular biology and Biotechnology, Sheffield, United Kingdom*
[6] *University of Sheffield, Biomedical Science, Sheffied, United Kingdom*

ABSTRACT TEXT

VHL is a multifunctional tumor suppressor. The lack of its function leads to various tumors, among which ccRCC provides the most serious outcome due to its resistance to chemotherapies and radiation therapies. Reflecting its best studied role of VHL in oxygen dependent regulation of HIFα subunit, HIF is highly activated in ccRCC. Although HIF promotes the progression of ccRCC, however, the precise mechanism by which the loss of VHL leads to tumor initiation remains unclear. There has been a number of studies demonstrating the HIF independent role of VHL that could also be important for ccRCC development, e.g. regulation of extracellular matrix, microtubule stabilization and DNA repair. The present study took advantage of two zebrafish *vhl* mutants, *vhl and vll*, and *Tg(phd3::EGFP)*^{i144} transgenic fish to investigate the roles of Vhl in DNA repair and apoptosis as crucial functions of Vhl in the tumor development. Using *vhl+/-;Tg(phd3::EGFP)*^{i144} as a unique tool to study the loss of heterozygosity at the *vhl* locus, we discovered that the role of human VHL in DNA repair is conserved in zebrafish *vll*. Interestingly, we also found that Hif activation strongly suppressed genotoxic stress induced DNA-repair defects and apoptosis in *vll* and *brca2* mutants, and in embryos lacking ATM activity. These results suggest the potential of HIF as a clinical modulator that can protect cells from accumulating DNA damage and apoptosis for the prevention of cancers and neurodegenerative disorders associated with accumulation of DNA damage. In addition, in more advanced ccRCC, targeting HIF could overcome the resistance of tumors to the chemo and radio therapies, and zebrafish *vhl-/-;vll-/-*embryos may well provide an excellent platform for drug discovery through chemical screening.

The role of P97 segregase in the repair of DNA-protein crosslink in vivo using CRISPR/Cas9 gene editing in zebrafish

SESSION: CANCER

Cecile Otten[1], *Christine Supina*[1], *Ivan Anticevic*[1], *Marta Popovic*[1]

[1] *Ruder Boskovic Institute, Marine and Environmental Research, Zagreb, Croatia*

ABSTRACT TEXT

DNA-Protein-Crosslinks (DPCs) are DNA lesions which occur when proteins become irreversibly covalently bound to DNA, thereby physically blocking all DNA transactions including chromatin remodeling, replication and transcription. DPC repair (DPCR) is a specialized DNA damage repair pathway in which crosslinked proteins are proteolytically cleaved and degraded. Recently, the specific roles of several enzymes in DPCR have been uncovered, and their dysfunction has been linked to ageing, cancer and neurodegenerative diseases. Among them, the P97/CDC48/VCP hexameric AAA ATPase is a well-known segregase which extracts proteins from various cellular compartments and targets them for degradation. In order to elucidate the specific role of P97 in DPCR *in vivo*, we are currently generating a P97 mutant with reduced ATPase activity (K524A mutation), as complete loss of P97 is embryonic lethal. To introduce the K524A mutation, we are using the CRISPR/Cas9 knock-in method developed by Hoshijima et. al. (Dev. Cell, 2016) to replace a stretch of endogenous DNA by a donor sequence of interest. Here, the donor sequence contains the mutation at the desired genomic location and a *cryaa:mVenus* fluorescent reporter for the selection of embryos with successful integration. In parallel, we utilize the human cell line RPE1 to assess the DPCR upon controlled loss of P97, as described in Kaulich et. al. (Nucleic Acids Res., 2015). First, the endogenous P97 locus is manipulated using Crisp/Cas9 to introduce a modified allele. Then the WT allele, the modified allele, or both can be targeted using allele-specific siRNAs. The DPCR is quantified by measuring the repair of a model DPC, hOgg1 using strand-specific primer extension qPCR as described in Chesner and Cambell (DNA Repair, 2018). Furthermore, we will investigate the epistasis of P97 with SPRTN, a central component of DPCR. Taken together, our results will unravel the role of P97 during the orchestration of DPC repair *in vivo*.

Using zebrafish to unravel the role of succinate dehydrogenase in tumour development

SESSION: CANCER

Margo Dona[1], *Selma Waaijers*[2], *Susan Richter*[3], *Antonius van Herwaarden*[2], *Graeme Eisenhofer*[3], *Richard Rodenburg*[4], *Henri Timmers*[1]

[1] *Radboud University Medical Center, Department of Internal Medicine, Nijmegen, Netherlands*
[2] *Radboud University Medical Center, Department of Laboratory Medicine, Nijmegen, Netherlands*
[3] *Institute of Clinical Chemistry and Laboratory Medicine, Department of Medicine III, Dresden, Germany*
[4] *Radboud University Medical Center, Department of Pedriatics, Nijmegen, Netherlands*

ABSTRACT TEXT

Background: Pheochromocytomas or paragangliomas (PPGLs) are chromaffin-cell tumours arising from the adrenal medulla and extra-adrenal paraganglia. Mutations in the *β-subunit of the succinate dehydrogenase* (*SDHB*) are associated with a high risk of malignancy for which no cure is available. To unravel the underlying pathogenic mechanisms and to evaluate therapeutic strategies, suitable *in vivo* models are needed but not available so far.

Aim: Generation and characterization of zebrafish *sdhb* mutants as a potential model for *SDHB*-related PPGL development.

Results: We have created zebrafish *sdhb* model that mimics the human tumour environment. Homozygous *sdhb*$^{-/-}$ larvae are viable, but exhibit a shorter lifespan. Biochemical analysis revealed decreased mitochondrial complex II activity and significant succinate accumulation in *sdhb*$^{-/-}$ larvae as compared to *sdhb*$^{+/-}$ and wild-type siblings. Although morphological analysis revealed no differences in amount and structure of the mitochondria, clear defects in energy metabolism and swimming behaviour were observed in *sdhb*$^{-/-}$ mutants using behavioural analysis. Besides, non-inflated swim bladders were observed in 60 % of the *sdhb*$^{-/-}$ mutants. Reactive oxygen species (ROS) levels were increased in homozygous *sdhb*$^{-/-}$ larvae indicative for oxidative stress. Transcriptomics analysis revealed significant differences in pathways involved in TCA-cycle and the electron transport chain. The previously identified hypermethylated regions of tumour suppressor genes in human *SDHB* tumours are for the majority also differentially expressed in our *sdhb* mutant larvae.

Conclusion: We successfully generated a first vertebrate animal model that mimics the metabolic effects of *SDHB*-associated PPGLs, characterization of *sdhb* zebrafish validates the relevance of this model for *SDHB*-associated PPGLs and reveals several therapeutic targets that can be evaluated in our newly developed semi high-throughput drug screening platform using zebrafish larvae.

HDAC8: a promising therapeutic target for acute myeloid leukemia

SESSION: CANCER

Marco Spreafico[1], *Alicja Maria Gruszka*[2], *Debora Valli*[2], *Mara Mazzola*[1], *Gianluca Deflorian*[3], *Arianna Quintè*[3], *Maria Grazia Totaro*[3], *Cristina Battaglia*[1], *Myriam Alcalay*[2,4], *Anna Marozzi*[1], *Anna Pistocchi*[1]

1 *University of Milan, Medical Biotechnology and Translational Medicine, Milan, Italy*
2 *IRCCS European Institute of Oncology, Experimental Oncology, Milan, Italy*
3 *IFOM, FIRC Institute of Molecular Oncology, Milan, Italy*
4 *University of Milan, Oncology and Hematology-Oncology, Milan, Italy*

ABSTRACT TEXT

The use of pan-histone deacetylase inhibitors (HDACi) for tumour treatment has gained an interest in recent years, albeit exhibiting low specificity, variable efficacy and side effects. Therefore, the identification of the potential of individual HDACs as anti-cancer target may improve therapy outcome, circumventing the adverse effects of pan-HDACi. Histone deacetylase 8 (HDAC8) is a class I HDAC that has been implicated with haematological malignancies, including acute myeloid leukemia (AML). In blasts from AML patients that carry the inversion of chromosome 16 (inv(16)), HDAC8 aberrantly inactivates p53 through its deacetylation, reducing apoptosis in the $CD34^+$ compartment and leading to the onset of leukemia. The inhibition of HDAC8 activity reduces p53 deacetylation and restores an appropriate $CD34^+$ cell number. Interestingly, the activity of HDAC8 was shown to be altered not only in inv(16) AML patients, but also in $CD34^+$ cells from several types of AML. It stands to reason that the use of commercially available specific HDAC8 inhibitors may constitute a promising therapeutic strategy to halt AML progression. Recently, we have assessed the efficiency of the highly-selective HDAC8 inhibitor, the PCI-34051, in different AML cell lines displaying high (HL60 and THP-1) or low activity (OCI-AML5) of HDAC8. Upon PCI-34051 treatment, high HDAC8 activity cells proliferation was arrested (HL60, THP-1) or cells underwent apoptosis (THP-1). In parallel, we overexpressed Hdac8 in a zebrafish model and observed an increase in myeloid population. The phenotype could be rescued by PCI-34051 treatment. Moreover, following HDAC8 deregulation both *in vitro* and *in vivo*, we observed a modulation of the canonical WNT pathway which is frequently dysregulated in AML insurgence. Our results suggest that selective inhibition of HDAC8 by PCI-34051 may represent a valuable therapeutic approach in the treatment of AML patients.

Germ Line, Early Development and Patterning II

Identification of Maternal-Effect Genes in Zebrafish by a Maternal Crispant Screen

SESSION: GERM LINE, EARLY DEVELOPMENT AND PATTERNING II

Cara Moravec[1], *Gabriella Voit*[1], *Jarred Otterlee*[1], *Francisco Pelegri*[1]

1 *University of Wisconsin–Madison, Genetics, Madison, USA*

ABSTRACT TEXT

In animals, early development is dependent on a pool of maternal factors, both RNA and proteins, which are required for basic cellular process and cell differentiation until zygotic genome activation. The role for a majority of these maternal expressed factors play in adult

fertility and early development is not fully understood. By exploiting the biallelic editing ability of CRISPR-Cas9 and the benefits of the zebrafish model, we aim to identify and characterize maternal-effect genes in a single generation, using a maternal crispant technique. We validated our ability to generate biallelic mutations in the germline by creating maternal crispants that phenocopied previously characterized maternal-effect genes: *motley/birc5b*, *tmi/prc1l*, and *aura/ mid1ip1*. Additionally, by targeting maternally expressed genes of unknown function in zebrafish, we identified two new maternal-effect zebrafish genes, *kpna7* and *fhcd3*. The genetic identity of these maternal crispants was confirmed by sequencing haploid progeny from F0-injected females, which allowed the sequence analysis of newly induced lesions in the maternal germ line. Analysis of the induced lesions shows minimal genetic variation within a clutch, with an average of two edited alleles per clutch. These findings are consistent with biallelic editing events occurring early in development in CRISPR-Cas9-injected embryos prior to the establishment of the germ cell precursors, leading to maternal-effect phenotypes in the offspring. Our studies show that maternal crispants allow for the effective identification and primary characterization of maternal-effect genes in a single generation, facilitating the reverse genetics analysis of maternal factors that drive embryonic development.

Circadian roles in reproduction

SESSION: GERM LINE, EARLY DEVELOPMENT AND PATTERNING II

Han Wang[1]

[1] *Soochow University, Center for Circadian Clocks, Suzhou, China*

ABSTRACT TEXT

The circadian clock generates and maintains 24-hour oscillations in almost all organs. However, the testis remains mysterious without a clear understanding of its circadian function, which has long been thought as the exception in the circadian system. Here, transcriptome analysis reveals more than 1, 000 rhythmically expressed genes in the zebrafish testis. Key circadian clock genes are expressed in spermatogonia and in particular, rhythmically in Sertoli cells, and regulate genes involved in retinoic acid (RA) signaling. Loss of Clock1a results in arrested spermatogonial differentiation and reduced fertilization, which can be rescued by time-of-day-specific RA treatment. Further, chronic perturbation of circadian regulation leads to similar reproductive defects. The circadian clock acts through RA signaling to synchronize spermatogonial differentiation via zbtb16a and to promote fertilization via izumo1. Together, our findings for the first time demonstrate that the testis clock ticks in a cell-specific manner and contribute to reproduction through RA signaling, highlighting circadian roles in male fertility.

Autophagy inhibition perturbed definitive hematopoiesis leading to aberrant myeloproliferation in Zebrafish models

SESSION: GERM LINE, EARLY DEVELOPMENT AND PATTERNING II

Kazi Md Mahmudul Hasan[1], *Xiangke Chen*[2], *Alvin Chun Hang Ma*[1]

[1] *The Hong Kong Polytechnic University, Department of Health Technology and Informatics, Kowloon, Hong Kong*

[2] *The University of Hong Kong, School of Biomedical Sciences, Pokfulam, Hong Kong*

ABSTRACT TEXT

Autophagy has been implicated as a lysosomal degradation pathway for intracellular protein and organelle clearance, cellular differentiation where uncoordinated-51 like autophagy activating kinases (ULK1/2) act as master regulators for autophagy initiation under starvation. However, the role of autophagy in normal hematopoiesis remains elusive. Here, we inhibited autophagy by TALEN mediated *ulk1b* and *ulk2* knockout in zebrafish embryos.

The overall autophagy processes were monitored by immunoblotting, high resolution confocal and light-sheet microscopy. Consequently, hematopoietic effects were scrutinized by q-PCR, flow cytometry and in-situ hybridization.

Dysfunction of the autophagy kinases *ulk1b/2* resulted in minimal cytosolic microtubule-associated protein 1A/1B-light chain 3 (Lc3I) conjugation to phosphatidylethanolamine (PE) to form LC3-II followed by decreased autophagosomal membrane recruitment. Consequently, light-sheet imaging showed that autophagy inhibition further suppresses autophagosomes and autolysosomes (co-localization of Lc3 puncta with lysotracker red) numbers without affecting autophagy flux. However, this autophagy inhibition modulated the hematopoietic lineage-specific concomitant upregulation of myeloid progenitors (*pu.1*), pan-leukocytes (*lcp1*), macrophages (*mpeg1.1*) and neutrophils

(*mpo*). Contrarily, both qualitative and quantitative analysis showed that aberrant inhibition of autophagy in *ulk1b* and *ulk2* deficient zebrafish embryos reduced erythroid (*gata1*), embryonic hemoglobin (*hbae1.1*) and hematopoietic stem cell (*cmyb*) numbers. Confocal imaging results showed that autophagy inhibition further arrests the number of co-localized green Lc3 puncta inside the myeloid cells in a tissue-specific manner.

These findings indicate that lack of autophagy throughout definitive hematopoiesis incorporates with myeloproliferation and anemia which further warrants the important role of autophagy to maintain normal HSC function during hematopoiesis.

Zebrafish pigment pattern formation – a problem solved or one ripe for discovery?

SESSION: GERM LINE, EARLY DEVELOPMENT AND PATTERNING II

Jennifer Owens[1,2], *Christian Yates*[2], *Robert Kelsh*[1]

[1] *University of Bath, Department of Biology & Biochemistry, Bath, United Kingdom*
[2] *University of Bath, Department of Mathematical Sciences, Bath, United Kingdom*

ABSTRACT TEXT

The generation of pattern in biology has been a problem that has fascinated experimentalists and mathematicians alike for decades, but usually biological understanding has lagged far behind the modelling. One key exception is adult pigment pattern formation in zebrafish. Recent advances in the cell biology and genetics of pigment pattern formation in the zebrafish has resulted in an extensive description of processes thought to contribute to the patterning process. Thus, formation of alternating light and dark stripes is critically dependent upon each of three different pigment cell types (yellow xanthophores, black melanophores and iridescent iridophores) which influence each other in a complex series of interactions. But how can we test if these biological rules are complete?

Previous mathematical approaches have usually asked if a specific class of model can reproduce the patterns seen in wild-type and mutant zebrafish, with less attention to the biological relevance of the assumptions made, and to the quantitative fit of the patterns generated to those seen in vivo. Here we reverse this strategy, developing an on-lattice model of pigment pattern formation built around the interaction rules defined experimentally, and incorporating parameter values as measured. We show by simulations that such a model is able to reproduce wild-type striped and multiple key mutant pigment patterns. Furthermore, we show how these patterns can be quantified to assess their fit to the biological observations. In general, we show that such a model provides an excellent fit to the known biology of stripe formation, indicating that most or even all the key rules appear to have been identified. However, other aspects, including detailed exploration of stripe maintenance, are ripe for discovery.

Immunity and Infection

New insights into lymphoid organization in fish: a lymphoid continuity between mucosal tissues and the discovery of a new lymphoid tissue identify the branchial cavity as a lymphoid nexus

SESSION: IMMUNITY AND INFECTION

Julien Resseguier[1], Jens Wohlmann[1], Mai-Eva N'Guyen-Chi[2], Håvard Bjørgen[3], Jean-Pierre Levraud[4], Shanawaz Khan[1], Irene Salinas[5], Geert Wiegertjes[6], Louis Du Pasquier[7], Gareth Wyn Griffiths[1], Erling Olaf Koppang[3], Pierre Boudinot[8]

1 *University of Oslo, Department of Biosciences, Oslo, Norway*
2 *University of Montpellier, UMR 5235: Laboratory of Pathogen-Host Interactions, Montpellier, France*
3 *Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Oslo, Norway*
4 *Institut Pasteur, Department of Developmental & Stem Cell Biology, Paris, France*
5 *University of New Mexico, Department of Biology, Albuquerque, USA*
6 *Wageningen University & Research, Department of Animal Sciences, Wageningen, Netherlands*
7 *University of Basel, Department Biozentrum, Basel, Switzerland*
8 *INRAE - French National Institute for Agriculture- Food and Environment, Molecular Virology and Immunology, Jouy-En-Josas, France*

ABSTRACT TEXT

The zebrafish is an important model to study human and fish pathologies that involve the immune system. Still, due to an incomplete characterization, the immune system of the zebrafish is often mistakenly perceived as lacking complexity, especially regarding Mucosal Associated Lymphoid Tissues (MALTs). In the natural environment fish mucosae are constantly exposed to high levels of potential pathogens, so one would expect fish MALTs to display a sophisticated organization. Recent studies have unraveled the existence of structured MALTs in fish such as the Interbranchial Lymphoid Tissue (ILT) in the gills. To further explore the mucosal immune system of adult zebrafish, we investigated the distribution of NK/T cells by labelling a highly-conserved epitope of the kinase ZAP70. Analysis of large 3D multi-field confocal images revealed a lymphoid continuity inter-connecting the thymus, the branchial cavity and other tissues. This arrangement could have significant implications for our understanding of natural infection routes and how MALTs share information upon antigen exposure. Also, by investigating this continuity we discovered a large structured mucosal lymphoid tissue that is embedded in the branchial cavity and which connects with each of the eight ILTs. Embedded in a vast network of reticulated epithelial cells, we found an abundance of immune cells such as NK/T lymphocytes, granulocytes, macrophages, dendritic cells and probably also B cells. Finally, we explored several features such as cell proliferation, hematopoiesis and lymphocyte recombination within this structure. Our data indicate that this tissue is not an additional thymus nor a reservoir of innate lymphoid cells, but is likely a bona-fide compartmentalized secondary lymphoid tissue. Collectively, these discoveries may change our perception of the fish immune system and have important implications for zebrafish disease models.

Reprogramming macrophages and neutrophils by infection and through loading with cargoes via protocells

SESSION: IMMUNITY AND INFECTION

Paco López-Cuevas[1], Can Xu[2], Charlotte Severn[1], MacCarthy-Morrogh Lucy[1], Ashley Toye[1], Stephen Mann[2], Paul Martin[1]

1 *University of Bristol, School of Biochemistry, Bristol, United Kingdom*
2 *University of Bristol, School of Chemistry, Bristol, United Kingdom*

ABSTRACT TEXT

Macrophages and neutrophils are immune cells that play key roles in the killing of both cancer cells and bacteria. We are investigating the possibility of reprogramming these cells to enhance their tumoricidal/bactericidal abilities. One reprogramming strategy is based on the

use of infection to make immune cells better at killing cancer. This approach was inspired by W. Coley's studies over a century ago that showed injection of bacterial toxins caused cancer regression in patients. The mechanism underpinning this cancer bacteriotherapy was not clear, but it was believed to be mediated by the immune system. We have used the translucent zebrafish model to investigate how various microbes and Coley's toxins can alter how leukocytes behave and their interactions with cancer cells. Our results have shown that infection modifies the behaviour, interaction and phenotype of leukocytes recruited to cancer cells.

As an alternative means of reprogramming leukocytes to be more able to kill cancer and bacteria we have tested the feasibility of using a novel protein-based microparticle (protocell) for the delivery of different cargoes to leukocytes to alter their phenotype towards a more pro-inflammatory "microbe/cancer killing" state. We have exploited the translucent zebrafish model to investigate how protocells flow in the circulation post injection, their uptake dynamics and biodistribution in leukocytes, and the subsequent trafficking and behaviours of loaded leukocytes. We have loaded protocells with either an endosomal TLR7/8 agonist drug or an inhibitor of an anti-inflammatory miR, miR223, and have shown that they effectively induce a prolonged pro-inflammatory state in zebrafish macrophages. Our in vitro data suggest that human macrophages also can be targeted with protocells thus enabling manipulation of these cells. Therefore, protocells hold promise as a new potential therapeutic tool for treating human pathologies where leukocyte phenotypes and functions are key.

Development of gamma/delta T-cells is regulated by cell localization and environmental signals in the thymus

SESSION: IMMUNITY AND INFECTION

Narges Aghaallaei[1], Advaita Dick[1], Eva Hasel[2], Thomas Thumberger[3], Jochen Wittbrodt[3], Baubak Bajoghli[1]

[1] *University Hospital Tübingen, Division of Translational Oncology, Tübingen, Germany*
[2] *European Molecular Biology Laboratory EMBL, Directors' Research, Heidelberg, Germany*
[3] *Heidelberg University, Centre for Organismal Studies COS, Heidelberg, Germany*

ABSTRACT TEXT

Two types of T-cells – αβ and γδ – are present in vertebrates and are defined by surface expression of either αβ or γδ T-cell receptor (TCR) complex. Both T-cell lineages, which have distinct functions in immunity, develop from a common lymphoid progenitor in the thymus. The choice between αβ and γδ fate is the first decision made by progenitors after they commit to the T-cell fate. Thus far, two models have been proposed to explain this biological process in mammals: The "stochastic" model proposes that lineage commitment is random, and the "instructive" model postulates that the strength of TCR signal determines the fate-choice. Given that our current view of T-cell lineage decision has been predominantly assembled from results obtained under culture conditions, the precise role of thymic microenvironments in this process is not yet clear. Interestingly, the relative number of γδ T-cells varies between 1 % and 40 % in species arguing for different mechanisms regulating this process, but how it is regulated in lower vertebrates is unknown. In this work, we studied the ontogeny of γδ T-cells in medaka. Our findings revealed that they develop in a specific thymic niche, where progenitor first colonize the thymus. Upon entry into the thymus, progenitors either stay within the same niche to further develop as γδ T-cells or migrate inwards the thymus to acquire the αβ T-cell phenotype. We also found that γδ T-cell development is enhanced when (1) the intrathymic positioning of thymocytes is genetically altered using a *ccr9b* mutant, and (2) the niche-specific expression of cytokines is manipulated. Collectively, our findings reveal that T-cell lineage decision in medaka is mainly determined by extrinsic factors. Our results also establish temporal residency of progenitors in a specialized thymic niche as a decisive factor for αβ and γδ T-cell development and provide insight into mechanisms controlling intrathymic cell positioning and fate decisions in lower vertebrates.

An evolutionary conserved program of TGFβ signaling-mediated microglia development in zebrafish

SESSION: IMMUNITY AND INFECTION

Giuliano Ferrero[1], *Magali Miserocchi*[2], *Giulia Boezio*[3], *Didier Steinier*[3], *Valérie Wittamer*[2]

[1] *Université Libre de Bruxelles, IRIBHM, Brussels, Belgium*
[2] *Université Libre de Bruxelles, Institute de Recherche Interdisciplinaire en Biologie Humaine et MoléculaireIRIBHM, Brussels, Belgium*
[3] *Max Planck Institute for Heart and Lung Research, Department of Developmental Genetics, Bad Nauheim, Germany*

ABSTRACT TEXT

Zebrafish provide a unique vertebrate model system for the analysis of hematopoietic cell development and functions due to the transparency of the embryo, the rapid development, and its powerful genetics. Contributing to establish zebrafish as a complementary tool for microglial investigations, we previously showed that microglia ontogeny in the zebrafish embryo closely parallels that of the mouse. Based on these observations, we have initiated experiments in zebrafish that combine state-of-the-art molecular, genetic and live imaging approaches, aiming to provide new insights into the molecular determinants underlying microglia development during vertebrate embryogenesis. Here, we show that *Transforming growth factor-β (*TGFβ*) receptor* signaling acts as an essential positive regulator of microglia ontogeny in the zebrafish embryo, in line with its established microglial maturation functions in the mouse model. Extending these findings, we demonstrate that pharmacological inhibition or genetic loss of TGFβ receptor activity specifically hinders the last steps of microglia ontogeny *in vivo*. Indeed, although microglial progenitors successfully seed the embryonic neuro-epithelium upon TGFβ receptor inhibition, they fail to differentiate, as reflected by the lack of induction and expression of microglia-specific markers *apoe* and *p2ry12*. Interestingly, live imaging analyses revealed that these undifferentiated cells display functional defects in the clearance of apoptotic bodies, which eventually accumulate in the embryonic brain. Functional dissection of the phagocytic phenotype through live imaging, as well as gene profiling of TGFβr-deficient microglia, are currently ongoing. Collectively, this work provides new insights into the process of TGFβ-mediated microglia cell fate and its implications for microglia homeostasis during development.

Regeneration II

Make do and make new: how zebrafish rapidly regenerates CNS injury

SESSION: REGENERATION II

Jan Kaslin[1]

[1] *Australian Regenerative Medicine Institute, Monash University, Clayton, Australia*

ABSTRACT TEXT

Zebrafish have a remarkable capacity to regenerate following spinal cord injury. While many factors controlling neurogenesis have been identified, the cellular mechanisms regulating global neural regeneration are largely unknown. We used in vivo imaging to pin-point specific cells and signals that control CNS regeneration in zebrafish. Surprisingly, we identified two temporally and mechanistically distinct waves of cellular regeneration in the spinal cord. The initial wave of regeneration relying on cell migration of neural precursors to the lesion site, enabling rapid functional recovery, and the activation of quiescent neural stem and progenitor cells (NCSs). This is then followed by the second wave of regeneration which largely driven by regenerative neurogenesis. Neurogenesis compensates for both the loss of tissue at injury site as well as the cells depleted from proximal areas due to early migration. Furthermore, we find that inflammation and leukocytes play a critical role in differentially regulating cell recruitment and activation of NSCs after injury. The two waves of regeneration demonstrate how the zebrafish are able to rapidly regain motor function after complete ablation, but also gradually replenish lost tissue

over time. Taken together, our data suggest that inflammation driven recruitment of neural precursors play an unanticipated role in neural repair.

Osteoblast cell migration during zebrafish fin regeneration

SESSION: REGENERATION II

Ivonne Sehring[1], *Gilbert Weidinger*[1]

[1] *Ulm University, Institute of Biochemistry and Molecular Biology, Ulm, Germany*

ABSTRACT TEXT

Bone regeneration in the zebrafish caudal fin is, at least in part, achieved by the plasticity of mature, differentiated osteoblasts. Amputation induces osteoblasts to dedifferentiate to bone progenitors, which migrate towards the amputation plane to form parts of the regeneration blastema. Directed cell migration requires a cell front and a cell rear along an axis approximately aligned with the direction of locomotion. We show that osteoblasts close to the amputation plane drastically change their morphology, as their shape changes from roundish to elongated with long protrusions, and that they align along the proximo-distal axis. Pharmacological interference with actomyosin dynamics impaired cell shape change as well as migration, indicating that a dynamic actomyosin cytoskeleton is required for osteoblast plasticity. In contrast, microtubuli, which are also an important force to drive cell shape changes and cell motility, are likely not involved, as interrupting microtubuli dynamics did not affect osteoblast migration. The migration of osteoblasts after amputation is directed towards the amputation site. Directional cell migration is usually initiated in response to extracellular cues such as chemokines or signals from the extracellular matrix. We show that inhibition of components of the complement system impaired both osteoblast cell shape changes and migration, suggesting that the complement system act as a guidance for directed osteoblast migration after amputation.

Interleukin-11 signaling limits scar formation by antagonizing endothelial-to-myofibroblast transdifferentiation during zebrafish heart regeneration

SESSION: REGENERATION II

Srinivas Allanki[1], *Yeszamin Onderwater*[1], *Alora Marks*[1], *Boris Strilic*[2], *Stefan Guenther*[3], *Didier Stainier*[1], *Sven Reischauer*[1]

[1] *Max Planck Institute for Heart and Lung Research, Department of Developmental Genetics, Bad Nauheim, Germany*
[2] *Max Planck Institute for Heart and Lung Research, Department of Pharmacology, Bad Nauheim, Germany*
[3] *Max Planck Institute for Heart and Lung Research, ECCPS Deep Sequencing Platform, Bad Nauheim, Germany*

ABSTRACT TEXT

In adult mammals, tissue damage after myocardial infarction induces excessive myofibroblast transdifferentiation and the formation of a permanent, functionally inert scar. However, the molecular mechanisms that orchestrate myofibroblast transdifferentiation and scarring remain poorly understood. Some vertebrates like zebrafish display a remarkable regenerative potential with only limited and transient fibrosis after tissue damage, including in the heart. Here, employing comparative expression profiling coupled with loss-of-function approaches, we identify the canonical Interleukin-11/Stat3 signaling axis as a core component of regeneration in zebrafish. Notably, animals lacking Interleukin-11 receptor (Il11ra) function reach adulthood without overt developmental defects, but exhibit strongly impaired regeneration across tissues. Furthermore, post cardiac cryoinjury, *il11ra* mutants exhibit excessive myofibroblast transdifferentiation and the formation of a permanent collagenous scar, similar to what is observed in adult mammals. Using zebrafish lineage-tracing approaches and human primary cell culture methods, we provide evidence that Interleukin-11 signaling limits endothelial-to-myofibroblast transdifferentiation and maintains a pro-regenerative niche to promote cardiac regeneration. Altogether, our data reveal a vital role for endothelial Interleukin-11/Stat3 signaling in containing injury-induced cardiac fibrosis.

Investigating the interaction between cardiac subpopulations during adult cardiac regeneration

SESSION: REGENERATION II

Phong Nguyen[1], *Sonja Chocron*[1], *Jeroen Bakkers*[1]

[1] *Hubrecht Institute, Hubrecht Institute, Utrecht, Netherlands*

ABSTRACT TEXT

Zebrafish heart regeneration following injury is achieved through the generation of de novo cardiomyocytes. This is achieved by pre-existing uninjured cardiomyocytes re-entering the cell cycle and initiating proliferation. While the generation of new cardiomyocytes is an important event during regeneration, the next steps by which how these cells migrate into and replace the fibrotic scar, as well as integrate into the healthy myocardium to produce a synchronously contracting heart is unknown. In this study, we attempted to investigate the dynamics of these newly generated cardiomyocytes during the regenerative phase. By using a number of transgenic lines and lineage tracing strategies, we found that the proliferated cardiomyocytes followed a defined set of cellular movements at specific timepoints during regeneration. Based on these cellular movements, we conducted single cell sequencing on these cardiomyocytes and revealed a number of subpopulations that interacted with the environment and other cell types. This may suggest a role for non-cardiomyocytes in aiding the cardiomyocytes to migrate into the injured area. We have now identified a number of candidate factors that may influence the migration of these de novo cardiomyocytes and are now investigating their potential role.

Cell Signalling and Metabolism

Yap regulates hematopoietic stem cell formation in response to the biomechanical forces of blood flow

SESSION: CELL SIGNALLING AND METABOLISM

Wade Sugden[1], *Vanessa Lundin*[2], *Lindsay Theodore*[1], *Patricia Sousa*[1], *Areum Han*[1], *Stephanie Chou*[1], *Paul Wrighton*[3], *Andrew Cox*[3], *Donald Ingber*[4], *Wolfram Goessling*[3], *George Daley*[1], *Trista North*[1]

[1] *Boston Children's Hospital/Harvard Medical School, Hematology/Oncology, Boston, USA*
[2] *Karolinska Institutet, Department of Medicine- Huddinge, Stockholm, Sweden*
[3] *Brigham and Women's Hospital/Harvard Medical School, Department of Medicine- Division of Genetics, Boston, USA*
[4] *Wyss Institute for Biologically Inspired Engineering/Harvard University, Vascular Biology Program, Boston, USA*

ABSTRACT TEXT

Hematopoietic stem cells (HSCs) are born from arterial endothelial cells in the dorsal aorta that adopt hematopoietic potential and commitment during development. Physical forces, namely wall shear stress (WSS) and cyclic stretch (CS), produced by hemodynamic blood flow are required to generate HSCs from arterial endothelium, but the mechanisms by which these forces are sensed and converted into a "stemness" regulatory program remain unknown. Using a novel "dorsal-aorta-on-a-chip" microfluidic platform, we demonstrate that exposure to WSS and CS increase expression of the hematopoietic transcription factor RUNX1 in human CD34+ hemogenic endothelial cells derived from induced pluripotent stem cells. CS specifically induced activity of the YAP transcription factor, a mechanically-activated regulator of organ size and pluripotency. We corroborate these findings in a zebrafish model, and demonstrate that loss or gain of YAP function *in vivo* can blunt or augment the production of HSCs, respectively, during definitive hematopoiesis. Importantly, by gene expression analysis and Notch pathway modulation, we find that YAP is responsible for the maintenance, not initiation, of the hematopoietic program in newly specified hemogenic endothelial cells. Molecularly, we identify a stretch-induced, RhoGTPase-dependent mechanotransduction network controlling YAP nuclear availability to regulate RUNX1 expression, both in zebrafish and human cells. Moreover, we show that small molecule stimulation of RhoGTPases can rescue HSC production in zebrafish embryos with no flow, and enhance the hematopoietic

potential of human iPSC-derived CD34+ endothelial cells grown in static culture. Together these findings uncover YAP as a transcriptional regulator of stem cell fate commitment during tissue morphogenesis, and suggest a pharmacologically amenable mechanotransduction pathway that could be exploited to improve the in vitro derivation of HSCs from human cells for therapeutic purposes.

Left side story: Cachd1, Wnt signalling and the habenulae

SESSION: CELL SIGNALLING AND METABOLISM

Gareth Powell[1], *Ana Faro*[1], *Yuguang Zhao*[2], *Heather Stickney*[1], *Ingrid Lekk*[1], *Gavin J. Wright*[3], *E. Yvonne Jones*[2], *Steve Wilson*[1]

[1] *University College London, Cell and Developmental Biology, London, United Kingdom*
[2] *University of Oxford, Division of Structural Biology, Oxford, United Kingdom*
[3] *Wellcome Trust Sanger Institute, Cell Surface Signalling Laboratory, Cambridge, United Kingdom*

ABSTRACT TEXT

Left-right asymmetry is a conserved feature of vertebrate brains, but the underlying developmental mechanisms remain poorly understood. We use the dorsal habenulae of zebrafish as a model system to study the establishment of laterality during embryogenesis. In zebrafish fry, the left habenula has a larger lateral subnucleus and responds preferentially to light stimuli whereas the right habenula has a larger medial subnucleus and responds more strongly to odour stimuli. Timing of neurogenesis in the left and right habenula influences asymmetry, with early events favouring lateral subnuclear fates. This programme of neurogenesis is regulated by Notch and Wnt signalling, as well as an as-yet-unidentified signal from the parapineal which promotes left-sided character.

Through a forward genetic screen, we identified the highly conserved orphan receptor, Cachd1, as being essential for the establishment of asymmetry in the habenulae: loss-of-function of *cachd1* leads to bilateral symmetry with both habenulae showing left-sided character. This phenotype is a result of precocious, symmetric neurogenesis and is epistatic to early ablation of the parapineal, suggesting that Cachd1 inhibits neurogenesis and is itself inhibited by the parapineal signal in the left habenula. We have employed different biochemical methods to identify binding partners for Cachd1 and determined that it can bind simultaneously to the Wnt co-receptors Lrp5/6 and Fzd family, suggesting it is able to regulate canonical Wnt signalling in the dorsal diencephalon. In support of this hypothesis, we find that loss of function of *lrp6* also causes bilateral symmetry in the dorsal habenulae.

We propose that *cachd1* is a novel regulator of Wnt signalling that maintains the balance of proliferation and neurogenesis in the dorsal diencephalon.

Multiple clocks regulate amino acid levels in zebrafish cells

SESSION: CELL SIGNALLING AND METABOLISM

Rima Siauciunaite[1], *Nicholas Simon Foulkes*[2]

[1] *Karlsruhe Institute of Technology, IBCS-BIP, Eggenstein-Leopoldshafen, Germany*
[2] *Karlsruhe Institute of Technology, Institute of Biological and Chemical Systems - Biological Information Processing, Eggenstein-Leopoldshafen, Germany*

ABSTRACT TEXT

The circadian system plays a pivotal role in orchestrating amino acid metabolism and makes it possible for daily rhythms in amino acid absorption, storage and transport to be temporally coordinated with sleep-wake cycle and feeding behaviour. At the molecular level the rhythmic expression of genes involved in amino acid biosynthesis is regulated by elements of the core clock mechanism, which is based on interconnected transcription/translation feedback loops. Hence, asynchrony between the external environment and endogenous circadian rhythms results in disruption of amino acid homeostasis and contributes to the development of metabolic disorders and cancer. Nevertheless, the major challenge remains to unravel the molecular pathways through which the circadian clock and metabolism respond to light and food. In this study, we are exploring crosstalk between the circadian clock and the regulatory circuits involved in amino acid metabolism in zebrafish (*Danio rerio*). By applying multi-omics approaches we have discovered for the first time that daily changes in amino acid concentration are infradian. How can amino acid levels cycle with an infradian period? Are there alternative clock mechanisms underlying temporal control of metabolism? The candidate gene approach suggests that Asparagines synthetase (ASNS) and Activating transcription factor 4 (ATF4) are involved in the regulation of these rhythms. Additionally, by a comparative approach involving blind cavefish as a model, we hope to gain unique insight into evolutionary advantages of interaction between circadian systems and infradian rhythms in order to regulate metabolism.

The increased concentration of 4-Hydroxynonenal in aldh3a1 zebrafish mutants disrupts pancreas development, leading to hyperglycaemia and retina hyaloid vasculature alteration

SESSION: CELL SIGNALLING AND METABOLISM

Bowen Lou[1], *Thomas Fleming*[2], *Peter Paul Nawroth*[2], *Jens Kroll*[1]

[1] *Heidelberg University, Department of Vascular Biology and Tumor Angiogenesis- European Center for Angioscience ECAS- Medical Faculty Mannheim, Mannheim, Germany*
[2] *Heidelberg University, Department of Internal Medicine I and Clinical Chemistry- Heidelberg University Hospital, Heidelberg, Germany*

ABSTRACT TEXT

Background and Aims: Aldehyde dehydrogenases (ALDH) are a group of aldehyde catalysing enzymes. Our previous work has identified a two-fold increased ALDH activity in glyoxalase 1 (Glo1) knockout zebrafish, which lead to hyperglycaemia by metabolic alteration. Besides, RT-qPCR based results identified elevated *aldh3a1* mRNA level in *glo1*$^{-/-}$ mutants. Thus, this project aims to generate Aldh3a1 knockout zebrafish and analyse its function in glucose homeostasis.

Materials and Methods: *aldh3a1*$^{-/-}$ zebrafish were generated using CRISPR/Cas9. Vasculature, pancreatic and β-Cell mass size were analysed in *Tg(fli1:EGFP)*, *Tg(hb9:GFP)* and *Tg(ins:nfsB-mCherry)* zebrafish larvae. mRNA expression was examined by RT-qPCR and RNA-seq analysis. 4-Hydroxynonenal (4-HNE) amount was measured by ELISA (BioVision) and whole-body glucose was measured by Glucose Assay Kit (Merck).

Results: Aldh3a1 knockout zebrafish were successfully generated and validated by significantly decreased ALDH activity. In *Tg(fli1:EGFP)* zebrafish larvae, loss of Aldh3a1 increased abnormal intersegmental vessels formation in the trunk and widened branch diameters in retina hyaloid vasculature. A combination of Aldh3a1 knockout and knockdown strategies identified a decreased pancreatic size in *Tg(hb9:GFP)* and reduced β-Cell mass dimension in *Tg(ins:nfsB-mCherry)* zebrafish larvae. Also, mRNA expression of *pdx1* and *insulin* were decreased and RNA-seq data have further confirmed disruption of the endocrine pancreas development in the *alda3a1* mutants. Consequently, *aldh3a1* $^{-/-}$ larvae exhibited hyperglycaemia by 40 % whole-body glucose elevation. Moreover, disruption of the pancreas in *alda3a1* mutants is driven by an increased 4-HNE amount and external 4-HNE in wild type zebrafish larvae mimics the phenotype in *aldh3a1* mutants.

Conclusion: These data show defective 4-HNE detoxification after Aldh3a1 loss, emphasising 4-HNE as a vital intermediate to regulate metabolic diseases via glucose homeostasis.

Neurobiology II

Molecular and functional characterizations of Gfi1ab role in the establishment of specific retinotectal connections

SESSION: NEUROBIOLOGY II

Shahad Albadri[1], *Fanny Eggeler*[1], *Elena Putti*[1], *Juliette Vougny*[2], *Diana Milicevic*[1], *Noe Testa*[2], *Filippo Del Bene*[1]

[1] *Institut de la Vision, Department of Development, Paris, France*
[2] *Curie Institute, Genetique et Biologie du Developpement, Paris, France*

ABSTRACT TEXT

With over 50 different combinations of dendritic and axonal morphologies, retinal ganglion cells (RGCs) form complex and specialized circuits with their target cells in the optic tectum, the brain's retino-recipient, to ensure the proper functioning of the visual system in zebrafish. The genetic events governing the specific connections between RGCs and their target cells remain however largely unknown to date. We herein study the role of the Growth factor inhibitor 1ab (Gfi1ab), a zinc finger transcription factor, in this process.

It was previously shown that Senseless, Gfi1ab *Drosophila* orthologue, governs the synaptic targeting of R8 types of photoreceptors into specific laminae in the medulla in the Drosophila brain. This function is achieved through the regulation of Capricious, a Leucine rich repeat (Lrr) cell adhesion molecule. In zebrafish and mouse, *gfi1ab* is sparsely expressed in RGCs. However to date, the role of Gfi1ab and its target genes have not been assessed in the context of neuronal circuit formation.

With the aim of unraveling Gfi1ab role in the vertebrate visual system, we generated a *Tg*(*gfi1ab:gal4/UAS:RFP*) BAC transgenic line. Our in-depth morphological characterization of *gfi1ab*-expressing RGCs reveals that *gfi1ab* is labels particular morphological subtypes of RGCs, which axons pre-branch within specific pre-tectal arborization fields and tectal laminae. The knock-out of *gfi1ab* within *Tg*(*gfi1ab:gal4/UAS:RFP*) genetic background and the zebrafish Capricious orthologues *neuronal lrr 2* and *3a* (*lrrn2* and *lrrn3a*) by CRISPR/Cas9, shows that Gfi1ab is crucial for the proper targeting of subtypes of RGCs to the optic tectum via Lrrn2 and Lrrn3a, similarly to Senseless in *Drosophila*.

Finally, we are currently investigating the physiological function ensured by Gfi1ab RGCs by calcium imaging. Together this study provides the first comprehensive map linking genetics, morphology and physiology of the circuits labeled by Gfi1ab in the visual system *in vivo*.

The olfactory epithelia: A novel neural immune tissue

SESSION: NEUROBIOLOGY II

Kathleen Whitlock[1,2], *Danissa Candia*[1], *Maria Fernanda Palominos*[1,2]

[1] *Universidad de Valparaiso, Instituto de Neurociencia, Valparaíso, Chile*
[2] *Universidad de Valparaíso, Centro Interdisciplinario de Neurociencia de Valparaíso CINV, Valparaíso, Chile*

ABSTRACT TEXT

The olfactory sensory neurons (OSNs) are unique: they are the only sensory neurons that interact directly with the external environment yet the first synapse lies within the central nervous system thus creating a potential gateway for the entry of damaging or infectious agents to the nervous system. In juvenile zebrafish we have shown both resident and non-resident neutrophils rapidly mobilize in response to copper-induced damage of the olfactory epithelia (OE). Strikingly, in the adult animal the principal populations of neutrophils in the brain are found in the OE and associated peripheral meningeal membranes. These populations have morphologies of both progenitors and differentiated neutrophils. Immediately after copper-induced damage, neutrophils increase in number and enter the olfactory bulb via the olfactory nerve suggesting the OE are the main source of neutrophils. We have analyzed of the lymphatic system in the adult brain of the adult zebrafish: the lamellae of the OE contain HEV-like cells while the OBs are wrapped in muLEC-like cells with extensive lymphatic vasculature extending along the ventral telencephalon to the ventral diencephalon. Overall these results support the conclusion that the OE is a specialized immune tissue. Finally we suggest that the lifetime renewal of peripheral and central olfactory neurons in vertebrates may be part of a protective strategy to eliminate potential transmission of infectious agents via this specialized neural portal to the brain.

Funding: FONDECYT-1160076 (KEW), Conicyt Fellowship 3657/2016 (MFP) ICM-ECONOMIA Instituto Milenio Centro Interdisciplinario de Neurociencias de Valparaíso PO9-022-F (KEW).

Synaptic silencing of fast muscle is compensated by rewired innervation of slow muscle

SESSION: NEUROBIOLOGY II

Fumihito Ono[1], *Buntaro Zempo*[1], *Yasuhiro Yamamoto*[1], *Williams Tory*[2]

[1] *Osaka Medical College, Department of Physiology, Takatsuki, Japan*
[2] *National Institutes of Health, Laboratory of Molecular Physiology, Bethesda, USA*

ABSTRACT TEXT

For decades, numerous studies have proposed that fast muscles contribute to quick movement, while slow muscles underlie locomotion requiring endurance. By generating mutant zebrafish whose fast muscles are synaptically silenced, we examined the contribution of fast muscles in both larval and adult zebrafish. In the larval stage, mutants lacked the characteristic startle response to tactile stimuli: bending of the trunk (C-bend) followed by robust forward propulsion. Surprisingly, adult mutants with silenced fast muscles showed robust C-bends and forward propulsion upon stimulation. Retrograde labeling revealed that motor neurons genetically programmed to form synapses on fast muscles are instead re-routed and innervate slow muscles, which led to partial conversion of slow and intermediate

muscles to fast muscles. Thus, extended silencing of fast muscle synapses changed motor neuron innervation and caused muscle cell type conversion, revealing an unexpected mechanism of locomotary adaptation.

Chemical Biology and Drug Discovery

Identification of bioactive compounds from fungi using zebrafish embryogenesis as read-out

SESSION: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Jelmer Hoeksma[1], *Jeroen den Hertog*[1]

[1] *Hubrecht Institute, Jeroen den Hertog lab, Utrecht, Netherlands*

ABSTRACT TEXT

Fungi are well known for the production of a wide variety of bioactive products. Commonly used drugs such as penicillin, cyclosporin and lovastatin are all produced by fungi. Even today, novel compounds from fungi are being discovered, proving that the fungal kingdom as a source of new compounds is far from depleted.

We performed a large-scale screen of filtrates from >10,000 fungal species on zebrafish embryos. Embryos were incubated continuously with filtered liquid medium on which fungi had grown from 6 hpf onwards and phenotypes were scored at 2dpf. In total, 1,526 filtrates induced various abnormal phenotypes including an undulating notochord and a wide variety of pigmentation, heart and tail defects. Subsequently, fungi inducing interesting phenotypes were selected and their bioactive compounds were purified through stepwise bioactivity-guided purification by liquid-liquid extraction and preparative HPLC. Intermediate products were tested on zebrafish embryos throughout the isolation process. Purified active fractions were analyzed using LCMS, UV-Vis spectrometry, high resolution mass spectrometry and NMR. Thus far, we have isolated and identified over 60 known fungal metabolites and several new bioactive compounds with novel activities in zebrafish using this method. Currently, we are exploring the potential of several identified compounds which have similar effects as known therapeutic drugs.

TRPswitch — a step function chemo-optogenetic ligand for the vertebrate TRPA1 channel

SESSION: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Pui Ying Lam[1], *Aditya Thawani*[2], *Enrique Balderas*[3], *Andrew White*[2], *Dipayan Chaudhuri*[3], *Matthew Fuchter*[2], *Randall Peterson*[1]

[1] *University of Utah, Department of Pharmacology and Toxicology, Salt Lake City, USA*
[2] *Imperial College London, Department of Chemistry, London, United Kingdom*
[3] *University of Utah, Department of Internal Medicine, Salt Lake City, USA*

ABSTRACT TEXT

Chemo-optogenetics has produced powerful tools for optical control of cell activity, but current tools suffer from a variety of limitations including low unitary conductance, the need to modify the target channel, or the inability to control both on and off switching. Using a zebrafish behavior-based screening strategy, we discovered "TRPswitch", a photoswitchable non-electrophilic ligand scaffold for the transient receptor potential ankyrin 1 (TRPA1) channel. TRPA1 exhibits high unitary channel conductance, making it an ideal target for chemo-optogenetic tool development. Key molecular determinants for the activity of TRPswitch were elucidated and allowed for replacement of the TRPswitch azobenzene with a next-generation azoheteroarene. The TRPswitch compounds enable reversible, repeatable, and nearly quantitative light-induced activation and deactivation of the vertebrate TRPA1 channel with violet and green light, respectively. The utility of TRPswitch compounds was demonstrated in larval zebrafish hearts exogenously expressing zebrafish Trpa1b, where heartbeat

could be controlled using TRPswitch and light. Therefore, TRPA1/TRPswitch represents a novel step-function chemo-optogenetic system with a unique combination of high conductance, high efficiency, activity against an unmodified vertebrate channel, and capacity for bidirectional optical switching. This chemo-optogenetic system will be particularly applicable in systems where a large depolarization current is needed or sustained channel activation is desirable.

The potential of Tetraselmis sp. CTP4 as a source for bone anabolic compounds in zebrafish

SESSION: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Alessio Carletti[1,2], Tamára F. Santos[2], Katkam N. Gangadhar[2], Nathana L. Cristofoli[2], Luísa Barreira[2], Hugo Pereira[2], João Varela[2], M. Leonor Cancela[1,2,3,4], Vincent Laizé[2], Paulo J. Gavaia[1,2]

1 *Department of Biomedical Sciences and Medicine DCBM, University of Algarve, Faro, Portugal*
2 *Centre of Marine Sciences CCMAR, University of Algarve, Faro, Portugal*
3 *Algarve Biomedical Center ABC, University of Algarve, Faro, Portugal*
4 *Centre for Biomedical Sciences CBMR, University of Algarve, Faro, Portugal*

ABSTRACT TEXT

Metabolic bone disorders such as osteoporosis affect millions of people worldwide and drugs currently available have a limited efficacy and have associated secondary effects. There is a compelling need for new compounds with bone anabolic properties acting through new and original mechanisms of action. In this regard, recent works have identified marine organisms as promising sources for bone anabolic compounds. A zebrafish (*Danio rerio*) screening pipeline was used to assess the bone anabolic property of extracts prepared from the microalgae *Tetraselmis* sp. strain CTP4. Osteogenic effects were first assessed in 6-days post-fertilization (dpf) larvae exposed to CTP4 extracts for 3 days. Morphometric analysis of alizarin red-S stained bone structures revealed that the ethanol extract has the potential to increase operculum mineralized area by 40 %. The exposure of reporter lines for *osterix* (*sp7:mCherry* marking early osteoblasts) and *osteocalcin* (*oc:GFP* marking mature osteoblast) to the ethanol extract also suggested that this osteogenic effect may result from a stimulation of the proliferation and maturation of osteoblast precursors. CTP4 ethanol extract was further fractionated through preparative HPLC and 15 fractions were re-tested in 6-dpf larvae. Nine of the new fractions were able to increase operculum growth to a higher extent than the original extract, and two of them induced ectopic mineralization of intervertebral disks. Aiming to identify the compound/s responsible for the osteogenic effect in the extract, the active fractions were analyzed by LC-MS for the tentative identification of possible lead compounds underlying the effect.

This work was financed by EU projects INTERREG Portugal-Spain 0055 ALGARED+ 5E, BLUEHUMAN (ATLANTIC AREA), BioMedaqu (H2020-MSCA-ITN), operational programmes CRESC Algarve 2020 and COMPETE 2020 through project EMBRC.PTALG-01-0145-FEDER-022121 and by PT projects OSTEOMAR (MAR2020) and UIDB/04326/2020 (FCT).

Establishing a locomotive zebrafish larvae bioassay using GABAA receptor modulators

SESSION: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Anke Wilhelm[1], Susan Bonnet[1], Steffen Hering[2]

1 *University of the Free State, Chemistry, Bloemfontein, South Africa*
2 *Chanpharm, Chanpharm, Vienna, Austria*

ABSTRACT TEXT

South Africa has a rich plant biodiversity and many plants have shown potential to be developed into possible lead compounds against diseases of the central nervous system (CNS) such as epilepsy, mood disorders, anxiety and insomnia. Gamma-aminobutyric acid type A (GABAA) receptors are major inhibitory neurotransmitter receptors in the CNS. Various drugs are currently on the market to treat epilepsy, although the lack of GABA receptor subtype selectivity warrants the need for novel drugs with less side effects. Through our collaboration with Prof Steffen Hering, a two-microelectrode voltage clamp assay with *Xenopus laevis* oocytes expressing GABAA receptors is used to identify GABAA receptor agonists as an initial screening.[1] Once a plant extract shows potential as a GABAA agonist, the bioactivity

of the plant extract is then confirmed using a locomotor activity model with zebrafish (*Danio rerio*) larvae.[2] Larval convulsions are provoked with the GABAA receptor antagonist pentylenetetrazol (PTZ) and GABAergic activity of the extracts can be tracked via their lowering of PTZ-provoked larval locomotion.[2] The bioactivity can then be localized to a specific fraction or isolated compound. This is not a new technique, however, to establish this bioassay from scratch in a new laboratory has various challenges. However, the bioassay has been a success at the University of the Free State which led to intellectual transfer from Europe to South Africa as well as training of students on a bioassay which holds the future of drug development in its hands, or fins rather. This bioassay serves as a multi-disciplinary tool for various research departments. The access to GABA receptor bioassays, combined with our access to information on African traditional medicine, makes this project unique.

1 Kim, HJ; Baburin, I; Khom, S; Hering, S; Hamburger, *Planta Med*. 2008; 74(5):521-526.
2 Moradi-Afrapoli, F; Ebrahimi, SN; Smiesko, M; Hamburger, M. *J. Nat. Prod.* 2017; 80(5):1548-1557.

Morphogenesis and Organogenesis II

Calcium Signaling during Primary Angiogenic Sprouting in Zebrafish

SESSION: MORPHOGENESIS AND ORGANOGENESIS II

Daniela Panáková[1], *Laura Bartolini*[1], *Motahareh Moghtadaei*[1]

[1] *Max Delbrück Center for Molecular Medicine, Cardiovascular and metabolic disease program, Berlin, Germany*

ABSTRACT TEXT

Similarities between the endothelial tip cell and the axonal growth cone are well established. The two cell types share not only a similar anatomical structure but also common molecular pathways, responding to the same molecular signals. Ca^{2+} signaling, especially through the L-type Ca^{2+} channel (LTCC), is crucial to regulate the axonal turning facilitating the response to the attractive or repulsive cues during axon growth. In endothelial cells (ECs), the increase in cytosolic Ca^{2+} concentration regulates a number of cell biological processes including migration and proliferation. Yet, the detailed understanding of the fluctuations in the intracellular Ca^{2+}concentrations during angiogenic sprouting *in vivo* is lacking. Moreover, the role of plasma membrane Ca^{2+} channels in angiogenesis is elusive. Here, we characterize Ca^{2+}osc illations during angiogenic sprouting of intersegmental vessels *in vivo* in zebrafish. We provide evidence Ca^{2+} transients differ between tip and stalk cells indicating their discernible physiologic states. Furthermore, we show the LTCC regulates EC migration during the primary angiogenic sprouting. The stimulation of the LTCC increases EC migration from the dorsal aorta (DA), resulting in an over-branching phenotype, while the reduced levels of the channel impair the EC migration and proliferation compromising the ISV formation. These phenotypes strongly correlate with the changes in Ca^{2+} oscillations. LTCC does not act in isolation, and synergizes with other plasma membrane Ca^{2+} channels, namely with the canonical transient receptor potential-1 (TRPC1) Ca^{2+} channel, promoting ISV development. Last, we show the changes in the Ca^{2+} fluxes through LTCC, but not TRPC1, affect the angiogenic VEGF and Dll4/Notch signaling. Taken together, our data reveal the importance of Ca^{2+} fluxes through the EC plasma membrane during angiogenesis, affecting the ECs migratory behavior and regulating angiogenesis.

Adaptive cell invasion of skin-derived ionocytes into hair cell-containing mechanosensory organs

SESSION: MORPHOGENESIS AND ORGANOGENESIS II

Daniela Münch[1,2], Julia Peloggia[1], Paloma Meneses-Giles[1], Andrés Romero-Carvajal[1,3], Melainia McClain[4], Y. Albert Pan[5,6,7], Alexander F Schier[8,9], Tatjana Piotrowski[1]

1 *Stowers Institute for Medical Research, Piotrowski Lab, Kansas City, USA*
2 *Technical University Braunschweig, Cellular and Molecular Neurobiology, Braunschweig, Germany*
3 *Pontificia Universidad Católica del Ecuador, Ciencias Biológicas, Quito, Ecuador*
4 *Stowers Institute for Medical Research, Microscopy- Imaging and Big Data, Kansas City, USA*
5 *Fralin Biomedical Research Institute at Virginia Tech Carilion, Center for Neurobiology Research, Roanoke, USA*
6 *Virginia Tech Carilion School of Medicine, Department of Psychiatry and Behavioral Medicine, Roanoke, USA*
7 *Virginia-Maryland Regional College of Veterinary Medicine- Virginia Tech, Department of Biomedical Sciences and Pathobiology, Blacksburg, USA*
8 *University of Basel, Biozentrum, Basel, Switzerland*
9 *Harvard University, Dept. of Molecular and Cellular Biology, Cambridge, USA*

ABSTRACT TEXT

Embryonic organ morphogenesis often requires cells to migrate long distances, coalesce and re-arrange in a spatiotemporally controlled manner. However, visualizing postembryonic migratory events that alter the cellular composition of pre-existing, functional organs remains challenging. Here, we report the discovery of Neuromast-associated ionocytes (Nm ionocytes), a previously uncharacterized cell type that invades mature mechanosensory organs of the zebrafish lateral line. This process, that we call 'Adaptive Cell Invasion', is dynamically regulated by changes in environmental stimuli, such as pH and salinity. Using high-resolution in vivo time lapse imaging and zebrabow lineage tracing, we characterize the translocation of these highly motile, skin stem cell-derived cells and show that they enter the sensory organ as individual closely associated pairs of cells. After extensive re-arrangement of the cells within the sensory organ that is accompanied by the extension of highly dynamic cellular protrusions, they anchor in a stereotypical position, in close association with mechanosensory hair cells. In fact, 3D reconstruction of Nm ionocyte ultrastructure by serial block face electron microscopy revealed the formation of a microvilli-containing apical crypt in close vicinity to the hair cell bundles and in direct contact with the hair cell microenvironment. Molecular analysis of these cells by scRNAseq and loss of function approaches show that Nm ionocytes maintain the proper ionic composition surrounding lateral line hair cells in response to changing aquatic environments. In sum, this study provides a detailed characterization of cells invading a mature sensory organ postembryonically. The possibility to trigger and modulate this invasive process by external stimuli, as well as the accessibility of the lateral line organ system to live cell imaging approaches makes Nm ionocytes an excellent model system to study cell migration and invasion in unprecedented detail.

Tissue-specific compensatory mechanisms for maintaining body size in polyploid Zebrafish

SESSION: MORPHOGENESIS AND ORGANOGENESIS II

Christopher Small[1], Tillmann Benfey[1], Bryan Crawford[1]

1 *University of New Brunswick, Biology, Fredericton, Canada*

ABSTRACT TEXT

Many whole genome duplication (WGD) events have occurred during evolutionary history and because these WGDs relax selection on copies of conserved genes, WGDs are often linked to the evolution of novel traits. Increased cell size is another consequence of increased genome size, but research has focused primarily on how duplicated genes evolve after WGD, rather than how changes in cell size affect tissue architecture. WGDs in plants result in increased cell size and a commensurate increase in the size of the organism. This pattern holds true in some animals such as nematodes, mollusks, and some arthropods, but ploidy transitions are embryonic lethal in mammals. Teleosts, amphibians, reptiles, and birds represent a middle ground; polyploids are often viable, but the correlation between cell size and body size is decoupled – although their cells are larger, polyploid individuals are no larger than their diploid counterparts. Using transgenic zebrafish with fluorescent histones, ploidy can be measured non-lethally very early in development to identify individuals for comparison. Comparing size and organization of the blood, muscle, and vasculature at the cellular level using immunohistochemistry and live-imaging

highlights how teleosts compensate for increases in ploidy and cell size at the tissue level to maintain body size. Furthermore, we show that while all tissues do indeed compensate for larger cells to maintain organ/tissue dimensions, the mechanisms are tissue-specific.

Twisting of the heart tube during cardiac looping is a tbx5-dependent and tissue-intrinsic process

SESSION: MORPHOGENESIS AND ORGANOGENESIS II

Federico Tessadori[1], *Fabian Kruse*[1], *Susanne van den Brink*[1], *Malou van den Boogaard*[2], *Vincent Christoffels*[2], *Jeroen Bakkers*[1,3]

[1] *Hubrecht Institute, Cardiac Development and Genetics, Utrecht, Netherlands*
[2] *Amsterdam UMC, Medical Biology, Amsterdam, Netherlands*
[3] *University Medical Center Utrecht, Pediatric Cardiology, Utrecht, Netherlands*

ABSTRACT TEXT

Organ laterality refers to the Left-Right (LR) asymmetry in disposition and conformation of internal organs, established in the developing embryo. The heart is the first organ to display visible LR asymmetries as it is positioned to the left side of the midline and undergoes rightward looping morphogenesis. Cardiac looping morphogenesis is tightly controlled by a combination of heart-intrinsic and -extrinsic mechanisms. As the mechanisms that drive cardiac looping are not well understood, we performed a forward genetic screen for zebrafish mutants with defective heart looping. We describe a new loss-of-function allele for *tbx5a*, which displays normal leftward positioning but defective rightward looping morphogenesis. By using live two-photon confocal imaging to map cardiomyocyte behavior during cardiac looping at a single-cell level we establish that during looping, ventricular and atrial cardiomyocytes rearrange in opposite directions towards the outer curvatures of the chambers. As a consequence, the cardiac chambers twist around the atrioventricular canal resulting in torsion of the heart tube, which is compromised in *tbx5a* mutants. Manipulations of cardiac looping by chemical treatment and *ex vivo* culture establishes that the twisting of the heart tube depends on intrinsic mechanisms and is independent from tissue growth by cell addition. Furthermore, the cardiac looping defect in *tbx5a* mutants is rescued in *tbx5a/tbx2b* double mutants, indicating that it requires proper tissue patterning. Together, our results establish that cardiac looping in zebrafish involves twisting of the chambers around the AV canal, which requires correct tissue patterning by Tbx5a.

Circuits and Behavior

Future state prediction error improves active avoidance behavior by adult zebrafish in virtual reality

SESSION: CIRCUITS AND BEHAVIOR

Makio Torigoe[1], *Tanvir Islam*[1,2], *Hisaya Kakinuma*[1,2], *Chi Chung Fung*[3], *Takuya Isomura*[4], *Hideaki Shimazaki*[5], *Tazu Aoki*[1], *Tomoki Fukai*[3], *Hotoshi Okamoto*[1,2]

[1] *RIKEN Center for Brain Science, Lab. for Neural Circuit Dynamics of Decision Making, Wako, Japan*
[2] *RIKEN Center for Brain Science, RIKEN CBS-Kao Collaboration Center, Wako, Japan*
[3] *Okinawa Institute of Science and Technology, Neural Coding and Brain Computing Unit, Onna-son, Japan*
[4] *RIKEN Center for Brain Science, Brain Intelligence Theory Unit, Wako, Japan*
[5] *Hokkaido University, Center for Human Nature- Artificial Intelligence- and Neuroscience CHAIN, Sapporo, Japan*

ABSTRACT TEXT

Selecting optimal actions in decision making according to the current sensory input is essential for animals. One prevailing model underlying this process is based on the idea that the ultimate aim of choice is to maximize utility or reward. In contrast, an alternative model has been proposed in which adaptive behavior requires animals to generate the internal model of their environment and to take actions

to minimize surprise about the state they encounter in comparison with the state predicted from the internal model. Which of these mechanisms animals adopt for decision making remains unknown.

We addressed this question using the closed-loop virtual reality-2photon calcium imaging system to train adult zebrafish, in which excitatory neurons expressed G-CaMP7, for active and passive avoidance. In the active avoidance, fish had to escape from the region surrounded by blue walls to the region in their front surrounded by red walls to avoid electric shock. In the passive avoidance, fish had to stay in the red wall region where the tasks started. Analysis of the dorsal pallium's neural activity by non-negative matrix factorization revealed neural ensembles assigning reward values to the surrounding walls' colors. Furthermore, conversion of the virtual reality system to the open-loop condition, where the scenery did not respond to the fish tail beat, revealed that one-third of learned fish generated another ensemble encoding the prediction error of the perceived scenery's status compared with the predicted favorable status. Intriguingly, the fish with both ensembles escaped more efficiently than the fish with the former ensemble alone, demonstrating that the latter ensemble guided zebrafish to take action to minimize this prediction error.

Altogether, zebrafish can use both of the two principles in active avoidance, *i.e.* value maximization and surprise minimization. In addition, our results support the first time that the future state prediction error improves behavior.

Functional Imaging and Optogenetic Analysis of Cells Derived from Three Germ Layers in the Larval Zebrafish Gut

SESSION: CIRCUITS AND BEHAVIOR

Daiji Takamido[1], Shin-ichi Okamoto[1], Shiori Satoh[2], Yumiko Mizumaki[2], Risa Wada[2], Ayumi Jimpo[2], Koichi Kawakami[3], Masataka Nikaido[1], Kohei Hatta[1]

[1] *University of Hyogo, Graduate School of Life Science, Akou, Japan*
[2] *University of Hyogo, Department of Science, Akou, Japan*
[3] *National Institute of Genetics, Laboratory of Molecular and Developmental Biology, Mishima, Japan*

ABSTRACT TEXT

Zebrafish larval gut could be a good model to investigate the motor function of digestive organs. Previously, we reported, in peristaltic reflex, strong periodic Ca2+ events in the circular smooth muscles and neurons. Meanwhile, other types of movements, more regular and higher in frequency have been observed, including retrograde waves in the proximal intestine (PI), anterograde waves in the mid-and distal intestine (MI, DI) and rapid contractions in the distal end of intestine (DE). In order to investigate the mechanism of their formation, we express Ca2+ indicator GCaMP3 in various cell types and search cells which fire in association with each wave. We also use photoconversion of Kaede to examine cell shapes. Here, we demonstrate a distinctive non-neuronal cell group acts strongly in conjunction with anterograde waves in MI and DI, which may correspond to the interstitial cells of Cajal or pacemaker cells. In addition, Ca2+ events were found in the endodermal tissues, some of which were associated with local gut wall movement. When endodermal cells expressing ChR2 were stimulated by irradiation with blue light in MI or DI, peristalsis reflex-like activity was induced. On the other hand, in PI, amplification of the retrograde waves was observed without changing its frequency by stimulation of endoderm or a neuron, while a strong local contraction perpendicular to the duct-axis were induced by stimulation of a neuron. Activation of nitrergic neurons inhibited rapid regular contractions in DE. In conclusion, various types of neurons and non-neuronal cells are associated with gut movements, and optogenetic stimulation of a single to small number of cells could manipulate such movements in vivo.

The left-right directional information in zebrafish is processed through the dorsal lateral habenula-interpeduncular nucleus pathway for decision making

SESSION: CIRCUITS AND BEHAVIOR

Bor-Wei Cherng[1,2], *Hitoshi Okamoto*[1,2]

[1] *RIKEN, Center for Brain Science, Wako- Saitama, Japan*
[2] *Graduate School of Arts and Sciences- The University of Tokyo, Department of Life Sciences, Tokyo, Japan*

ABSTRACT TEXT

Exploiting directional information is essential to adapt environment. However, the underlining mechanism remains unclear. We address the issue by designing a behavioral paradigm with an automated training system, unbiasedly monitored the learning process and selection pattern of information by reward reinforcement training. Zebrafish learn to exploit the information by associating the reward with the internal directional rule (choice from left or right), or external cued rule (choice from blue or red). With the repeated rule-shifts, zebrafish keep their behavioral flexibility to adapt to the exchanged information. The time to re-apply the rules is decreased as a function of the repeated number for rule shifts, revealing the rule memory retrieval. Intriguingly, we found that the zebrafish with the silenced pathway in the lateral subregion of the dorsal habenula (dHbL) to the dorsal interpeduncular nucleus (dIPN) specifically impairs the internal-directional rule learning. As dHbL-dIPN is known to be the potentiated winner circuit under conflict behavior, this result indicates that the dHbL-dIPN circuit engages the dual function in adopting internal-directional information and determining social hierarchy.

Exploring the role of the P2y12 receptor in the zebrafish brain

SESSION: CIRCUITS AND BEHAVIOR

Sarra Zenagui[1], *Adrian Ekehorn Gimdal*[1], *Kettunen Petronella*[1]

[1] *University of Gothenburg, Neuroscience and Physiology, Gothenburg, Sweden*

ABSTRACT TEXT

Microglia are resident immune cells that play a crucial role in central nervous system (CNS) function. During the past years, research has provided a better understanding of these immune cells and emphasized their importance in brain development and diseases. However, some mechanisms underlying their function remain unclear. The purinergic receptor P2Y12 is expressed by microglia cells and has been proposed to be involved in different CNS properties such as neuroinflammation and modulation of behaviors. In this study, we used a newly developed *p2y12* CRISPR mutant and wild-type zebrafish to better understand the role of P2y12 for brain function. First, we compared *in silico* human and zebrafish P2Y12 protein structures to identify conserved amino acids, especially those involved in ligand recognition. Secondly, in order to assess the impact of *p2y12* mutation on microglia clearance of dead cells during development, we analyzed brain confocal images of larvae from both genotypes. Finally, we compared larvae locomotion behavior at 5, 6, 7 and 8 days post fertilization to identify any differences in overall activity in *p2y12* mutants and controls. Our findings show the importance of P2y12 for clearance of dead cells, implying that P2y12 could underlie diseases caused by microglia dysfunction.

Disease Models II

Adult pdx1-deficient zebrafish display diabetes-induced vasculopathy in the retina

SESSION: DISEASE MODELS II

Jens Kroll[1], *Lucas Wiggenhauser*[2], *Haozhe Qi*[2], *Lena Metzger*[2], *Katrin Bennewitz*[2], *Hans-Peter Hammes*[3]

[1] *Medical Faculty Mannheim- Heidelberg University, Vascular Biology, Mannheim, Germany*
[2] *Heidelberg University, Vascular Biology, Mannheim, Germany*
[3] *Heidelberg University, 5. Medical Department, Mannheim, Germany*

ABSTRACT TEXT

Background: Diabetic retinopathy is a common microvascular complication of diabetes mellitus. Zebrafish provide similar retinal physiology compared to humans and could enhance the available set of screening organisms for new interventions and help to uncover possible new disease mechanisms. We established a new genetic animal model in embryonic and adult zebrafish to study diabetic retinopathy via gene knockout of pdx1.

Methods: Pdx1-deficient zebrafish were generated using CRISPR/Cas9 mediated genome editing. The transgenic lines Tg(hb9:GFP) and Tg(fli1:EGFP) were utilized to investigate pancreatic and vascular changes, respectively. Metabolic profiling and assessment of the hyaloid vasculature was done at the larval stage at 5 dpf and 6 dpf. Adult zebrafish were sacrificed for blood sugar measurements, histology, microarrays and retinal preparations.

Results: pdx1-/- embryos showed reduced pancreatic size and increased whole body glucose levels. Adult pdx1-/- zebrafish have increased blood sugar values 1 hour and 2 hours after feeding. Homozygous pdx1-/- gene knockout in zebrafish led to structural and functional changes of the hyaloid vasculature in 6 dpf old larvae. The adult retinal vasculature analysis uncovered increased sprouting angiogenesis in pdx1+/- and pdx1-/- zebrafish and changes in the vascular architecture. Metabolome analysis of the tissue uncovered alterations in the amino acid metabolism linked to nitric oxide production. Lastly, the hyperbranching and hypersprouting alterations responded to anti-angiogenic and anti-diabetic therapies.

Conclusion: pdx1 knockout successfully lead to a diabetic condition with increased blood sugar values and impaired pancreas development in juvenile and adult pdx1-/- zebrafish. Both larval and adult mutant zebrafish exhibited specific alterations in the vasculature of the eye. These findings in total suggest that zebrafish are susceptible to pdx1 knockout-mediated diabetic microvascular complications in the retina.

The nuclear gene rpl18 regulates erythroid maturation via JAK2-STAT3 signaling in zebrafish model of Diamond-Blackfan anemia

SESSION: DISEASE MODELS II

Cheng Chen[1], *Mengjia Lu*[1], *Shuo Lin*[2], *Wei Qin*[1]

[1] *Peking University Shenzhen Graduate School, State Key Laboratory of Chemical Oncogenomics, Shen, China*
[2] *University of California- Los Angeles, Department of Molecular- Cell and Developmental Biology, Los Angeles, USA*

ABSTRACT TEXT

Diamond–Blackfan anemia (DBA) is a rare, inherited bone marrow failure syndrome, characterized by red blood cell aplasia, developmental abnormalities and enhanced risk of malignancy. However, the underlying pathogenesis of DBA is yet to be understood. Recently, mutations in the gene encoding ribosomal protein (RP) L18 were identified in DBA patients. RPL18 is a crucial component of the ribosomal large subunit but its role in hematopoiesis remains unknown. To genetically model the ribosomal defect identified in DBA, we generated a *rpl18* mutant line, using CRISPR/Cas9 system, in zebrafish molecular characterization of this mutant line demonstrated that Rpl18

deficiency mirrored the erythroid defects of DBA, namely a lack of mature red blood cells. Rpl18-deficiency caused an increase in p53 activation and JAK2-STAT3 activity. Furthermore, we found inhibitors of JAK2 or STAT3 phosphorylation could rescue anemia in *rpl18* mutants. Our research provides a new *in vivo* model of Rpl18 deficiency and suggests involvement of signal pathway of JAK2-STAT3 in the DBA pathogenesis.

Zebrafish calreticulin loss of function model as a potential therapeutic target for human CALR mutated myeloproliferative neoplasms

SESSION: DISEASE MODELS II

Kazi Md Mahmudul Hasan[1], *Xiangke Chen*[2], *Alvin Chun Hang Ma*[1]

[1] *The Hong Kong Polytechnic University, Department of Health Technology and Informatics, Kowloon, Hong Kong*
[2] *The University of Hong Kong, School of Biomedical Sciences, Pokfulam, Hong Kong*

ABSTRACT TEXT

Myeloproliferative neoplasms (MPNs) are clonal hematopoietic disorders characterized by JAK2, CALR, or MPL driver mutations upon activation of JAK/STAT signaling pathway. MPNs are classified into essential thrombocythemia (ET), primary myelofibrosis (PMF) and polycythemia vera (PV) where calreticulin (*CALR*) exon 9 driver mutations occurring in approximately 30 % of ET and PMF patients. However, lack of in vivo model system makes it difficult to endorse preclinical trials to find out suitable and potential drug targets against *CALR* mutated MPNs.

We have taken advantages of the in silco genome editing and functional genomics to establish a robust *calr* loss of function zebrafish model which strikingly defended the human mutant *CALR* effects without affecting their normal hematopoietic functions.

Our results demonstrated that loss of normal *calr* function slightly perturbed definitive hematopoiesis by increased pan leukocytes (*lcp1*), neutrophils (*mpx* and *lyz*), macrophages (*mpeg1.1*) expression and decreased lymphocytes (*rag1*), erythrocytes (*gata1*), embryonic hemoglobin (*hbae1.1*) levels without affecting hematopoietic stem cells (*cmyb* and *cd41*^{low}) and common myeloid progenitors (*pu.1*). Interestingly, overexpression of human *CALR* mutation (*CALR*$^{T\text{-}I}$ and *CALR*$^{T\text{-}II}$) strikingly causes hematopoietic defects in wild type siblings (*calr*$^{+/+}$) through aberrant HSCs proliferation, pan leukocytes, granulocytes expansion and severe anemia. Overall survival percentages attenuated significantly in *calr*$^{+/+}$ groups (14 %) at three days post fertilized embryos comparing with the null functional *calr*$^{-/-}$ mutants (56 %). Furthermore, mutated *CALR* overexpressing zebrafish *calr*$^{-/-}$ mutants treated with the chemotherapeutic drug ruxolitinib significantly ameliorated *CALR* mutagenicity and potentiate overall survival rate.

These findings indicated that zebrafish *calr* loss of function model can be a novel therapeutic target for the treatment of human *CALR* mutated MPNs.

Molecular mechanisms of neural stem cell plasticity and regenerative neurogenesis in Alzheimer's disease model of zebrafish brain: from zebrafish to humans

SESSION: DISEASE MODELS II

Prabesh Bhattarai[1], *Mehmet Ilyas Cosacak*[1], *Hilal Celikkaya*[1], *Tohid Siddiqui*[1], *Kerstin Brandt*[1], *Stanislava Popova*[1], *Uwe Freudenberg*[2], *Yixin Zhang*[3], *Carsten Werner*[2], *Caghan Kizil*[1]

[1] *German Center for Neurodegenertive Diseases DZNE- Helmholtz Association, AG Kizil, Dresden, Germany*
[2] *Institute für Polymerforschung IPF Dresden- Leibniz Institute, AG Werner, Dresden, Germany*
[3] *CMCB- B-CUBE- TU Dresden, AG Zhang, Dresden, Germany*

ABSTRACT TEXT

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and is incurable. The exact cause and how to revert the disease manifestation are unknown. The neurocentric view of AD, which proposed that the neurons are the centrally affected cells and

a treatment could be possible by focusing on these cell types, evolved into a more complex paradigm where many non-neuronal cell types are now thought to be causative to the onset of AD: immune cells, neurovascular unit, and neural stem cells. All these cell types malfunction in AD and contribute to the complex pathological output. In our lab, we hypothesized that providing new neurons into the diseases brain would be a way to circumvent the effects of AD. One specialized cell type that is capable of doing so is the endogenous neural stem cells, which could act as reservoirs for new neurons; however, lose their ability to do so in disease conditions. Therefore, we are investigating the molecular mechanisms through which human neural stem cells could be coaxed to be plastic – proliferative and neurogenic – again in AD, and we do so by using zebrafish – an organism with extraordinary neuro-regenerative ability. We believe that we can design novel therapeutic ways to fight AD from a neuro-regenerative perspective with the findings in zebrafish. I will present (1) our zebrafish model of AD and its regenerative ability, (2) the molecular programs that we identified to enable a regenerative response after Alzheimer's-like loss of neurons, (3) first single cell sequencing-based analyses of the adult zebrafish brain in AD conditions, (4) a novel 3D culture method for experimentally modeling human neural stem cell plasticity, and (5) our current efforts to translate the findings in zebrafish to humanized systems.

Relevant publications

- Cosacak et al. (2019). Cell Reports.
- Celikkaya et al (2019) Frontiers in Cellular Neuroscience.
- Papadimitriou et al. (2018) Developmental Cell.
- Bhattarai et al. (2016) Cell Reports.

A functional role of microglia in epilepsy

SESSION: DISEASE MODELS II

Alexandre Brenet[1], Julie Somkhit[1], Rahma Hassan-Abdi[1], Constantin Yanicostas[1], Nadia Soussi-Yanicostas[1]

[1] *INSERM U1141-NeuroDiderot, Hospital Robert Debre, Paris, France*

ABSTRACT TEXT

Epilepsy is one of the most common neurological disorders, affecting approximately 1 % of people worldwide across the age-span. Epilepsy is the fourth most common neurological disease, affecting more than 65 million people worldwide. It is characterized by spontaneous recurrent seizures. Despite many studies about the involvement of microglial cells in the pathophysiology of epilepsy and their comorbidities, it is still unclear whether microglial functions are beneficial or harmful for hyper excited neurons.

By combining a genetic models of epilepsy in zebrafish with transgenic lines expressing fluorophores in microglial cell as well as a fluorescent calcium sensor in neurons, we monitored neuronal activity as well as microglial phenotypes in real-time. We also looked at the effect of microglial depletion on the neuronal activity through calcium imaging and local field potential recordings. Finally we assessed the consequences of the brain hyperactivity on the locomotor behavior of the larvae.

Our work being published shows real-time microglial dynamic in a living hyper-excited zebrafish brain and demonstrates the modulation of the brain activity by microglial cells, underlying the interest of targeting these cells in order to develop new anti-antiepileptic treatments.

Recent publications of the team related to the present work

- Brenet A, Hassan-Abdi R, Somkhit J, Yanicostas C, Soussi-Yanicostas N. ***Cells.*** **2019**;8(10):1199.
- Van Steenwinckel J, Schang A-L, Krishnan ML, et al. **Brain. 2019**;142(12):3806-3833.
- Hassan-Abdi R, Brenet A, Bennis M, Yanicostas C, Soussi-Yanicostas N. **Front Neurosci. 2019**;13.
- Mairesse J, Zinni M, Pansiot J, et al. ***Glia. 2019***;67(2):345-359. doi:10.1002/glia.23546
- Samarut É, Swaminathan A, Riché R, et al. ***Epilepsia.*** **2018**;(September):2061-2074.
- Swaminathan A, Hassan-Abdi R, Renault S, et al. ***Curr Biol.*** **2018**;28:1924-1937.
- Brenet A, Somkhit J, Hassan-Abdi R, et al. ***Preprint.*** doi.org/10.1101/2019.12.15.876649

Stem Cells

A csf1rb mutation uncouples two waves of microglia development in zebrafish

SESSION: STEM CELLS

Giuliano Ferrero[1], *Magali Miserocchi*[1], *Elodie Di Ruggiero*[1], *Valerie Wittamer*[1]

[1] *Université Libre de Bruxelles, Iribhm, Brussels, Belgium*

ABSTRACT TEXT

In vertebrates, the ontogeny of microglia, the resident macrophages of the central nervous system, initiates early during development from primitive macrophages. While murine embryonic microglia then persist through life, in zebrafish these cells are transient, as they are fully replaced by an adult population originating from larval hematopoietic stem cell (HSC)-derived progenitors. *Colony-stimulating factor receptor 1* (*csf1r*) is a fundamental regulator of microglia ontogeny in vertebrates, including zebrafish which possess two paralogous genes: *csf1ra* and *csf1rb*. While previous work showed invalidation of both genes completely abrogates microglia development, the specific contribution of each paralog remains largely unknown. Here, using a fate-mapping strategy to discriminate between the two microglial waves, we uncover non-overlapping roles for *csf1ra* and *csf1rb* in hematopoiesis, and identified *csf1rb* as an essential regulator of adult microglia development. Notably, we demonstrate that *csf1rb* positively regulates HSC-derived myelopoiesis, resulting in macrophage deficiency, including microglia, in adult mutant animals. Overall, this study contributes to new insights into evolutionary aspects of Csf1r signaling and provides an unprecedented framework for the functional dissection of embryonic versus adult microglia in an *in vivo* model.

Unveiling the heterogeneity of vertebrate adult Neural Stem Cells

SESSION: STEM CELLS

David Morizet[1], *Alessandro Alunni*[1], *Laure Bally-Cuif*[1]

[1] *CNRS UMR3738-Institut Pasteur, Developmental and Stem Cell Biology, Paris, France*

ABSTRACT TEXT

The vertebrate brain harbors neural stem cells (NSCs) that persist into adulthood to ensure the life-long production of functional neurons. These NSCs are mainly quiescent, a feature important for their maintenance into old age. The adult zebrafish pallium hosts NSCs with similar characteristics to their mammalian counterparts but present in large number, making it a powerful model to unravel NSC properties. Using this model, our lab recently demonstrated the existence of subpopulations of NSCs that differ in their quiescence depths[1] and/or are hierarchically organized along a cascade leading to activation and neuronal production[2]. However the molecular signature and functionally relevant markers for these subpopulations remain unknown.

In order to get a better description of the heterogeneity of quiescent NSCs we used single-cell RNA-sequencing with the 10x Genomics platform. We recovered over 17k cells, including more than 3k quiescent NSCs, making it the most extensive dataset on the adult zebrafish forebrain and on adult NSCs to date. This allowed us to discover new cell subtypes, and in particular highlighted the existence of previously undescribed glial sub-clusters that differ in their level of quiescence and/or commitment. To uncover functional interactions between clusters and identify potential master regulators of NSC quiescence, we developed and adapted tools applied to scRNA seq analysis in mammals for use with zebrafish. Applying these tools to a reiterated scRNAseq analysis upon transient blockade of Notch signaling, which poises NSCs for activation, further revealed the existence of a rare population of resistant NSCs, and identified their putative regulator of quiescence and *E(spl)* genes. These results together provide molecular signatures for NSC heterogeneities, including for a novel, Notch-independent, deeply quiescent NSC sub-population.

1. *et al. Cell Rep.* **17**, 1383–1398 (2016).
2. *et al. Sci. Adv.* **in press**, (2020).

Transposable elements induce a RIG-I-like receptor-mediated inflammation to regulate HSPC emergence

SESSION: STEM CELLS

Stylianos Lefkopoulos[1,2], Aikaterini Polyzou[1], Thomas Clapes[1], Veronica Bergo[1,3], Pierre Cauchy[1], Carolina Jerez-Longres[1], Megumi Onishi-Seebacher[4], Na Yin[1], Natalia-Adriana Martagon-Calderón[1], Kathryn S. Potts[5], Lhéanna Klaeylé[1], Feng Liu[6], Teresa V. Bowman[5], Thomas Jenuwein[4], Marina Mione[3], Eirini Trompouki[1]

1 *Max Planck Institute of Immunobiology and Epigenetics, Department of Cellular and Molecular Immunology, Freiburg, Germany*
2 *University of Freiburg, Faculty of Biology, Freiburg, Germany*
3 *University of Trento, Centre for Integrative Biology, Trento, Italy*
4 *Max Planck Institute of Immunobiology and Epigenetics, Department of Epigenetics, Freiburg, Germany*
5 *Albert Einstein College of Medicine, Department of Developmental and Molecular Biology, Bronx- NY, USA*
6 *Institute of Zoology- Chinese Academy of Sciences, State Key Laboratory of Membrane Biology, Beijing, China*

ABSTRACT TEXT

Inflammatory signaling is a key regulator of hematopoietic stem and progenitor cell (HSPC) generation, yet the mechanisms driving these signals are largely unexplored. Here we report that pharmacological induction of transposable element (TE) expression with a histone deacetylase (HDAC) inhibitor enhances HSPC generation in zebrafish embryos. Overexpression of one TE, namely a *sine3-1a*, recapitulates the phenotype. To address how TE transcripts signal to determine cell fate, we speculated that the RIG-I-like receptor (RLR) family, a viral RNA sensor family (*rig-I*, *mda5* and *lgp2*), is involved in TE transcript recognition and hematopoiesis. Indeed, *rig-I* and *mda5* morphants and crispants show impaired HSPC formation, while Lgp2-deficient embryos exhibit increased HSPC numbers. Simultaneous deficiency in *lgp2* and *rig-I* or *mda5* rescues HSPCs in the latter morphants. Expression and chromatin accessibility analyses in endothelial and hemogenic endothelial cells, showed that Rig-I or Mda5 loss leads to impaired inflammatory signaling which explains the HSPC reduction in these morphants, while loss of Lgp2 enhances inflammation and HSPC numbers. Since LGP2 was preciously reported to interact with TRAF6, we then checked whether this interaction was involved in our phenotype. Indeed, an optimal dose of *traf6* morpholino impairs HSPC formation. A sub-optimal dose of the same morpholino in *lgp2* morphants abrogates the increase of HSPCs, suggesting the phenotype of Lgp2 is Traf6-dependent. Finally, to verify our hypothesis that TEs activate RLRs to modulate HSPC emergence, we used sub-optimal doses of *rig-I* or *mda5* morpholino in HDAC inhibitor treated or *sine3-1a* overexpressing embryos and found that this dose abrogates the HSPC increase caused by the drug or the TE overexpression. Our work uncovers a previously unknown role for TE transcripts, which signal through RLRs to fine-tune inflammatory signals necessary for HSPC generation.

Redox biology of neural stem cell: understanding the role of antioxidants during retinogenesis

SESSION: STEM CELLS

Shahad Albadri[1], Marion Thauvin[2], Sacha Reichman[1], Olivier Goureau[1], Sophie Vriz[2], Filippo Del Bene[1]

1 *Institut de la Vision, Department of Development, Paris, France*
2 *Collège de France, Homéoprotéines et Plasticité Cellulaire, Paris, France*

ABSTRACT TEXT

In the past, the majority of research on reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) has classically focused on their deleterious effect. However several reports have recently pointed at a physiological role for redox signaling in stem cell biology. One model is that ROS may play the role of a "*stem cell rheostat*" by acting as a link between extrinsic cues and cellular response to intrinsic programs. In this context, we here assess the *in vivo* role of redox signaling for retinal stem and progenitor cell (RSCs and RPCs). The zebrafish retina presents a unique advantage in that it contains at its periphery a stem cell niche called the ciliary marginal zone (CMZ). The CMZ is composed of RSCs and RPCs are spatially distributed in distinct domains. Using an H_2O_2 sensor line, we observed that H_2O_2 levels coincide with the proliferative state of RPCs. Furthermore we show that the fine regulation of H_2O_2 in RPCs by the metabolic enzyme Catalase is crucial to mediate the switch of RPCs from proliferation to differentiation. Our results reveal for the first time the physiological role for redox signaling in RPC differentiation and CMZ homeostasis. Currently, we are asking how Catalase and Sod2 enzymes, both expressed in the CMZ, are themselves regulated in this context. Our research is focused on the family of Cap'N'Collar (CNC) TFs, shown to play a cytoprotective role in several stem cell populations. This function is ensured via their transcriptional activity on antioxidant-response elements present in the

cis-region of many genes encoding for metabolic enzymes like Catalase and Sods. While their role during retinogenesis has not been addressed so far, CNC TFs have been implicated in neurodegenerative and oxidative stress -associated diseases including retinopathies, sparking high interest for their therapeutic potential in the last years. Using loss and gain-of-function approaches, we are exploring these questions, investigating their role in RSCs and RPCs.

Toxicology

New insights into the osteotoxicity of benzo[α]pyrene in zebrafish

SESSION: TOXICOLOGY

Marco Tarasco[1], Paulo J. Gavaia[1,2], João Cardeira-da-Silva[3], Anabela Bensimon-Brito[3], Fabrice P. Cordelières[4], Maria J. Bebianno[5], Didier Stainier[3], M. Leonor Cancela[1,2,6], Vincent Laizé[1]

1 *Centre of Marine Sciences, Universidade do Algarve, Faro, Portugal*
2 *Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal*
3 *Max Planck Institute for Heart and Lung Research, Department of Developmental Genetics, Bad Nauheim, Germany*
4 *Bordeaux Imaging Center, UMS 3420 CNRS - University of Bordeaux - US4 INSERM, Bordeaux, France*
5 *Centre of Marine and Environmental Research, University of Algarve, Faro, Portugal*
6 *Algarve Biomedical Center and Centre for Biomedical Research, University of Algarve, Faro, Portugal*

ABSTRACT TEXT

Persistent and ubiquitous organic pollutants (POPs), such as the polycyclic aromatic hydrocarbon benzo[α]pyrene (BaP), represent a major threat to all aquatic organisms but also to human health. Beside some well-documented adverse effects on the development and reproduction of aquatic organisms, BaP was recently shown to also affect fish bone formation and skeletal development through mechanisms that remain poorly understood. In this work, several zebrafish bone-related assays were used to evaluate the osteotoxic effects of BaP. Waterborne exposure to BaP induced a dose-dependent reduction of the size of the opercular bone in 6 days post-fertilization (dpf) larvae and increased the incidence of skeletal deformities in 30 dpf juveniles. The amount of mineralized bone was also reduced in adult zebrafish exposed to BaP during the regeneration of both their caudal fin and scales, through mechanisms that involved increased bone resorption by osteoclasts. Reporter lines *Tg(Ola.sp7:mCherry)* and *Tg(Ola.oc:EGFP)* and transcriptomic analysis were used to unveil the cellular and molecular dynamics underlying BaP osteotoxicity. We found that the expression of genes involved in the xenobiotic signaling pathway and bone matrix formation were altered upon BaP exposure. Our work provides novel data on the cellular and molecular players involved in BaP osteotoxicity and confirms the suitability of the zebrafish as a model organism for bone-related developmental and ecotoxicological studies.

This work was funded by Portuguese national funds from the Foundation for Science and Technology (FCT) through projects UIDB/04326/2020 and UIDP/00350/2020. Marco Tarasco was supported by the FCT through the PhD grant SFRH/BD/128634/2017.

Automated feature recognition in zebrafish embryos for chemical hazard characterisation

SESSION: TOXICOLOGY

Stefan Scholz[1], *Elisabet Teixido*[2], *Tobias Kießling*[3], *Willy Oesterheld*[1], *Chih Lai*[4]

[1] *Helmholtz Centre for Environmental Research - UFZ, Bioanalytical Ecotoxicology, Leipzig, Germany*
[2] *University of Barcelona, Pharmacology, Barcelona, Spain*
[3] *TKS3, Scientific Software Solutions, Leipzig, Germany*
[4] *University of St. Thomas, Graduate Program in Software- School of Engineering, St. Paul, USA*

ABSTRACT TEXT

Morphological alterations for chemical hazard characterisation in zebrafish embryos are typically estimated from visual assessment by an experienced observer using a microcope. This assessment can be biased by the experience and potential fatigue of the observer. Furthermore, subtle changes may not be recognised and the reproducibility for a quantitative assessment and pattern analysis is limited. Using an automated assessment based on the FishInspector software (Teixido et al. 2019, Tox. Sci., 167, 438–449.) we obtained EC50 patterns of morphological alterations for 26 different test compounds. Similarity was observed between compounds that shared similar mode of actions such as interference with retinoic acid metabolism or ACCase inhibition. Variability in patterns may be explained by discrepancies between the pharmacological mode of action used for grouping of chemicals and the MoA provoking the developmental phenotype.

The feature recognition occasionally required user correction and the number of features that could be detected was limited. Therefore, the previously detected features were used to train feature-specific models using the Matlab Computer Vision Toolbox and the VGG-19 pre-trained Convolutional Neural Network. Most of the features such as body contour or yolk revealed very high recognition superior to the previously used segmentation based approach. Given the versatility of the model-based approach new features can be trained in a step wise approach using initially a relatively small number of manually labeled image with subsequent rounds of automated recognition and gradually reduced need for user interaction. This approch allows to train images from various orientation of embryos and is very flexible regarding different image characteristics and user preferences. While the approach was developed for toxicological research it is applicable in other areas such as functional genetics, e.g. for the unbiased screening of mutants or knock-outs.

Toxicity assessment and behavioral effects of parabens in zebrafish early-life stages

SESSION: TOXICOLOGY

Carmine Merola[1], *Monia Perugini*[1], *Giovanni Angelozzi*[1], *Tyrone Lucon-Xiccato*[2], *Cristiano Bertolucci*[2]

[1] *University of Teramo, Faculty of Bioscience and Agro-Food and Environmental Technology, Teramo, Italy*
[2] *University of Ferrara, Department of Life Sciences and Biotechnology, Ferrara, Italy*

ABSTRACT TEXT

Parabens are esters of *p*-hydroxybenzoic acid used as preservatives in personal care products, food and pharmaceuticals due to their antimicrobial activity. Recently, data on human exposure and ecotoxicological studies raised concerns regarding their safety. Thus, this study aimed to enrich the knowledge about the neurotoxicological effects of parabens using zebrafish (*Danio rerio*) as animal model. Fish embryo acute toxicity tests (FET tests) were performed on zebrafish fertilized eggs according to OECD guideline n. 236. And methylparaben (MeP), ethyl-paraben (EtP) and butylparaben (BuP) were tested. The toxicity of parabens was found in the order of BuP > EtP > MeP. Moreover, parabens exposure led to developmental abnormalities and teratological effects including misshaped yolk sac, reduction in blood circulation, reduced heartbeat, blood stasis, pericardial edema and deformed notochord as well as delay in hatching rate. To study behavior, circadian photic entrainment, average locomotor activity, and thigmotaxis were recorded automatically using DanioVision equipment. Zebrafish larvae exposed to sublethal concentrations of parabens showed able to synchronize their behavior to photic conditions with a typical diurnal pattern, i.e., higher activity during light phases compared to dark phases. However, high sublethal concentrations of parabens led to alternation in the average locomotor activity, namely activity suppression for BuP and hyperactivity for EtP and MeP. In addition, exposure to higher concentrations of BuP and EtP caused increased thigmotaxis response in larvae. The present study detected sublethal

effects and behavioral changes in zebrafish early-life stages exposed to parabens, then further surveys are needed to understand new potential modes of action of these endocrine-disrupting chemicals on central nervous system.

Zebrafish as a model for investigating noise-Induced physiological stress and hearing loss

SESSION: TOXICOLOGY

Raquel Vasconcelos[1]

[1] *University of Saint Joseph, Institute of Science and Environment, Macao, Macao*

ABSTRACT TEXT

The zebrafish, *Danio rerio*, has become an important model to investigate the mechanisms underlying inner ear development, hair cell regeneration, and to test ototoxic environmental agents. However, there is no information available on the natural soundscape of this species, and neither on the acoustics of zebrafish large-scale housing systems. Although anthropogenic noise is known to cause hearing dysfunction and physiological stress, limited knowledge is available on the mechanisms underlying noise-induced hearing loss in fish.

The goals of this project were: 1) characterize the natural soundscape of zebrafish and compare it with the captive conditions; 2) evaluate the impact of noise on zebrafish hearing and inner ear; and 3) investigate the effects of acoustic stress in early development.

Our findings from field recordings in Southwest India showed that the species' natural soundscapes were quite diverse in spectral composition, presenting quiet noise windows matching with the best hearing range. Contrastingly, housing systems revealed higher noise levels with potential to cause auditory masking.

Lab experiments involved exposure of zebrafish to white noise versus silent control for 24 h, and measurement of auditory sensitivity through the auditory evoked potential recording technique. Results revealed noise level-dependent temporary auditory threshold shifts and increased response latency. Hearing impairment and recovery was accompanied by significant hair cell loss followed by regeneration.

In addition, noise treatments in larval zebrafish induced changes in mortality, yolk sac consumption, and cardiac rate.

We provide first evidence of zebrafish adaptation to the acoustic conditions in the wild, first baseline data of noise-induced hearing loss, as well as new insights on how acoustic stress can impact fish in early ontogeny.

List of Poster Abstracts

Cancer

Cell Signaling

Chemical Biology and Drug Discovery

Circuits and Behavior

Disease Models

Education and Outreach

Emerging Techologies

Epigenetics, Gene Regulation and Genomic Resources

Evolution

Genomic Resources

Germ Line, Early Development and Patterning

Husbandry and Aquaculture

Immunity and Infection

Metabolism

Morphogenesis and Organogenesis

Neurobiology

Patterning

Regeneration

Stem Cells

Toxicology

Cancer

P 001: Extracellular vesicles release by colorectal cancer cells and modulation of innate immune response in zebrafish

TOPIC: CANCER

Marco Cafora[1], Luca Ferrari[2], Federica Rota[2], Mirjam Hoxha[2], Simona Iodice[2], Maria Dolci[3], Letizia Tarantini[2], Serena Delbue[3], Valentina Bollati[2], Anna Pistocchi[1]

[1] *Università degli Studi di Milano, Department of Medical Biotechnology and Translational Medicine, Segrate, Italy*
[2] *Università degli Studi di Milano, EPIGET LAB- Department of Clinical Sciences and Community Health, Milan, Italy*
[3] *Università degli Studi di Milano, Department of Biomedical- Surgical and Dental Sciences, Milan, Italy*

ABSTRACT TEXT

Tumor cells proliferate, infiltrate, and metastasize by forming a metastatic niche that can be defined as a supportive and receptive microenvironment, which evades the surveillance by the immune system. Extracellular vesicles (EVs) are important components of the metastatic niche and are crucial in infiltration, metastasis, and immune tolerance processes during tumorigenesis. Several membrane-bound determinants of tumor-derived EVs are involved in cancer infiltration and in immune tolerance. Among these, a growing interest has been turned to human endogenous retroviruses (HERVs) that are often associated with the progression of cancers such as colorectal cancer. In the present study, we investigated the hypothesis that EVs derived from colorectal cancer cell lines are involved in the modulation of the innate immune response, a central step in the formation of the metastatic niche. We conducted our experiments in two different colorectal cancer cell lines, which represented two different stages in cancer development: Caco-2, derived from a non-metastatic epithelial colorectal adenocarcinoma; SK-CO-1, derived from a metastatic colorectal adenocarcinoma (ascites). We evaluated the cellular production of total EVs and HERV-positive EVs and tested their effect on innate immune response by injecting them in zebrafish embryos. The injection of EVs derived from both Caco-2 and SK-CO-1 cells in zebrafish embryos significantly lowered the expression of the pro-inflammatory cytokine IL1-beta and an increase in expression of the anti-inflammatory cytokine IL-10 indicating a role of EVs in modulating innate immune response.

P 002: Investigating MMEJ and cNHEJ role and importance during embryogenesis in zebrafish

TOPIC: CANCER

Mathieu Carrara[1], Anne-Laure Gaillard[1], Alice Brion[2], Evelyne Duvernois-Berthet[2], Giovannangeli Carine[2], Concordet Jean-Paul[2], Guillaume Pézeron[1]

[1] *Muséum National d'Histoire Naturelle - CNRS, Laboratory of Molecular physiology and adaptation - UMR7221, Paris, France*
[2] *Muséum National d'Histoire Naturelle - CNRS, Laboratoire Structure et instabilité des génomes - UMR7196, Paris, France*

ABSTRACT TEXT

Eukaryotic cells have multiple DNA double strand break (DSB) repair pathways at their disposal, the two principal pathways being canonical Non-Homologous End Joining (cNHEJ) and Homologous Recombination (HR). Microhomology Mediated End-Joining (MMEJ) was originally considered as a backup pathway, used when NHEJ and HR are not available. However, recent research showed that MMEJ is essential for surviving DNA DSBs during zebrafish early development. Our project aims to understand the importance and regulation of DNA DSB-repair pathways during zebrafish embryonic development, by focusing on the competition between cNHEJ and MMEJ.

For this purpose, we developed two strategies that allow us to study this two pathways : (i) an inducible CRISPR/Cas9 expression system with deep sequencing of target sequences and (ii) and *in vivo* fluorescent reporter system. We are currently analyzing relative activity of cNHEJ and MMEJ pathways in a temporal- and/or cell-type-specific manner in a WT context and in mutant fish lines which are defective for cNHEJ or MMEJ. This work will allow a better understanding of genotoxic stress consequences during vertebrate embryogenesis. It will also help to establish zebrafish as a model for in vivo DNA repair research.

P 003: Heat-sensitive poly-acrylamide nanoparticle for cancer treatment

TOPIC: CANCER

Patrizia Colucci[1,2], Marta Damora[3], Alice Usai[2], Elena Landi[2], Lieselot Deleye[3], Luciana Dente[2], Francesco De Angelis[3], Vittoria Raffa[2], Francesco Tantussi[3]

[1] *University of Siena, Department of Biotechnology- Chemistry and Pharmacy, Siena, Italy*
[2] *University di Pisa, Department of Biology, Pisa, Italy*
[3] *Istituto Italiano di Tecnologia, n/a, Genova, Italy*

ABSTRACT TEXT

The development of nanoformulated drugs as anticancer treatment is becoming increasingly popular. The use of nanocarrier to vehiculate molecules already exploited in the clinical route allows to overcome the disadvantages associated to the classical administration of therapies. In fact, nanocarriers allow to perform a selective tumour targeting, avoiding the side effects due to unspecific treatment. Moreover, they protect molecules from degradation, allowing to reduce the therapeutic dosage, and promote the cellular internalization of drugs, eluding the multi-drug resistance mechanisms of tumours.

In the present work, the attention is focused on the development of a new promising nanoformulation characterized by a thermal-induced release of the drug in a temporal and local specific manner. Specifically, we synthesized poly-acrylamide based nanoparticles containing doxorubicin as antineoplastic drug and coated with folic acid (DOX:PAA-NP-FA), useful to improve the selective targetability of the system. We investigated the efficacy of our DOX:PAA-NP-FA both *in vitro*, on HeLa cells and *in vivo*, on zebrafish larvae xenografted with human pancreatic cancer cell line Mia Paca-2. Our results showed an efficient uptake of DOX:PAA-NP-FA by the cancer cells and a weak cytotoxic effects at physiological conditions. In contrast, when exposed to the temperature of 41 °C, DOX:PAA-NP-FA undergo a solid-gel phase transition and are able to induce apoptosis of cancer cells, as demonstrated *in vivo* in zebrafish xenografted models. Notably, no statistically significant increase in the apoptosis rate was observed in the zebrafish xenografted group receiving a free doxorubicin standard treatment.

Such time-controlled and physically-activated cargo release makes this drug delivery system safer and appealing to be exploited in combined therapies for the local treatment of solid cancers.

P 004: Fast, in vivo model for drug-response prediction in patients with B-cell precursor acute lymphoblastic leukemia

TOPIC: CANCER

Anton Gauert[1], Nadine Olk[1], Helia Pimentel-Gutiérrez[1], Lasse Jensen[2], Yihai Cao[3], Angelika Eggert[1], Cornelia Eckert[1], Anja Heeren-Hagemann[1]

[1] *Charité-Universitätsmedizin Berlin, Pediatric Oncology/Hematology, Berlin, Germany*
[2] *Linköping University, Department of Medical Sciences, Linköping, Sweden*
[3] *Karolinska Institute, Department of Microbiology- Tumor and Cell Biology, Stockholm, Sweden*

ABSTRACT TEXT

Only half of patients with relapsed B-cell precursor (BCP) acute lymphoblastic leukemia (ALL) currently survive with standard treatment protocols. Predicting individual patient responses to defined drugs prior to application would help therapy stratification and could improve survival. With the purpose to aid personalized targeted treatment approaches, we developed a human-zebrafish xenograft (ALL-ZeFiX) assay to predict drug response in a patient in 5 days. Leukemia blast cells were pericardially engrafted into transiently immunosuppressed *Danio rerio* embryos, and engrafted embryos treated for the test case, venetoclax, before single-cell dissolution for quantitative whole blast cell analysis. Bone marrow blasts from patients with newly diagnosed or relapsed BCP-ALL were successfully expanded in 60 % of transplants in immunosuppressed zebrafish embryos. Response of BCP-ALL cell lines to venetoclax in ALL-ZeFiX assays mirrored responses in 2D cultures. Venetoclax produced varied responses in patient-derived BCP-ALL grafts, including two results mirroring treatment responses in two patients treated with venetoclax with refractory BCP-ALL (Gauert et al., 2020). We demonstrate proof-of-concept for our 5-day ALL-ZeFiX assay with primary patient blasts and the test case, venetoclax, which after expanded testing for further targeted drugs could support personalized treatment decisions within the clinical time window for decision-making.

P 005: Establishing a Ewing Sarcoma model in zebrafish

TOPIC: CANCER

Sarah Grissenberger[1], *Luisa Morelli*[1], *Anna Poetsch*[2], *Nathan Sheffield*[3], *Heinrich Kovar*[4], *Martin Distel*[1]

[1] *St. Anna Children's Cancer Research Institute, Innovative Cancer Models, Vienna, Austria*
[2] *St. Anna Children's Cancer Research Institute, N/a, Vienna, Austria*
[3] *Center for Public Health Genomics- University of Virginia, Public Health Sciences, Virginia, USA*
[4] *St. Anna Children's Cancer Research Institute, Molecular Biology of Solid Tumors, Vienna, Austria*

ABSTRACT TEXT

Ewing sarcoma (EwS) is a malignant bone and soft tissue tumor in children and adolescents. The formation of EwS is caused by a chromosomal translocation, leading in 85 % of cases to the expression of the oncogene EWS-FLI1. This aberrant transcription factor is the main driver of the disease and leads to massive transcriptional deregulation and malignant transformation. Although the genetic mechanism that drives EwS is well understood, an animal model adequately mimicking the disease is still lacking. One reason, why modeling attempts have remained difficult, is the elusive cell-of-origin of EwS. Several cell types including neural crest progenitor cells and mesenchymal stem cells have been proposed, but could not be confirmed as EwS cell-of-origin yet.

Zebrafish with mosaic expression of human EWS-FLI1 have been reported to develop EwS-like tumors, however at very low frequency. We reason, that targeting EWS-FLI1 to the proper cell-of-origin will greatly enhance tumor formation. Towards this goal, we are following an unbiased approach in which we use human EwS specific enhancers to unravel the identity of the cell-of-origin.

Furthermore, we have already established a Cre-inducible zebrafish effector strain, harboring human EWS-FLI1. By crossing this strain to different Cre-driver strains, we will target EWS-FLI1 expression to distinct cell types found in our enhancer screen, but also to proposed cell-of-origin candidates, like neural crest and mesenchymal stem cells.

If our approach is successful, a zebrafish EwS model will help to understand tumor initiation and progression and furthermore, will be a valuable tool to develop novel therapeutic strategies.

P 006: Bioluminescent cancer xenotransplantation model in zebrafish

TOPIC: CANCER

Martina Hason[1], *Jovana Jovičić*[1], *Petr Bartůněk*[1]

[1] *Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of Cell Differentiation, Prague, Czech Republic*

ABSTRACT TEXT

Zebrafish (*Danio rerio*) is a valuable non-mammalian model organism widely used for the study of development as well as disease. Modeling cancer in zebrafish has many advantages compared to the traditional murine model. Zebrafish is a robust model for cancer research mainly because of its cost-effective maintenance, high fecundity, dynamic visualization of tumor cell growth *in vivo* and the possibility of chemical high-throughput screening of animals at reasonable costs. Aspects of human cancerogenesis could be recapitulated and followed *in vivo* in zebrafish at the cellular level.

We aim is to utilize a new fast and reliable readout for tracking cancer cell growth *in vivo* in zebrafish embryos. We have employed NanoLuc™, an engineered luciferase subunit from the deep-sea shrimp *Oplophorus gracilirostris*, for this purpose. We performed allograft transplantations (tumor cells derived from a recipient of the same species) and xenograft transplantations (tumor cells derived from another species) of cancer cell lines into zebrafish embryos. This assay allows fast, simple and quantitative analysis of cancer cell growth *in vivo* with high sensitivity and less background compared to conventional fluorophores. The visualization of cancer cell migration could be further enhanced by using immune-deficient zebrafish lines in the transparent *casper* background. A set of kinase inhibitors has been selected for a pilot screen and is being tested for activity *in vitro* as well as *in vivo*. In summary, our luminescent xenotransplantation model could provide a powerful tool for critical evaluation of human cancer pathology in zebrafish. Our setup would be suitable for medium-throughput screens as the embryos fit into a single well of a 96-well plate. The results of our kinase inhibitor screen could provide us with new leads useful in anticancer therapy.

P 007: Head and Neck cancer niche and drug sensitivity in 3D collagen-based scaffold and zebrafish model

TOPIC: CANCER

Giacomo Miserocchi[1], *Claudia Cocchi*[1], *Sebastiano Calpona*[1], *Giandomenico Di Menna*[1], *Alessandro De Vita*[1], *Chiara Liverani*[1], *Chiara Spadazzi*[1], *Alberto Bongiovanni*[1], *Silvia Carra*[2], *Franco Cotelli*[3], *Laura Mercatali*[1], *Toni Ibrahim*[1]

[1] *Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori- IRST IRCCS, Osteoncology and Rare Tumors Center, Meldola, Italy*

[2] *Istituto Auxologico Italiano IRCCS, Laboratory of Endocrine and Metabolic Research, Milano, Italy*

[3] *Università degli Studi di Milano, Department of Biosciences, Milano, Italy*

ABSTRACT TEXT

Head and Neck cancers (HNCs) represent the sixth most common non-skin cancers worldwide. As other cancers, HNCs are dynamic masses that remodel the 3D extracellular matrix and interact constantly with stroma components. We have used collagen-based scaffolds culture system and zebrafish embryos to study the implication of a 3D microenvironment on phenotype and drug response of HNC cell lines.

We have synthesized 3D scaffolds composed of bovine collagen type I. Two HNC cell lines (UPCI:SCC090 and UM-SCC6) were seeded and paraffin embedded for immunohistochemistry analysis. Drug sensitivity to Cisplatin, 5' Fluorouracil, Cetuximab and Gemcitabine were performed treating 2D and 3D cultures at plasma peak concentrations. Zebrafish embryos were injected at 48 hpf in the perivitelline space with cells obtained by 2D and 3D cultures.

Both cell lines displayed the capability to colonize the whole scaffold area. UM-SCC6 acquired a mesenchymal-like phenotype with homogeneous distribution along the collagen fibers. Conversely, UPCI:SCC090 developed a dense clustered organization. Both cell lines displayed high resistance to each treatments when seeded on the scaffolds. We characterized a new method for the recovery and preparation of cells from 3D culture for embryos injection. Cells recovered by both culture methods survived inside zebrafish embryos since the end of experiments (3 dpi). The 89 % of embryos injected with UM-SCC6 grown on collagen scaffold displayed cell migration and distant metastasis. Differently, 2D culture cells invaded and grew at second sites only in the 33,5 % of embryos.

We have evaluated 3D collagen-based scaffold cultures and zebrafish embryos as a new combination models that might have an impact to preclinical research, improving the existing standard culturing techniques. This systems provide an innovative method for the evaluation of HNCs drug efficacy and the study of tumor microenvironment features.

P 008: The role of proteases in DNA-protein crosslink repair

TOPIC: CANCER

Marta Popovic[1], *Christine Supina*[1], *Ivan Anticevic*[1], *Cecile Otten*[1]

[1] *Ruder Boskovic Institute, Department for Marine and Environmental Research, Zagreb, Croatia*

ABSTRACT TEXT

DNA-protein crosslinks (DPCs) have adverse effects on the organismal level including cancer, premature aging and neurodegenerative diseases. DPCs are severe DNA lesions which occur when a protein becomes irreversibly covalently linked to DNA. Due to their bulky nature, DPCs impair all DNA transactions and DPC repair (DPCR) is therefore an essential cellular pathway. They are one of the most abundant DNA lesions with only abasic (AP) sites occurring more frequently (approx. 10,000 events/human genome). Our discovery of proteolysis-coupled DPCR centred on SPRTN protease led to recognition of DPCR as a separate DNA damage repair pathway. SPRTN initiates DPCR by proteolytic cleavage of crosslinked proteins, followed by removal of protein remnants from DNA backbone via different downstream factors. While SPRTN removes a vast majority of cellular DPCs in the S-phase, another potential protease, ACRC has been recently linked to DPCR when SPRTN is not present. We show that 3D structure of the protease core of ACRC is very similar to that of SPRTN and includes two α-helices bearing three Zn-binding histidines and a catalytic glutamate residue, a characteristic of all Zn-dependent metalloproteases. Our goal is to biochemically characterize ACRC, investigate mechanisms behind its role in DPCR and its relation to SPRTN. To this end, we are identifying ACRC substrates and cleavage kinetics with purified components in vitro. In vivo, in RPE1 cells and in zebrafish embryos, we are measuring DPCR with a newly developed system in ACRC and/or SPRTN deficient genetic background created using CRISPR/Cas9 gene manipulations. Our study will reveal the mechanisms of ACRC action and its contribution to DPCR on the cellular and organismal

level. Considering that both SPRTN and ACRC have been linked to cancerogenesis and potential therapies, we aim to show unequivocal link between DPCR and cancer emergence on the organismal level, with the aim of developing efficient cancer therapies.

P 009: Bevacizumab blocks tumor development by modulating tumor macrophages

TOPIC: CANCER

Cátia Rebelo De Almeida[1], *Rita Fior*[1]

[1] *Champalimaud Center for the Unknown, Cancer Development and Innate Immune Evasion, Lisboa, Portugal*

ABSTRACT TEXT

VEGF-A is one of the most potent pro-angiogenic factor and is often upregulated in a variety of tumors. Several therapies were developed to neutralize VEGF signaling, such as bevacizumab. Although VEGF signaling is mostly related to angiogenesis, VEGF-A has also been shown to regulate tumor cell survival and migration. However, research is mainly focused on the impact of anti-angiogenic therapies on endothelial cells, not exploring their effect on other cell populations present in the tumor microenvironment (TME). Here, we investigated the impact of Bevacizumab, an anti-VEGF-A therapy, on innate immune cell populations present in the TME by using zebrafish larvae xenografts, where the influence in the tumor microenvironment can be readily analyzed. By using a sensitive triple negative breast cancer cell line model, in which Bevacizumab impairs angiogenesis and shrinks tumor size, we show that Bevacizumab can modulate the innate immune cell populations present in the TME. Bevacizumab can polarize the tumor-associated macrophages towards a pro-inflammatory M1-like phenotype. Strikingly, depletion of macrophages, genetically or chemically, with L-Clodronate leads to the same phenotype as bevacizumab, i.e impairment of angiogenesis and reduction of tumor size, suggesting that anti-VEGF-A therapy can act through tumor associated macrophages and have a secondary effect on angiogenesis.

P 010: Development of a zebrafish xenograft model of cancer and metastasis

TOPIC: CANCER

Federica Ruscitto[1], *Chiara Priami*[1], *Enrica Migliaccio*[1], *Pier Giuseppe Pelicci*[1,2]

[1] *European Institute of Oncology, Department of Experimental Oncology, Milan, Italy*
[2] *University of Milan, Department of Oncology and Hemato-Oncology, Milan, Italy*

ABSTRACT TEXT

Cancer is a complex disease, characterized by high inter- and intra-tumor heterogeneity. Cancer progression is often accompanied by metastatic spreading. Metastasis is the leading cause of cancer-related mortality, with mechanisms still largely unknown. Although mice are commonly used to study cancer *in vivo*, other faster and easy-to-use alternatives are needed. The zebrafish larva (ZL) is a powerful system to perform xenotransplantation of human cancer cell lines and patient-derived samples. Some of its advantages are: (i) short experimental time frame; (ii) small size and transparency, which allow to perform imaging with unrivaled in vivo resolution; (iii) lack of adaptive immunity; (iv) small number of cells necessary for implantation; (v) large number of animals that can be manipulated in a single round. This study describes the development of a reliable 7-day-long ZL xenograft assay, which enables to evaluate implantation, proliferation and metastatic potential of cancer cells. We established the micro-transplantation in the peri-vitelline space and in the circulation of 48 hpf ZL of two fluorescent human breast epithelial cell lines: the metastatic MDA-MB-231 and the non-tumorigenic MCF10A as control. These modalities of microinjection recapitulate both early and late stages of the metastatic cascade, that is followed by live confocal microscopy over a period of 4 days. We also optimized imaging-based approaches to measure proliferative index and tumor volume. Then we transplanted the metastatic melanoma line B16-F10, observing different dynamics of proliferation and metastasis formation and demonstrating the feasibility of ZL xenograft model to highlight differences among distinct cell lines. Our model - combining cell labeling, micro-transplantation and imaging in a rapid and low-cost assay - fits into the current scenario of personalized oncology, which claims for rapid *in vivo* tools to predict patient's tumor behavior and guide therapeutic decisions.

P 011: The role of ACRC/GCNA in the repair of DNA-protein crosslinks

TOPIC: CANCER

Christine Supina[1], *Ivan Anticevic*[1], *Jovica Loncar*[1], *Marta Popovic*[1]

[1] *Ruder Boskovic Institute, Division for Marine and Environmental Research, Zagreb, Croatia*

ABSTRACT TEXT

DNA-protein crosslinks (DPCs) are DNA lesions formed when a protein becomes irreversibly covalently bound to DNA. DPC formation is very common in cells, as it can arise from endogenous factors, such as aldehydes and reactive oxygen species produced during cellular metabolism and exogenous sources (ionizing radiation, UV, chemotherapeutics). DPCs present a physical blockage to all DNA transactions and if left unrepaired cause genomic instability, premature aging and liver cancer in mice and humans. Proteases Wss1 in yeast and SPRTN in mammals initiate the removal of DPCs through the proteolytic digestion of crosslinked proteins. After SPRTN proteolysis of DPCs, peptide remnant remains crosslinked to the DNA backbone, and is subsequently removed by downstream factors. Considering that SPRTN is a replication-specific protease, it is probable that another protease acts in lowly proliferative cells where DPCs pose a threat to transcription progression. Phylogenetic analysis of the SPRT family in metazoans indeed identified a SPRT-like protein family, ACRC (acidic repeat containing). In line with the phylogenetic proximity, the 3D structure of the protease core within the Sprt domain of ACRC is very similar to that of SPRTN. The goal of our study is to determine if ACRC is proteolytically active and what is its relation to SPRTN, using zebrafish model. We compared both proteases using phylogenetic and syntenic analysis and in regard to mRNA and protein expression across different tissues in human and zebrafish. To address functionality of ACRC, protein purification and protease functional assays are under way. Most importantly, we are addressing the role of ACRC *in vivo* using CRISPR/Cas9 gene manipulation to introduce mutation in the ACRC putative protease active site with the aim of creating an enzymatic dead version of ACRC. Our study will reveal actual contribution of ACRC to the DPC removal on the organismal level.

P 012: Heterogeneous cell subpopulations characterise melanoma residual disease

TOPIC: CANCER

Jana Travnickova[1], *Sonia Wojciechowska*[1], *Ava Khamseh*[1], *Alessandro Brombin*[1], *Marie E. Mathers*[2], *Chris P. Ponting*[1], *E. Elizabeth Patton*[1]

[1] *MRC Institute of Genetics and Molecular Medicine- MRC Human Genetics Unit & Cancer Reaseatch UK Edinburgh Centre, University of Edinburgh, Edinburgh, United Kingdom*

[2] *Western General Hospital, Department of Pathology, Edinburgh, United Kingdom*

ABSTRACT TEXT

Melanoma is the most lethal type of skin cancer due to its frequent rate of recurrence and eventual resistance to current drugs. After targeted therapy or immunotherapy, most patients relapse due to the presence of therapy persisting cells, also called residual disease cells. The transcriptional identity and their role in melanoma recurrence remains to be fully understood.

We generated genetic melanoma models in zebrafish with a conditional *mitfa* allele combined with BRAF and/or p53 mutations. Loss of MITF activity causes melanoma regression while restoring MITF activity leads to melanoma recurrence at the same site of original tumour. Using fluorescent reporter transgenic lines, we discovered minimal residual disease with no MITF activity at the regression site. Transcriptomic analysis reveals these residual cells are enriched in neural crest stem cell and mesenchymal cell signatures. Critically, single cell RNA-seq analysis demonstrates that these cells show high heterogeneity, and include populations that transdifferentiate during regression and a G0-like population that pre-exists in the primary tumour. Our work now focuses on enlightening the ability of these cells to contribute to melanoma recurrence by following the individual cell subpopulations and their fate during melanoma recurrence. This will allow identifying and testing their vulnerabilities, which could lead to novel therapeutic targets.

P 013: Patient-derived tumor xenografts: proliferation, invasiveness and predictiveness

TOPIC: CANCER

Alice Usai[1], Ida E. R. Montesanti[1], Gregorio Di Franco[2], Niccola Funel[2], Luca E. Pollina[3], Enrico Vasile[4], Luca Morelli[2], Vittoria Raffa[1]

[1] *University of Pisa, Department of Biology, Pisa, Italy*
[2] *University of Pisa, Department of Translational Research and New Technologies in Medicine and Surgery, Pisa, Italy*
[3] *University of Pisa, Department of Surgical- Medical- Molecular Pathology and Critical Area, Pisa, Italy*
[4] *University of Pisa, Division of Medical Oncology, Pisa, Italy*

ABSTRACT TEXT

Cancer incidence has increased in the past decades. Despite great advances in precision medicine, there is still a lack of methods to personalize the cancer therapy. Animal "Avatars" and co-clinical trials represent an emerging concept for implementing schemes to predict individual patient response.

In this study, patient-derived xenograft (PDX) in 2dpf zebrafish embryos have been derived from human fresh tumor fragments taken from surgical specimen. This method has performed to maintain both the cancer microenvironment and the intratumour heterogeneity, and it has been validated in a clinical study enrolling 120 adult patients with solid cancers (hepato-biliary-pancreatic cancer and gastro-intestinal cancer) (XenoZ, NCT03668418). Experimental data confirm that the PDX is able to engraft in the host, to survive, spread and migrate, with retention of the primary tumor phenotype based on histology.

Moreover, we run chemotherapy sensitivity assays on 10 PDXs derived from gastric cancers, 35 PDXs derived from colon cancers and 25 PDXs derived from pancreatic cancers. We evaluated disease regression by adapting the "Response evaluation criteria in solid tumors (RECIST)" to the fish trial. Interestingly, our experimental data have shown good agreement with observations registered in the common clinical practice. For patients affected by colon cancer, we found a superiority of the chemotherapy treatment when a combination of drugs are used (FOLFOX, FOLFIRI and FOLFOXIRI). A complete response was never observed for patients affected by pancreatic cancers because of its high aggressiveness. For patients affected by gastric cancer, we found an excellent response to FOLFIRI that can be considered an acceptable first-line treatment for advanced gastric cancers.

In conclusion, this work would pave the way to establish a simple, reliant, less expensive and more physiological model of human cancers as a valuable tool for implementing schemes of personalized medicine in oncology.

Cell Signaling

P 014: Nuclear matrix associated protein, BANP, is required for proper cell-cycle progression and neuronal survival in zebrafish retinas. Swathy Babu and Ichiro Masai

TOPIC: CELL SIGNALING

Swathy Babu[1]

[1] *Okinawa Institute of Science and Technology- Japan, Developmental Neurobiology Unit, Onna, Japan*

ABSTRACT TEXT

BTG3-associated nuclear protein (BANP), is a nuclear matrix attachment region-binding protein. *In vitro* studies on human and mouse BANP demonstrated that BANP functions as a tumor suppressor and regulates p53-mediated DNA damage response linked to apoptosis, cell cycle, and cancer metastasis. Furthermore, BANP is also reported to have fundamental function during development. The homozygous

banp mutant mouse is embryonic lethal, hence the mechanism by which BANP regulates developmental process is unknown. To understand this mechanism, we focus on a zebrafish *banp* gene. We performed a large scale mutagenesis of zebrafish, and identified zebrafish *banp* mutant, which shows defects in retinal development. During development, multi-potent progenitor cells generate six major classes of retinal neurons. We found that the early born-retinal cell types such as retinal ganglion cells are normally produced in zebrafish *banp* mutants. However, in the late stage of retinal development, cell cycle progression is arrested in M phase and progenitor cells subsequently undergo apoptosis, suggesting that BANP is required for mitotic progression and neuronal survival. Although p53 expression is enhanced in *banp* mutants, cell-cycle arrest and apoptosis are not rescued in the *banp; p53* double mutants. Thus, mitotic arrest and apoptosis might also be induced by p53-independent mechanism upon knocking out BANP. Being a tumors suppressor as well as an essential factor during development, understanding regulatory function of banp is of great significance. To elucidate BANP-related signaling network, we are comparing protein expression profile, mRNA expression as well as active promoters between wild type and *banp* mutant retinas, and found that energy metabolism is enhanced in *banp* mutants. Thus, BANP maintains proper level of energy metabolism, and is required for cell-cycle progression and neuronal survival in developing retinas.

P 015: Vangl2 Regulates Cytoneme-mediated Wnt Transport in Early Zebrafish Neural Plate Patterning

TOPIC: CELL SIGNALING

Lucy Brunt[1], *Steffen Scholpp*[1]

[1] *University of Exeter, Living Systems Institute, Exeter, United Kingdom*

ABSTRACT TEXT

Secreted Wnt proteins activate a signalling network that regulate key functions in development, such as cell proliferation, differentiation, cell polarity and cell migration. The hallmark of the Wnt network is a relatively small population of cells, which produce the signal to orchestrate behaviour of a larger, neighbouring cell group. This group of cells establishes a Wnt concentration gradient from source to target cells. How Wnt proteins are able to communicate with distant target cells is, however, not understood.

Recent work from our lab has shown that actin-based cellular protrusions known as cytonemes traffic Wnt8a molecules between cells in zebrafish to establish neural plate patterning. At the contact site Wnt cytonemes stimulate signalosome clustering which leads to activation of the Wnt signalling pathway in the target cell. However, a thorough analysis of mechanisms regulating controlled cytoneme-mediated Wnt signal trafficking, is still needed.

Through microinjection of fluorescently-tagged constructs into the early zebrafish embryo and subsequent confocal imaging, we track Wnt-positive cytonemes *in vivo*. We have found that Wnt Planar Cell Polarity (PCP) components such as Vangl2, localise to cytoneme tips with Wnt8a and are essential, along with receptor tyrosine kinase Ror2, for regulation of Wnt cytoneme formation and stability. Overexpression of Vangl2 in the embryo produces longer and more stable cytonemes. Consistently, blockage of Vangl2 function reduces the length of cytonemes. This change in cytoneme behaviour impacts the signalling gradient, resulting in changes to the sharpness and presence of tissue boundaries of the neural plate. Therefore, we have been able to uncover novel functions for a key player of the PCP pathway for regulation of Wnt cytoneme behaviours, such as cytoneme length and stability. This control of cytoneme behaviour allows controlled Wnt signal activation in target cells, for accurate and discrete tissue patterning.

P 016: fgf and fgfr expression in regenerating zebrafish fins

TOPIC: CELL SIGNALING

Nicole Cudak[1], *Sebastian Keil*[1], *Franziska Knopf*[1]

[1] *CRTD and Center for Healthy Aging- UKD, TU Dresden, Dresden, Germany*

ABSTRACT TEXT

Zebrafish have the potential to regenerate composite tissues after loss, such as of the fins. Inhibition of Fgf signaling leads to impaired fin regeneration which shows its importance for this process. Here, we study the expression of Fgf ligands and their receptors (Fgfrs) that might contribute to regeneration. More precisely, we performed RT-PCR and whole mount RNA in situ hybridization (ISH) on a high number of signaling components to provide an overview about their sites of expression. Subsequently, we performed cryosectioning

in order to assess expression in more detail. As previously reported, *fgf3* and *fgf10a* are expressed in the distal and proximal blastema at 3 days post amputation. Also *fgf2* was expressed in the blastema. Other *fgfs*, such as *fgf20a* and *fgf24* are expressed in the basal layer of the wound epidermis (BLWE). In contrast, *fgf8a, fgf8b* and *fgf13b* were not expressed in regenerating fins. Examination of *fgfr* expression revealed a broad but slightly different expression for *fgfr1a, fgfr1b and fgfr2 (fgfr1a in* blastema, and lateral BLWE, *fgfr1b* and *fgfr2* only in blastema). In contrast, *fgfr3* appears to be expressed in bone forming cells. *fgfr4* is clearly detected in this domain, too, and is also found in the blastema and stump osteoblasts. In summary, our work adds information on *fgf* and *fgfr* expression during fin regeneration and gives an overview on those that might facilitate regeneration.

P 017: Regulation of erythro-thrombocytic differentiation in zebrafish

TOPIC: CELL SIGNALING

Ivana Dobiasovska[1], Ondrej Svoboda[1], Bjoern Schuster[2], Petr Bartunek[1]

[1] *Institute of Molecular Genetics of the Czech Academy of Sciences, Department of Cell Differentiation, Prague, Czech Republic*
[2] *Institute of Molecular Genetics of the Czech Academy of Sciences, CZ-Openscreen, Prague, Czech Republic*

ABSTRACT TEXT

Hematopoiesis is a process of formation, development and self-renewal of all blood cellular components, essential for maintaining homeostasis of all vertebrate organisms. Highly specialized cells rise from the common progenitors through the tightly regulated process of cell differentiation driven by specific factors. Elucidation of how these factors control cell differentiation is necessary to understand mechanisms of hematopoiesis.

Here, we aim to characterize new potential regulators involved in erythropoiesis and thrombopoiesis. Based on our previous gene expression profiling experiments in chicken, we identified several gene candidates that were differentially expressed during the differentiation of erythrocytes and thrombocytes, such as *c1qntf7* and *nkx2-7*. We tested function of both these genes in zebrafish that represents suitable and evolutionary conserved model of hematopoiesis. When ectopically expressed, we observed multiple phenotypes at different levels of blood development, suggesting their importance during blood maintenance.

In summary, our candidate genes potentially provide new insights into mechanisms underlying hematopoietic cell differentiation and might represent evolutionary conserved regulators responsible for blood development.

P 018: Mapping cardiac calcium dynamics in zebrafish embryos with genetically encoded calcium indicators based on FRET

TOPIC: CELL SIGNALING

Salgado-Almario Jussep[1], Vicente Manuel[1], Domingo Beatriz[1], Vincent Pierre[2], Griesbeck Oliver[3], Juan Llopis[1]

[1] *Universidad de Castilla-La Mancha, Centro Regional de Investigaciones Biomédicas CRIB y Facultad de Medicina, Albacete, Spain*
[2] *Sorbonne Université, CNRS- Biological Adaptation and Ageing, Paris, France*
[3] *Max Planck Institute of Neurobiology, Tools for Bio-Imaging, Martinsried, Germany*

ABSTRACT TEXT

Alterations in Ca2+ cycling in the heart may lead to arrhythmias and heart failure. Zebrafish has become a popular vertebrate in vivo model in cardiovascular research because action potential morphology and heart rate are quite similar to those of humans. In recent studies, genetically encoded calcium indicators (GECIs) that change their single fluorescence intensity with Ca2+ concentration, like GCaMPs, have been used to study Ca2+ changes in zebrafish heart. Since these signals are strongly affected by motion, contraction must be stopped with chemical myosin inhibitors or with tnnt2 morpholino oligomers. In contrast, FRET biosensors ameliorate motion artifacts by ratioing two emission channels acquired simultaneously.

We screened FRET GECIs based on troponin C (TN-XXL and Twitch series) with different Ca2+ affinities and decay times, expressed transiently under the control of the heart-specific promoter cmlc2, to compare their functionality and sensitivity. The shape and kinetic parameters of cytosolic Ca2+ during the cardiac cycle were characterized in atrium and ventricle. Among the biosensors evaluated, Twitch-4 showed the best dynamic range and robustness in 3 days post-fertilization embryos. Thus, we generated a transgenic line

expressing Twitch-4 in the heart, which was able to report the expected alterations in Ca2+ levels and cycling in response to several drugs with known effects. Nifedipine (L-type Ca2+ channel blocker) almost completely abolished Ca2+ transients whereas the L-type Ca2+ channel activator Bay K8644 increased their amplitude; the beta-adrenergic antagonist propranolol decreased heart rate, the rise slope and the decay slope of Ca2+ transients; and dofetilide, which inhibits the rapid delayed-rectifier K+ current, induced a 2:1 arrhythmia. This new zebrafish transgenic line is thus an excellent model to characterize in vivo cardiac Ca2+ physiology, pathophysiology and to perform drug screening without the need to stop heart beating.

P 019: A role for zebrafish SelO in unfolded protein response

TOPIC: CELL SIGNALING

Ting Liang[1], *Seok-Yong Choi*[1]

[1] *Chonnam National University Medical School, Biomedical Sciences, Gwangju, Korea Republic of*

ABSTRACT TEXT

Selenocysteine (Sec) is a cysteine analogue with a selenium-containing selenol group replacing a sulfur-containing thiol group and the 21st amino acid in ribosome-mediated protein synthesis. Sec was discovered by Thressa Stadtman in 1976. Selenoproteins are proteins that contain one or more Sec residues and have 25 subtypes, one of which is Selenoprotein O (SelO). SelO has been reported to be a redox-active mitochondrial protein that transfers AMP from ATP to serine, threonine, and tyrosine residues on protein substrates (AMPylation). To determine a functional role for SelO, we generated *selo* null zebrafish mutant (*selo*$^{-/-}$) using CRISPR/Cas9 technology and exposed *selo*$^{-/-}$ zebrafish embryos to thapsigargin, an inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase and inducer of unfolded protein response (UPR). Upon thapsigargin treatment, *selo*$^{-/-}$ embryos exhibited stronger apoptosis than wild-type embryos, suggesting that SelO may be involved in the UPR. We are now i) investigating which domain of SelO, either an AMPylase domain or a Sec domain (CXXU), is more important in the UPR and ii) identifying UPR-related target proteins (substrates) of SelO.

P 020: Csf1 signaling in zebrafish myelopoiesis

TOPIC: CELL SIGNALING

Tereza Mikulasova[1], *Martina Hason*[1], *Petr Bartunek*[1]

[1] *Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of Cell Differentiation, Prague, Czech Republic*

ABSTRACT TEXT

Colony-stimulating factor 1 receptor (CSF1R), also known as macrophage colony-stimulating factor receptor (M-CSFR) is a class III receptor tyrosine kinase coded by c-*fms* proto-oncogene. It is activated by two distinct ligands – CSF1 (M-CSF) and IL-34. CSF1 signaling plays an important role in the survival, proliferation, differentiation and activation of mononuclear phagocyte cells, such as monocytes, macrophages, Langerhans cells, microglia or osteoclasts. Mutation in both, receptor and ligands, leads to an absence or decreased numbers of these cells.

Due to the whole genome duplication that occurred in the evolution of teleost fish, there are two paralogs of Csf1 receptor (Csf1ra/b) and Csf1 ligand (Csf1a/b) in zebrafish. This brings a higher complexity to the study of Csf1 signaling in zebrafish, where it is necessary to understand if the function of these paralogs diversified or they still play a role in the same processes. Csf1 signaling in zebrafish has been so far connected with the role in skin pattern formation and in microglia development and migration.

The aim of this study is to uncover the role of Csf1 signaling in zebrafish myelopoiesis. For this purpose, we are analyzing the effect on myeloid cells *in vivo* after *csf1a*, *csf1b* and *il34* overexpression or knock-out. We also want to understand the ligand-receptor binding specificity and their spatio-temporal expression pattern. To address this, we are also overexpressing the ligands in Csf1r mutants. As we expressed and purified zebrafish Csf1a, Csf1b and Il34 recombinant proteins, we are also studying their role on the myeloid differentiation in *ex vivo* cultures from zebrafish kidney marrow. Studying the precise role of the cytokines and receptors of this signaling pathway can lead to a better understanding of how the hematopoiesis events are conserved in the evolution of vertebrates.

P 021: A novel role of the E3 ubiquitin-ligase Mindbomb1 in the regulation of Planar Cell Polarity

TOPIC: CELL SIGNALING

Vishnu Muraleedharan Saraswathy[1], Priyanka Sharma[1], Ahshai Janardhana Kurup[1], Sophie Polès[1], Morgane Poulain[1], Maximilian Fürthauer[1]

[1] *Institut de Biologie Valrose, Cnrs UMR 7277, Nice, France*

ABSTRACT TEXT

Planar Cell Polarity (PCP) signaling is essential for embryonic morphogenesis. Here we report a novel function of the E3 Ubiquitin-ligase Mindbomb1 (Mib1) in PCP signaling. Mib1 has been extensively studied for its role in Delta/Notch signaling where it promotes internalization of Delta ligands to trigger Notch activation. In addition to Notch ligands, proteomic studies have identified additional Mib1-interacting proteins, but *in vivo* evidence for Mib1 functions beyond Notch signaling has remained limited.

While using a validated morpholino to inhibit *mib1* function, we realized that *mib1* morphants presented morphogenetic defects that suggest an essential function of *mib1* in PCP. Accordingly, *mib1*-dependent morphogenetic defects can be rescued through the injection of the PCP downstream mediator RhoA. The analysis of two *mib1* null mutant alleles, one of which was newly generated in the current study, confirmed a genetic requirement of *mib1* for PCP. In contrast, no PCP defects were observed for mutants for *mib1*^{ta52b}, the allele most commonly used for the analysis of *mib1* function in Notch signaling. The role of *mib1* in PCP is therefore distinct from its well-known function in Delta/Notch signaling.

Mib1 can promote substrate ubiquitination through its three RING Finger (RF) domains. While RNAs encoding WT Mib1 or the ta52b RF3 point mutation are equally capable of rescuing the morphogenetic defects of *mib1*-depleted embryos, no rescuing activity is observed for a truncated form of Mib1 that lacks all three RF domains. These observations suggest that Mib1 controls the ubiquitination and endocytic internalization of a PCP pathway component. We have identified the target of Mib1 and will present the mechanism through which Mib1 controls PCP. In addition to identifying Mib1 as an essential component if the PCP pathway, our analysis reveals that compensatory interactions can buffer the PCP gene regulatory network against perturbations of individual components.

P 022: Visualization of cytosolic and mitochondrial calcium transients in skeletal muscle and heart of zebrafish embryos with bioluminescent aequorin

TOPIC: CELL SIGNALING

Manuel Vicente Ruiz[1], Jussep Salgado-Almario[1], Joaquim Soriano[1], Miguel Burgos[1], Beatriz Domingo[1], Juan Llopis[1]

[1] *University of Castilla-La Mancha, Department of physiology, Albacete, Spain*

ABSTRACT TEXT

The bioluminescent photoprotein aequorin was the first genetically-encoded calcium ion (Ca2+) indicator (GECI) able to detect Ca2+ levels *in vivo*. Its fusion with GFP and mitochondrial targeting result in GFP-aequorin (GA, expressed in the cytoplasm) and mitoGFP-aequorin (mitoGA, trapped in the mitochondrial matrix), respectively. GA and mitoGA expressed transiently in the trunk of 24 hpf embryos, reconstituted with substrate coelenterazine-*f*, -*fcp*, -*h* and -*hcp*, allowed continuous monitoring of skeletal muscle Ca2+ for hours. Two periods of high Ca2+ activity in cytosol and mitochondria were observed, reflecting the spontaneous contractions observed by transmitted light. Mitochondrial Ca2+ levels tracked cytoplasmic changes but showed a slower decay. The uncoupler FCCP and the mitochondrial calcium uniporter inhibitor, DS16570511, abolished mitochondrial Ca2+ transients. Thus, these probes can be used to uncover the role of mitochondria in shaping intracellular Ca2+ transients during muscle contractions. We also generated a *cmlc2*-GA transgenic zebrafish line to study Ca2+ dynamics in the heart of 72 hpf zebrafish embryos. Since Ca2+ levels in the heart rise and decay continuously during the cardiac cycle, most functional aequorin was spent during its reconstitution with the substrate coelenterazine. Thus, a protocol with the Ca2+ channel blocker nifedipine was devised to allow GA reconstitution with various coelenterazine analogs. After nifedipine washout, hearts resumed beating and Ca2+ transients were recorded continuously for at least 60 min. Since Ca2+ modulating drugs changed the dynamics of systolic transients, this model can potentially be used for drug screening and to study the pathophysiology of cardiac disorders.

P 023: Mitofusin 2 regulates neutrophil adhesive migration and the actin cytoskeleton

TOPIC: CELL SIGNALING

Wenqing Zhou[1], Alan Y. Hsu[1], Yueyang Wang[2], Ramizah Syahirah[1], Tianqi Wang[1], Jacob Jeffries[1], Xu Wang[1], David Umulis[3], Qing Deng[1]

1 *Purdue Univerisity, Biological Sciences, West Lafayette, USA*
2 *Purdue University, Biological Sciences, West Lafayette, USA*
3 *Purdue Univerisity, Agricultural & Biological Engineering, West Lafayette, USA*

ABSTRACT TEXT

Neutrophils rely on glycolysis for energy production. How mitochondria regulate neutrophil function is not fully understood. Here, we report that mitochondrial outer membrane protein Mitofusin 2 (Mfn2) regulates neutrophil homeostasis and chemotaxis in vivo. *Mfn2*-deficient neutrophils are released from the hematopoietic tissue, trapped in the vasculature in zebrafish embryos, and not capable of chemotaxis. Consistently, human neutrophil-like cells deficient with MFN2 fail to arrest on activated endothelium under sheer stress or perform chemotaxis on 2D surfaces. Deletion of Mfn2 results in a significant reduction of neutrophil infiltration to the inflamed peritoneal cavity in mice. Mechanistically, MFN2-deficient neutrophil-like cells display disrupted mitochondria-ER interaction, heightened intracellular calcium levels, and elevated Rac activation after chemokine stimulation. Restoring mitochondria-ER tether rescues the abnormal calcium levels, Rac hyperactivation, and chemotaxis defect resulted from MFN2 depletion. Finally, inhibition of Rac activation restores chemotaxis in MFN2-deficient neutrophils. Altogether, we identified that MFN2 regulates neutrophil migration via maintaining mitochondria-ER interaction to suppress Rac activation and uncovered a previously unrecognized role of MFN2 in regulating cell migration and the actin cytoskeleton.

Chemical Biology and Drug Discovery

P 024: Establishing a link between the microbiome and aryl hydrocarbon receptor system in zebrafish neurobehavioral development

TOPIC: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Luísa Bertotto[1], Leah Wehmas[2], Carmen Wood[3], Gleta Carswell[2], Xia Meng Howey[1], Shaza Gaballah[1], Brian Chorley[2], Nils Klüver[4], David Leuthold[4], Nicole Schweiger[4], Stefan Scholz[4], Tamara Tal[2,4]

1 *ORISE, United States Environmental Protection Agency, Research Triangle Park, USA*
2 *United States Environmental Protection Agency, Center for Computational Toxicology and Exposure, Research Triangle Park, USA*
3 *United States Environmental Protection Agency, Center for Public Health and Environmental Assessment, Research Triangle Park, USA*
4 *Helmholtz Centre for Environmental Research – UFZ, Department of Bioanalytical Ecotoxicology, Leipzig, Germany*

ABSTRACT TEXT

Individual susceptibility to xenobiotic exposure is variable. One factor that might account for this is the microbiome, which encompasses all microorganisms, and their encoded genes and associated functions, which colonize a host organism. We have previously shown that larval axenic (i.e. microbe-free) zebrafish exhibit dark-phase hyperactivity relative to conventionally colonized and conventionalized zebrafish. To understand mechanisms by which microbes influence neurobehavioral development, unbiased RNA sequencing was performed in head tissue isolated from axenic (AX), axenic colonized on day 1 (AC1), or conventionally colonized (CC) zebrafish at 10 days post fertilization (dpf). No differentially expressed transcripts were identified when comparing CC and AC1 colonized cohorts. In contrast, there were 504 differentially expressed genes (>2-fold, 0.5 FDR) when comparing both colonized groups to the axenic cohort. The Aryl

Hydrocarbon Receptor (AHR) was one predicted upstream regulator. There are three AHRs in zebrafish: AHR1a, AHR1b, and AHR2. To determine the essentiality of AHR-dependent signaling for neurobehavioral development, CRISPR/Cas9 gene editing was used to create sets of AHR1a, AHR1b, and AHR2 F0 mosaic zebrafish. Similar to axenic zebrafish, AHR2 F0 mosaics exhibited dark-phase hyperactivity relative to a scrambled control. In comparison, AHR1b mosaics exhibited light-phase specific hypoactivity while AHR1a mosaic larvae presented with control-like behavioral activity. Confirmation of these phenotypes in stable mutant lines is ongoing. These data demonstrate the importance of host-associated microbes for neurodevelopment and raise the question of whether microbial products and xenobiotics converge via AHR to affect neurobehavioral development. *This abstract does not necessarily reflect EPA policy.*

P 025: Drug repurposing for Duchenne Muscular Dystrophy using Worm and Fish phenotype-based Drug Screening

TOPIC: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Estefania Carrillo[1], *Claudia Maios*[1], *James Doyle*[1], *Alex Parker*[1], *Éric Samarut*[1]

[1] *University of Montréal Hospital Research Center CRCHUM, Department of Neurosciences, Montréal, Canada*

ABSTRACT TEXT

Duchenne Muscular Dystrophy (DMD) is a rare genetic disorder and the most frequent among muscular diseases. It is due to the deficiency of a protein called dystrophin and is characterized by progressive weakness and degeneration of the skeletal muscles. Since no cure for DMD has yet been found, there is an urgent need to identify new therapies. With this aim, we believe that drug repurposing is a powerful alternative approach to bring potential new therapies to the bedside.

Our drug discovery approach is based on unbiased phenotype-based screening of already approved molecules using simple DMD genetic avatars: worms and fish. Using those, we screened more than 4,500 molecules against the motility defect of *dmd*-mutant worms and identified 20 that could significantly ameliorate their symptoms. We are now currently validating their effect on *dmd*-mutant zebrafish that depict decreased swimming ability and muscle integrity defects. Interestingly, we identified one lead compound that is able to fully restore the swimming behaviour of *dmd*-mutant zebrafish after chronic exposure. We are now confirming the efficacy of our lead compound to restore muscle defects and prolong survival of *dmd*-mutant zebrafish. The next step will be to validate its efficacy in a mouse model of DMD and eventually to translate up to the clinic.

P 026: Screening of a small molecule library for chemicals that increase motile cilia using zebrafish and mice

TOPIC: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Gopalakrishnan Chandrasekaran[1], *Hyunjin Ryu*[2], *Hao Lu*[3], *Sudipto Roy*[3], *Jin Hee Ahn*[4], *Myung Ae Bae*[5], *Eun-Young Choi*[2], *Seok-Yong Choi*[1]

[1] *Chonnam National University Medical School, Department of Biomedical Sciences, Gwangju, Korea Republic of*
[2] *University of Ulsan College of Medicine, Department of Biomedical Sciences, Seoul, Korea Republic of*
[3] *Agency for Science- Technology and Research ASTAR, Institute of Molecular and Cell Biology, Singapore, Singapore*
[4] *Gwangju Institute of Science and Technology, Department of Chemistry, Gwangju, Korea Republic of*
[5] *Korea Research Institute of Chemical Technology, Bio & Drug Discovery Division, Daejeon, Korea Republic of*

ABSTRACT TEXT

Cilia are classified into primary (non-motile) cilia and motile cilia. Ciliopathies are a group of genetic disorders caused by dysfunctional cilia. Motile cilia play a vital role in the clearance of mucus and foreign matter along the trachea and bronchi, circulation of cerebrospinal fluid, movement of ova from the ovary and sperm motility. Defects in the motile cilia result in hydrocephalus, chronic respiratory infections, and infertility. As such, restoration of motile cilia with respect to structure and function is critical to the treatment of motile ciliopathies. However, there has been no effective therapeutic modalities for motile ciliopathies to date. To develop a small molecule that can increase motile cilia, we performed a small molecule library screen using *Tg(foxj1a:gfp)* zebrafish. Foxj1 transcription factor is a master regulator of motile ciliogenesis. We identified several hit compounds, whose efficacy was further confirmed with anti-acetylated α-tubulin immunostaining of WT zebrafish treated with the compounds. One such hit compound effectively rescued the ependymal motile cilia in a zebrafish genetic model with decreased motile cilia. Furthermore, the efficacy of the hit compound was tested using multi-ciliated cells differentiated

ex vivo from primary mouse tracheal epithelial cells. Finally, administration of the hit compound to the chronic obstructive pulmonary disease (COPD) mice model restored tracheal motile cilia.

P 027: Exploring neuroactive drug responses through inter-individual variation

TOPIC: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Jennifer Fitzgerald[1], *Colette Vom Berg*[1]

[1] *Eawag- Swiss Federal Institute of Aquatic Science and Technology, Environmental Toxicology, Dübendorf, Switzerland*

ABSTRACT TEXT

The degree of response to diseases, stressors, but also to therapeutic drugs is dependent on the susceptibility of the individual; however, the underlying reasons for these response differences remain largely unclear. Inter-individual differences in drug responses can have widespread consequences ranging from therapeutic success to life-threatening toxicities. In particular, for neuroactive drugs individual effects are difficult to predict, due to the complex etiology of neurological and mental disorders and the multitude of unknown molecular drug targets. By investigating inter-individual differences, we aim to identify molecular targets of neuroactive drugs, whilst exploring the reasons underlying variability in drug responses. We use behavioral measures to sort chemically exposed individual zebrafish larvae based on their sensitivity. Larval locomotor behavior is widely used as a read-out for the assessment of external challenges to the nervous system; however, it is highly variable and difficult to predict at the individual level. Yet, in an initial analysis of unexposed larvae, we found that locomotor activity of an individual becomes consistent from 6 to 7 dpf, with variability lowest when fish encounter sudden darkness. Using this information, we carried out exposures to neuroactive drugs and sorted the larvae into tolerant and susceptible populations based on their response. Individuals from the different sensitivity categories are currently being subjected to transcriptome analysis to explore the molecular mechanisms that underpin these sensitivity differences. In addition, differentially regulated genes might point to drug response pathways. Overall, these data will provide mechanistic understanding of drug responses that can benefit drug discovery processes and human health, whilst additionally supporting environmental risk assessment, as high quantities of neuroactive drugs and other man-made chemicals are frequently detected in the aquatic environment.

P 028: In vivo screening assay for the identification of compounds enhancing epicardium formation in zebrafish

TOPIC: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Eleonora Lupi[1,2], *Jochen Gehrig*[2], *Nadia Mercader*[1]

[1] *University of Bern, Institute of Anatomy, Bern, Switzerland*

[2] *Acquifer is a Division of Ditabis, Digital Biomedical Imaging System AG, Pforzheim, Germany*

ABSTRACT TEXT

The zebrafish is increasingly used to study developmental and regenerative processes of various tissues and organs including the heart. Despite structural differences, the zebrafish heart shares similarities with humans on the genetic and histological level. The epicardium (the external layer of the heart), has a crucial role in cardiac formation and regeneration. It consists of an external monolayer deriving from a cluster of precursor cells called Proepicardium. We hypothesized that an increased number of proepicardial and epicardial cells would enhance regenerative capacity of cardiac tissue. Therefore, we designed zebrafish chemical screening experiments and automated imaging workflows to search for modifiers of epicardial cells number using a set of 43 compounds of epigenetic modulators. We will present our results related to molds for larvae positioning, establishment of the imaging pipeline, segmentation of epicardial cells and first results of the effect of chemical compounds on epicardium formation.

P 029: Zebrafish screening pipeline to identify osteoactive compounds in marine extracts

TOPIC: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Joana T. Rosa[1], Alessio Carletti[1,2], Tatiana Varela[1,2], Paulo J. Gavaia[1,2], M. Leonor Cancela[1,2,3,4], Vincent Laizé[1]

[1] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal*
[3] *Centre for Biomedical Research, University of Algarve, Faro, Portugal*
[4] *Algarve Biomedical Center, University of Algarve, Faro, Portugal*

ABSTRACT TEXT

Skeletal disorders such as osteoporosis affect millions of people worldwide and current treatments are poorly effective or trigger secondary effects. Farmed fishes also suffer from a large variety of skeletal malformations that hamper growth and survival. Both situations result in a massive economic burden for the society and have to be solved. Although still largely unexplored, marine biodiversity represents a promising source of natural bioactives, in particular compounds with the capacity to improve skeletal status. Zebrafish, a well-established animal model in biomedicine and aquaculture, was used to screen marine extracts for molecules with pro- or anti-osteogenic activities. Fractions (n=160) prepared from a variety of marine organisms - cyanobacteria, actinobacteria, planctomycetes, microalgae, seaweeds and halophytes – using different solvents were first assessed for their capacity to increase the growth of the opercular bone[1]. From those, 24 fractions were shown to increase operculum area up to 60 % and 10 were further tested for their effect on bone regeneration using the zebrafish caudal fin system[2]. Several fractions increased the mineralized area of newly formed rays, but also affected thickness and patterning. Fractions were also tested *in vitro*[3] and several of them were found to stimulate (up to 3.5 folds) extracellular matrix mineralization. Our data confirms the potential of marine organisms as a source of osteogenic and mineralogenic compounds, but also the suitability of the zebrafish as a first approach for bioactive screening. Promising extracts will be further fractionated towards the identification of osteoactive compounds.

1 Tarasco 2017 DOI 10.1016/J.CBPC.2017.04.006
2 Cardeira 2016 DOI 10.1038/srep39191
3 Pombinho 2004 DOI 10.1007/s00441-003-0830-1

Financed by projects ALGARED+ (INTERREG), BLUEHUMAN (ATLANTIC AREA), BioMedaqu (H2020-MSCA-ITN), OSTEOMAR (MAR2020) and UIDB/04326/2020 (FCT). Thanks to CIIMAR-BBE (Porto) & CCMAR-MARBIOTECH (Faro).

P 030: Effect of the algal alkaloid caulerpin on zebrafish reproductive performance and its parental transfer to offspring

TOPIC: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Valentina Schiano[1], Carla Lucini[1], Paolo De Girolamo[1], Livia D'Angelo[1,2], Antonio Terlizzi[3], Serena Felline[4], Adele Cutignano[5], Ernesto Mollo[5]

[1] *University of Naples Federico II, Veterinary Medicine and Animal Production, Napoli, Italy*
[2] *Stazione Zoologica Anton Dohrn, Biology and Evolution of Marine Organisms BEOM, Napoli, Italy*
[3] *University of Trieste, Department of Life Sciences, Trieste, Italy*
[4] *The National Interuniversity Consortium For Marine Sciences CoNISMa, CoNISMa, Roma, Italy*
[5] *Institute of Biomolecular Chemistry, National Research Council, Pozzuoli, Italy*

ABSTRACT TEXT

Biological invasions are crucial environmental issues with negative ecological and economic impacts. In addition, recent studies emphasize the impact of bioactive metabolites from invasive species as drivers of changes in marine ecosystems (Defranoux and Mollo 2020). The exotic green alga *Caulerpa cylindracea* invaded the Mediterranean Sea and became a preferential food of the native commercial fish *Diplodus sargus*. The main algal metabolite, the alkaloid caulerpin (CAU), which had already shown a panel of biological activities of interest in pharmacology and biotechnology, was found to enter the food chain in the invaded environment and accumulate in fish tissues (Mollo et al. 2015, and references therein). Furthermore, we recently found that CAU accumulated in the gonads of *D. sargus*, potentially affecting fish reproduction. Hence, to deepen the effects of CAU on fish fertility we decided to employ zebrafish as an experimental model. CAU was added to commercial dry food and administered daily to groups of adult fish at an equal age and sex ratio. Liquid Chromatography–Mass

Spectrometry was used to assess CAU occurrence both in the laid eggs and embryos. As a result, it was demonstrated an unprecedented parental transfer of CAU to fish offspring, strongly supporting that the metabolite was accumulated in the fish gonads and maternally transferred. CAU oral administration to adult fish was also accompanied by a higher rate of fertility, egg hatching, and embryos/larvae survival, compared to the control group. These findings suggest that CAU can improve fish reproductive performance, paving the way for desirable exploitation of invasive algal biomasses as a source of functional feed additives of interest in aquaculture.

P 031: Identification of Novel Bioactive Natural Compounds using In Vivo Zebrafish Phenotypic Assays

TOPIC: CHEMICAL BIOLOGY AND DRUG DISCOVERY

Vasiliki Tsata[1], Adamantia Agalou[1], Efstathia Ioannou[2], Vassilios Roussis[2], Niki Chondrogianni[3], Dimitris Beis[1]

[1] *Biomedical Research Foundation Academy of Athens, Developmental Biology, Athens, Greece*
[2] *National and Kapodistrian University of Athens, Department of Pharmacy- Section of Pharmacognosy and Natural Product Chemistry, Athens, Greece*
[3] *National Hellenic Research Foundation, Chemical Biology, Athens, Greece*

ABSTRACT TEXT

Identification of new Bioactive Natural Products (BNPs) that may serve as potential drug lead compounds is a constant challenge. Large-scale screens with zebrafish embryos, as alternatives to mammalian models, allow *in vivo* monitoring of complex cell behavior and physiological parameters. Therefore zebrafish-based assays are gaining high popularity and wide-usage in both academic and industrial drug discovery efforts as valuable whole animal platform for various stages of BNPs bioprospection. Abnormal pigmentation correlates with various aesthetic problems, as well as health diseases, including melanoma. We identified an extract from the Greek hawthorn *Crataegus pycnoloba* as a potent inhibitor of melanin synthesis and used activity-based fractionation to identify active subfractions and eventually three single compounds that inhibit melanogenesis. Finally, we identified a molecular mechanism that elucidates how their activity is mediated.

We are currently focusing on extracts and metabolites from macroalgae. Unlike terrestrial organisms that have been the subject of intensive research, marine benthic organisms are much less studied. Nevertheless, a number of secondary metabolites with valuable properties for the cosmetology sector have already been reported from macroalgae. Their active ingredients find applications worldwide not only in the food industry, but also in cosmetics. However, the rich biodiversity of the Eastern Mediterranean basin remains largely underexplored. To this end, we initiated the ALGOSMETIC project aiming to discover metabolites with anti-aging, melanogenesis-inhibitory and/or wound-healing activities from macroalgae of the Greek seas. We present results from a large-scale chemical screen of several macroalgal species (that are either found in dense populations or there is aquaculture know-how), collected from numerous different geographic areas and at different time periods.

Circuits and Behavior

P 032: Mood-like states in Fish: Judgement bias and Scototaxis tests to measure long-term emotional responses in Zebrafish

TOPIC: CIRCUITS AND BEHAVIOR

Jhon Buenhombre[1], *Amauri Gouveia Jr*[2], *Pêssi Sousa*[1], *Erika Daza-Cardona*[3]

1 *UFPA-Instituto de Ciências Biológicas, Pós-Graduação em Neurociências e Biologia Celular, Belém, Brazil*
2 *UFPA-Instituto de Ciências Biológicas, Núcleo de Teoria e Pesquisa do Comportamento, Belém, Brazil*
3 *UFC, Ciências da Saúde, Sobral, Brazil*

ABSTRACT TEXT

Zebrafish is becoming a promising model-organism to study neuroscience and behaviour and the environment where it is kept is very important for their welfare and to increase the validity and reproducibility of preclinical data. However, information about environmental influences in long-term emotional states in zebrafish (*Danio rerio*) is sparse. Here we used a Judgement bias, a Dark/light and a Salinity tests to evaluate long-term environmental effects on emotional states and coping responses. We used 72 zebrafish (*Danio rerio*) wild type that were divided into six groups of environmental manipulation that varied in terms of time presentation and level of enrichment. First fish were trained for 12 days to discriminate between two stimuli: a green colour that was rewarded and a red colour that was not. Afterwards their environment was manipulated according to their group for 4 days. We then ran the judgement bias, followed by the Dark/light and the Salinity tests. The results showed that Zebrafish learn to discriminate between two colour stimuli and remember the association after four days. Also fish whose environment is enriched and maintained constant are better at coping with stress, but that response was not mirrored in the Judgement bias. Fish that experimented a sudden environmental increase showed a negative bias and a consequently negative response towards stressful stimuli. Similarly, fish that experienced a gradual decrease have a negative coping response but without a corresponding negative bias. Our results show that constant environmental enrichment has positive effects in coping with stress but can produce negative effects if it is suddenly increased or gradually decreased. Also, we show that judgement bias can be used to measure negative influences in long-term emotional states, but overall, the dark/light test was more sensitive to affective states.

P 034: Phenotypic correlations and strain differences of motivational and cognitive components of social behavior in zebrafish

TOPIC: CIRCUITS AND BEHAVIOR

Claudia Gonçalves[1], *Magda Teles*[1,2], *Kyriacos Kareklas*[1], *Rui F. Oliveira*[1,2,3]

1 *Instituto Gulbenkian de Ciência, Lisboa, Portugal*
2 *ISPA - Instituto Universitário, Lisbon, Portugal*
3 *Champalimaud Research, Lisbon, Portuga*

ABSTRACT TEXT

Sociality is often characterised as a single phenotypic trait, but it relies on motivational, cognitive and physiological elements that may implicate several discrete components. In turn, these components may rely on phenotypic traits specific to the social domain or generalised across social and non-social contexts. By regarding the genetic contribution that largely underlies individual variation in social phenotypic traits, we tested these hypotheses in males and females across six common wild-type zebrafish laboratory strains. Social tendency towards a shoal was examined, together with cognitive performance and motivational tendencies (exploration and novelty-seeking) in both a social and non-social recognition test (conspecific and object). Additionally, an open-field test was used to quantify anxiety levels, a likely generalised physiological and motivational modulator of behaviour. Fish from all strains exhibited social and non-social recognition and shoal preference. However, component analysis indicated four separate sources of behavioural variation: General Boldness (social and non-social novelty-seeking), General Memory (social and non-social recognition), General Interaction (social and non-social exploration

with sociality) and Anxiety. Strains differed in Anxiety and General Interaction and in their sexual dimorphism. Combined, our findings reveal that sociality is discrete from social cognitive performance and mostly implicates motivational components selected for generality across social and non-social contexts.

P 035: A systematic review of the neurobehavioral effects of Cannabidiol (CBD) in Zebrafish

TOPIC: CIRCUITS AND BEHAVIOR

Gustavo Gonzalez-Cuevas[1]

[1] *Idaho State University, Biomedical and Pharmaceutical Sciences, Meridian, USA*

ABSTRACT TEXT

Cannabidiol (CBD), the main non-addictive constituent of the Cannabis Sativa plant, has emerged as a potential therapeutic tool for treating a wide variety of neuropsychiatric disorders. However, very little is known regarding the precise brain mechanisms, pharmacokinetics, developmental toxicity, effective dosage, drug interactions, and behavioral consequences of CBD administration. Since Zebrafish, which are cheap and amenable to high-throughput behavioral screens, have been recently recognized as an ideal model organism in behavioral neuroscience, a deeper understanding of the effects of CBD on behavior and the brain can be gained by integrating previous empirical evidence. Therefore, the overarching goal of this systematic review is to comprehensively synthesize existing research on the neurobehavioral effects of CBD in Zebrafish. Implications of these findings are discussed and recommendations for future research delineated.

P 036: Insecticides trigger olfactory-mediated aversive responses in larval zebrafish

TOPIC: CIRCUITS AND BEHAVIOR

Sarah Könemann[1], *Stéphanie Meyer*[2], *Colette vom Berg*[1]

[1] *Swiss Federal Institute for Aquatic Science and Technology, Environmental Toxicology, Dübendorf, Switzerland*

[2] *École Polytechnique Fédéral de Lausanne, Civil and Environmental Engineering, Lausanne, Switzerland*

ABSTRACT TEXT

Via spray drift, run-off or leaching, insecticides inevitably find their way into surface water bodies, which leads to the exposure of aquatic non-target organisms, such as fish. The presence of insecticides in the water might not only lead to neurotoxic effects but also alter fish behavior, affecting the survival of the individual as well as the whole population. To detect chemicals in the environment, fish use different sensory modalities, such as olfaction, gustation, and chemosensation. Sensory signals are further integrated in the brain to generate an appropriate behavioral response, such as positive (attraction) or negative chemotaxis (avoidance). This study investigates whether different classes of insecticides induce chemotaxis in zebrafish larvae and which brain areas are responsible for generation of the measured behavioral response. The behavior of zebrafish larvae was tracked with an automated video recording system, while the test substance was added to one side of the test chamber. Olfactory-deficient larvae were used to assess whether the response is mediated by the olfactory system. To further analyze underlying brain regions, neuronal activity is visualized by antibody staining of the activity indicator pERK. The larvae responded with significant aversion to the neonicotinoid imidacloprid and the organophosphate diazinon at a concentration of 10 µM, while for the other tested insecticides no significant response was measured. In olfactory-deficient larvae the aversive responses were omitted, indicating that imidacloprid and diazinon were detected through olfaction. We are currently testing whether the brain activity patterns correspond to the areas reported to be involved in attractive and aversive responses, such as habenula, dorsal raphe nucleus, locus coeruleus, spinal cord and hindbrain. Overall, our study will advance our understanding of the impact of chemicals on fish behavior and their underlying mechanisms.

P 037: Zebrafish exhibit visual attentional pop-out effects in their behavior

TOPIC: CIRCUITS AND BEHAVIOR

Sarah Stednitz[1], *Jennifer Li*[2], *Drew Robson*[2], *Li Zhaoping*[1]

[1] *Max Planck Institute for Biological Cybernetics, Sensory & Sensorimotor Systems, Tuebingen, Germany*
[2] *Max Planck Institute for Biological Cybernetics, Systems Neuroscience & Neuroengineering, Tuebingen, Germany*

ABSTRACT TEXT

How do animals decide what parts of the environment are important - and how do they do it so quickly? Our senses drive our interactions with the world, but our sensory organs produce far more data than could ever be analyzed in full. Further complicating the problem, many sensory stimuli are entirely irrelevant and require no response, and as such survival is best enhanced by attending only to relevant cues. To overcome this sensory bottleneck, the brain evolved methods for directing attention to salient objects without the need for higher-order feedback that is costly when a rapid reaction is required. While empirically testing the underlying neural mechanisms behind visual attention selection in mammals has been historically challenging due to technical limitations, the zebrafish (*Danio rerio*) is perhaps an ideal model system for interrogating visual computations. They exhibit a range of visual behaviors very early in life, possess evolutionarily conserved regions of the brain dedicated to visual computation, and are amenable to live imaging using genetically encoded fluorescent indicators of neuronal activity. By 4 days post fertilization, zebrafish larvae begin to pursue and capture prey, requiring the rapid selection of targets and subsequent orienting to the prey. However, the stimulus features governing visual target selection in non-mammalian vertebrates are not fully described. Despite the clear advantages of using zebrafish to study the neural mechanisms of behavior, attentional behavior is poorly studied compared to the vast literature on such topics in primates and other mammals. Therefore, to characterize visual attention we analyze responses to artificial stimuli in a novel behavioral assay.

P 038: Basal ganglia output to cortex bi-directionally encodes positive and negative values for goal-directed behavior in adult zebrafish

TOPIC: CIRCUITS AND BEHAVIOR

Yuki Tanimoto[1], *Makio Torigoe*[1], *Tanvir Islam*[1], *Ryo Aoki*[2], *Hisaya Kakinuma*[1], *Hitoshi Okamoto*[1]

[1] *RIKEN Center for Brain Science, Laboratory for Neural Circuit Dynamics of Decision Making, Wako- Saitama, Japan*
[2] *RIKEN Center for Brain Science, Laboratory for Neural Circuits and Behavior, Wako- Saitama, Japan*

ABSTRACT TEXT

Animals can learn multiple external cues that predicts future punishment or reward, and respond to them properly. However, how recognition of the punishment or reward predicting cues is reflected to the behavior is largely unknown. We recently have established a closed-loop virtual-reality system for 2-photon calcium imaging of adult zebrafish, and recorded activities of the dorsal pallium, zebrafish cerebral cortex, in the course of learning of Go/No-go task (Torigoe et al., submitted). In this task, a fish needs to move from blue- to red-region in Go trials and needs to stay in red-region in No-go trials to avoid electrical shock. During these tasks, we observed emergence of neural ensembles encoding dangerous blue-cue, safe red-cue, and an error of current situations from expected ideal situations.

Here, we report a putative role of the dorsal entopeduncular nucleus (dEN), zebrafish pallidum, for integration of value information from the dangerous and safe situations. Neuropeptide Y-positive neurons in the dEN, homologous to the mammalian globus pallidus internus, have dense projection directly to the entire dorsal pallium. We recorded activities of the NPY-positive axon termini from the dEN during the Go/No-go task in the virtual reality. Each of the dEN axon terminus primarily responded to the electrical shock and then started to be activated by dangerous blue-cue and inhibited by safe red-cue during learning, suggesting that the dEN bi-directionally encode positive and negative values assigned to the colors. Based on the result, we will discuss a possible neural circuit of the zebrafish basal ganglia, where positive values from striatal direct-pathway and negative values from the striatal indirect-pathway are integrated in the dEN to facilitate the behavioral response by the dorsal pallium. Such an integration circuit may be responsible for proper behavioral responses of animals to punishment or reward predicting external cues.

Disease Models

P 039: Zebrafish as a model for Bloom syndrome

TOPIC: DISEASE MODELS

Tamás Annus[1], *Dalma Müller*[1], *Bálint Jezsó*[2], *Gábor Harami*[3], *Máté Varga*[1], *Mihály Kovács*[3]

[1] *Eötvös Loránd University, Department of Genetics, Budapest, Hungary*
[2] *Eötvös Loránd University, Department of Anatomy- Cell and Developmental Biology, Budapest, Hungary*
[3] *Eötvös Loránd University, Department of Biochemistry, Budapest, Hungary*

ABSTRACT TEXT

Also known as the "guardians of the genome", RecQ helicases play crucial roles in genome integrity maintenance through their involvement in various DNA metabolic pathways. Aside from being conserved from bacteria to vertebrates, their importance is also reflected in the fact that in humans impaired function of certain RecQ helicase orthologs are known to cause severe sets of symptoms such as Bloom, Werner and Rothmund-Thomson syndromes. Zebrafish is a promising model organism for the study of RecQ helicases, as all five human paralogs have single zebrafish orthologs.

In order to gain a better insight into the specific roles of RecQ helicases in the genome maintenance of vertebrates, we create and characterize zebrafish models for mutations in the five RecQ helicase genes (*recql, blm, wrn, recql4* and *recql5*). The first such gene we have examined more closely is *blm*. As mentioned above, humans afflicted with BLM malfunction exhibit Bloom syndrome, a recessive autosomal disorder characterized by short stature, skin rashes, reduced fertility, increased risk of carcinogenesis and shortened life expectancy brought on by genomic instability. To determine whether zebrafish is indeed a viable model for Bloom syndrome we analyzed lifespan, fertility, histology and DNA repair efficiency in our mutant strain.

Our results show that our model recapitulates major hallmarks of the human disease. Moreover, some functions of zebrafish Blm bear additional importance in germ line development, and thus in sex differentiation. Therefore, our model will be a valuable tool for further understanding the developmental and molecular attributes of this rare disease, along with providing novel insights into the role of genome maintenance proteins in somatic DNA repair and fertility.

P 040: Inactivation of cpamd8 reveals early eye developmental defects in zebrafish larvae: A putative model for anterior segment dysgenesis type 8

TOPIC: DISEASE MODELS

Jose-Daniel Aroca-Aguilar[1], *Juan-Manuel Bonet-Fernández*[1], *Ana-Isabel Ramírez*[2], *Susana Alexandre-Moreno*[1], *Jesús-José Ferre-Fernández*[1], *Raquel Atienzar-Aroca*[1], *Juan-José Salazar*[2], *Julio Escribano*[1]

[1] *University of Castilla-La Mancha Medical School, Genetics, Albacete, Spain*
[2] *Universidad Complutense de Madrid/OFTARED., Instituto de Investigaciones Oftalmológicas Ramón Castroviejo, Madrid, Spain*

ABSTRACT TEXT

CPAMD8 has been recently associated with anterior segment dysgenesis type 8 (ASD-8) in human patients although both gene function and underlying molecular mechanism are mostly unknown. In an effort to better understand the role of this gene in ASD we analysed the expression of the zebrafish *cpamd8* orthologous gene in 96 hpf larvae by fluorescent-whole mount immunohistochemistry (FWIHC). 3D reconstruction revealed periocular labeling in the optic cup edge and mesenchymal-like cells in the dorsoposterior quadrant of the eye. Both confocal optical sections and immunohistochemical analysis of tissue sections showed cpamd8 positive signals in the periocular mesenchyme of developing dorsal and ventral iridocorneal angles. We also inactivated this gene in zebrafish embryos by CRISPR/Cas9 genome editing using two gRNA pairs targeting exons 4 (*cpamd8ex4*) or 25 (*cpamd8ex25*). Analysis of 50–200 *F0* mosaic embryos in each experiment showed the presence of two different 100 bp-deletions, associated with similar abnormal gross *F0* phenotypes which included microphthalmia, pharyngeal maldevelopment and pericardial, periocular and brain edema in 7–25 % of crispant embryos. Toluidine blue staining of embryo head sections confirmed the presence of microphthalmia, periocular and brain edemas, and clearly showed

pharyngeal cartilage underdevelopment, progressive dorsal and ventral iridocorneal angles hypoplasia with decreased number of iris stroma and mesenchymal-like cells and increased size of corneal epithelium cells. Transmission electron microscopy of dorsal and ventral iridocorneal angles demonstrated a reduction of the anterior chamber size, corneal epithelium thickening and irregular organization of collagen fibers in the corneal stroma. These data indicate that *cpamd8* disruption results in early anterior segment maldevelopment that recapitulate some ASD-8 features.

Funding: ISCIII PI15/01193; JCCM SBPLY/17/180501/000404 & PREJCCM2016/28; OFTARED RD16/0008/0019 & RD16/0008/0005.

P 041: Dissecting the molecular networks of cardiomyocyte proliferation to guide heart regeneration

TOPIC: DISEASE MODELS

Alena Boos[1], *Severin Dicks*[2], *Melanie Börries*[2], *Wolfgang Rottbauer*[1], *Steffen Just*[1]

[1] *Ulm University Medical Center- Internal Medicine II, Molecular Cardiology, Ulm, Germany*
[2] *Faculty of Medicine Albert-Ludwigs University of Freiburg- Medical Center, Institute of Medical Bioinformatics and Systems Medicine, Freiburg, Germany*

ABSTRACT TEXT

Myocardial infarction (MI) is a life-threatening disease of the human heart and leads to reduction of functional cardiac tissue. The myocardium of the adult mammalian heart is incapable to recover structure and function after damage by proliferating of surviving cardiomyocytes, leading to impaired contractile function and heart failure.

Although, current therapeutic approaches could reduce the mortality in MI patients significantly, they are limited due to unknown molecular-based strategies to induce myocardial regeneration. Unlike the mammalian heart, the zebrafish heart can naturally undergo heart regeneration after injury mediated by proliferation of resident cardiomyocytes. In large-scale forward mutagenesis screens, we isolated five zebrafish mutant lines with either cardiac hyperplasia or hypoplasia and dissected the underlying genetic defects.

The focus of the project is to investigate networks underlying endogenous cardiomyocyte proliferation by bioinformatics analyses of transcriptome datasets of these mutant lines. Therefore, RNA-Seq data are generated from whole embryos and hearts of each fish line. The differentially expressed genes of the hypo- and hyperplastic lines are compared in order to find common networks and hub nodes, which regulate cardiomyocyte proliferation. To transiently or stably inactivate network components potentially controlling cardiomyocyte proliferation Morpholino-antisense or CRISPR/Cas9 technologies will be used and screened for cardiac phenotypes to analyze their role specifically in cardiomyocyte proliferation.

Using the zebrafish, we want to understand the underlying genetic and molecular networks of embryonic cardiomyocyte proliferation. To find a common mechanism of cardiomyocyte proliferation the identified embryonic networks will be compared with adult heart regeneration networks. The translation of this mechanism into adult mammalian heart can be an approach to induce regeneration of the damaged cardiac tissue after MI.

P 042: Chd7 regulates lipid metabolism and swim bladder inflation in zebrafish

TOPIC: DISEASE MODELS

Maximilian Breuer[1], *Gabrielle Fortier*[1], *Priyanka Jamadagni*[1], *Charlotte Zaouter*[1], *Betelhem Kassa*[1], *Kessen Shunmoogum Patten*[1]

[1] *INRS - Armand-Frappier Santé Biotechnologie Research Centre, Biology, Laval, Canada*

ABSTRACT TEXT

The chromodomain helicase remodelling enzyme, CHD7, is a key regulator of several pathways from neural crest cell differentiation to stem cell maintenance. Mutations in CHD7 have been directly linked to CHARGE syndrome- a syndrome characterized by multisystemic symptoms, including craniofacial dysmorphisms and heart defects.

Our study shows that *chd7*$^{-/-}$ zebrafish present with characteristics of a dyslipidemia. Specifically, we identify accumulation of neutral lipid droplets in the swim bladder and vascular system of developing larvae. Along these lines we identified a collection of dysregulated lipid metabolism enzymes along with ppary regulated lipid metabolism. Following, we could link lipid metabolism to swim bladder

development and observe decreased size and non-inflated swim bladder during larval development dependent on proper lipid metabolism in the surfactant system. In detail we could show that loss of elongation factor 1, *elovl1*, and the fatty acid binding protein 7b, *fabp7b*, are dependent for the lipid metabolism of the swim bladder and subsequently lead to failure to inflate the swim bladder.

Our study is the first to investigate the multisystemic role of CHD7 in correlation to lipid metabolism and swim bladder development and its fundamental underlying pathway.

P 043: Craniofacial dysmorphism in CHARGE syndrome due to dysregulation of serotonin receptor HTR2B

TOPIC: DISEASE MODELS

Maximilian Breuer[1], *Priyanka Jamadagni*[1], *Kessen Shunmoogum Patten*[1]

[1] *INRS - Armand-Frappier Santé Biotechnologie Research Centre, Biology, Laval, Canada*

ABSTRACT TEXT

CHARGE syndrome is a severe multisystemic developmental disorder that is most commonly caused by mutations in the ATP-dependent chromatin remodelling enzyme CHD7. To understand the function of CHD7, we generated a *chd7* mutant in zebrafish by CRISPR/Cas9 mediated mutagenesis. This model has proven highly efficient in replicating the characteristics observed in CHARGE syndrome including craniofacial defects.

Using an unbiased transcriptomic analysis (RNA-Seq), we identified a significant downregulation of the 5-hydroxytryptamine receptor 2b (*Htr2b*) in these *chd7* mutant zebrafish. Interestingly, this member of the serotonin receptor family is closely associated with behavioral defects including impulsiveness and aggressiveness, as well as developmental defects in heart and jaw development, which are characteristics observed in CHARGE syndrome.

To gain more insights in the role of *htr2b* in CHARGE syndrome pathogenesis, we assessed its function further in zebrafish. We found that *htr2b* is expressed in the branchial arches, giving rise to the jaw, via whole-mount *in situ* hybridization. Htr2b inhibition via the specific inhibitor RS-12744 partially mimics the morphological phenotypes. More specifically, the inhibition of Htr2b results in defective development of the palatoquadrate in the jaw as determined by alcian blue and calcein staining, with high similarity to the morphology observed in *chd7* mutants. To further understand the mechanism involved we generated *htr2b* and *htr2b/chd7* mutants to determine its role in the defective craniofacial development that is often observed in CHARGE syndrome patients. First results indicate a high dependency on Htr2b for successful palatoquadrate development, which may underlie the craniofacial phenotype in CHARGE syndrome.

Altogether, our data show an implication of dysregulated HTR2B functions upon loss of function of CHD7 in CHARGE syndrome pathogenesis and suggest that HTR2B may serve as a potential therapeutic target.

P 044: ALS gene, C9orf72, loss of function Zebrafish model shows motor and synaptic defects

TOPIC: DISEASE MODELS

Zoe Butti[1], *Jean Giacomotto*[2], *Kessen Patten*[1]

[1] *INRS - Centre Armand Frappier, Biology, Laval, Canada*

[2] *Queensland Brain Institute- University of Queensland, Neuroscience, Brisbane, Australia*

ABSTRACT TEXT

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motoneurons causing muscular atrophy, paralysis and ultimately to death. To this day, no curative treatment exists. Understanding the physiopathological mechanisms will help develop new efficient treatments. In 2011, an expansion of a repetition of a hexanucleotide (GGGGCC) in the first intronic region of the *C9orf72* gene has been discovered as the first genetic cause of ALS. To investigate the role of *C9orf72* loss of function in ALS, we used synthetic micro-RNAs to specifically target the zebrafish C9orf72 gene (C9-miRNA) and have developed a stable zebrafish C9-miRNA line with reduced expression of *C9orf72*. Upon loss of function of *C9orf72*, we observed that zebrafish C9-miRNA mutants display severe motor deficits starting at 6 days postfertilization (6 dpf) and a majority die premature as of 15 dpf. Analysis of the neuromuscular junctions using

specific presynaptic and postsynaptic markers znp1 and alpha-bungarotoxin respectively, revealed a significant decrease in the number of synaptic contacts in the C9-miRNA mutant line at 6 dpf correlating with a decreased synaptic vesicles turnover. Among the few fishes that survived till adulthood, we observed a significant motoneuron and muscle atrophy. We are currently performing electrophysiology studies to characterize the synaptic defects. Altogether, our zebrafish C9-miRNA replicates aspects of ALS and we are investigating further the role of reduced C9orf72 in ALS pathogenesis.

P 045: The role of HNF1 and HNF4 in zebrafish pancreatic development and function

TOPIC: DISEASE MODELS

Pablo Cabezas-Sainz[1], *Jose Bessa*[1]

[1] *Instituto de Investigação e Inovação em Saúde i3S- Universidade do Porto, Vertebrate- development and regeneration, Porto, Portugal*

ABSTRACT TEXT

Maturity onset diabetes of the young (MODY) it is a very early onset of diabetes. Many of the genes that are highly important for pancreatic function were identified because coding mutations caused this type of very early and penetrant form of diabetes. HNF1 and HNF4 are two protein coding genes that encode hepatocyte nuclear factors (HNFs), which mutations are known to be associated to MODY, showing their important role in pancreas development and function. HNF1 and HNF4 are known to work as transcription factors, binding to enhancers, controlling the transcription of target genes. However, which genes are controlled by HFN1 and HFN4 and what are the molecular consequences of their loss-of-function, is yet to be fully explored in vivo. In this work we have used epigenetic marks of enhancer activity, obtained from zebrafish pancreas, to identify putative binding sites of HFN1 and HFN4, allowing us to predict regulatory landscapes controlled by these factors. We are currently using mutants for these two genes to better understand the impact in the regulatory landscapes of pancreatic genes and their molecular consequences. We will present our most recent data from this project.

P 046: Dissecting the role of the InterPhotoreceptor Matrix proteoglycan IMPG2 in zebrafish retinal development and function

TOPIC: DISEASE MODELS

Maria Elena Castellini[1], *Andrea Messina*[2], *Alessandra Speccher*[1], *Lucia Poggi*[1], *Simona Casarosa*[1]

[1] *University of Trento, CIBIO, Trento, Italy*
[2] *University of Trento, Cimec, Rovereto, Italy*

ABSTRACT TEXT

Retinitis pigmentosa (RP) is one of the most commonly inherited retinal dystrophies, characterized by progressive degeneration of rod and cone photoreceptors. The genetic background of RP is heterogeneous, as are inheritance modes. Recent studies have reported that nonsense mutations in the interphotoreceptor matrix proteoglycan 2 (IMPG2) gene are associated with autosomal recessive RP in humans. This gene encodes the proteoglycan IMPG2, secreted by photoreceptors in the interphotoreceptor matrix, the extracellular matrix that surrounds retinal photoreceptor outer segments and ellipsoids. IMPG2 is synthesized by rods and cones and it is secreted in the IPM. We investigated IMPG2 function and expression in zebrafish, where the protein is present in two isoforms, IMPG2a and IMPG2b. RT-qPCR experiments performed on zebrafish embryos at different developmental stages revealed that IMPG2a and IMPG2b expression starts from 3 days post fertilization (dpf). In adults, both isoforms have an eye-specific expression. Western blot analyses showed a similar expression pattern for the proteins. Furthermore, immunohistochemistry experiments performed on retina sections showed that the expression of IMPG2 is specifically found in the outer segment of photoreceptors. Microinjection of antisense morpholinos oligonucleotides (MOs), specific for each of the two isoforms provided preliminary evidence that IMPG2 is involved in eye development and RPE pigmentation in zebrafish. Moreover, morphant embryos show an increase in cell proliferation in the ciliary marginal zone (CMZ). Currently, we are generating a zebrafish line carrying the human IMPG2 protein truncations, by using CRISPR/Cas9 technology. This will allow us to study the adult phenotype and perform large-scale testing of therapeutic compounds to discover a possible treatment for this type of retinopathy.

P 047: Zebrafish as a pre-clinical platform for haemorrhagic stroke research

TOPIC: DISEASE MODELS

Siobhan Crilly[1], Alexandra Njegic[2], Adrian Parry-Jones[1], Stuart Allan[1], Paul Kasher[1]

[1] *The University of Manchester, Faculty of Biology- Medicine and Health, Manchester, United Kingdom*
[2] *The University of Manchester, School of Biological Sciences, Manchester, United Kingdom*

ABSTRACT TEXT

Our understanding of the disease mechanisms underlying intracerebral haemorrhage (ICH) is incomplete. Furthermore, no specific medications exist for ICH patients – partly due to limitations associated with current pre-clinical mammalian systems and a failure to translate. It is essential, therefore, that we develop new tools for translational ICH research.

Zebrafish larvae are transparent, allowing for easy visualisation of brain tissue in intact animals. Their small size, high fecundity and fast developmental rates are amenable to rapid throughput genetic and drug screening. Furthermore, unlike rodent models, zebrafish larvae can reliably exhibit spontaneous ICH. By using live imaging, our recently published work indicates that ICH in zebrafish larvae induces quantifiable brain injury, locomotor and neuroinflammatory phenotypes, which recapitulate key features of the human pathology (Crilly et al, *F1000 Res*, 2018; Crilly et al, *J Vis Exp*, 2019). Using fluorescent brain cell death and inflammation reporter assays, we are performing cell-specific transcriptomic analysis and pharmacological intervention studies in zebrafish to identify candidate therapeutic targets that can reduce brain injury following ICH.

Our data indicate that the pathological consequences of brain bleeding are conserved between zebrafish and humans, thereby validating the use of this model as an important new tool for translational ICH research.

P 048: Antisense oligonucleotide-based treatment of retinitis pigmentosa caused by USH2A exon 13 mutations: from proof-of-concept in zebrafish to clinical application

TOPIC: DISEASE MODELS

Erik De Vrieze[1], Hester van Diepen[2], Kalyan Dulla[2], Ralph Slijkerman[1], Silvia Albert[3], Margo Dona[1], Hee Lam Chan[2], Jiayi Miao[2], Hanka Venselaar[4], Jingjing Zang[5], Stephan Neuhauss[5], Theo Peters[1], Sanne Broekman[1], Hannie Kremer[1], Peter Adamson[2], Erwin van Wijk[1]

[1] *Radboud University Medical Center, Otorhinolaryngology, Nijmegen, Netherlands*
[2] *ProQR Therapeutics, ProQR Therapeutics, Leiden, Netherlands*
[3] *Radboud University Medical Center, Stem Cell Technology Center, Nijmegen, Netherlands*
[4] *Radboud University Medical Center, Centre for Molecular and Biomolecular Informatics, Nijmegen, Netherlands*
[5] *University of Zürich, Institute of Molecular Life Sciences, Zürich, Switzerland*

ABSTRACT TEXT

Mutations in *USH2A*, encoding usherin, are the most common cause of syndromic and non-syndromic retinitis pigmentosa (RP). Two founder mutations in exon 13 collectively account for ˜34 % of *USH2A*-associated RP cases. Skipping of exon 13 from the *USH2A*transcript during pre-mRNA splicing presents a potential treatment modality. The resulting transcript is predicted to encode a shortened usherin protein (usherinΔexon13) with residual function. The available *Ush2a* knock-out mouse model presents with a mild and late-onset retinal degeneration. We therefore generated a zebrafish model for *USH2A*-associated retinal dysfunction, carrying protein-truncating germline lesions in exon 13 of the *ush2a* gene. We observed loss of usherin in the photoreceptor cells of *ush2a*mutant larvae, and a reduction in USH2 complex members whirlin and Adgrv1 at the photoreceptor periciliary membrane. Electroretinogram (ERG) recordings revealed a significant decrease in both a- and b-wave amplitudes in *ush2a* mutant larvae as compared to strain- and age-matched wild-type larvae. Next, we investigated the functionality of the usherinΔexon13 in the retina of *ush2a* mutant larvae. Morpholino-induced skipping of *ush2a* exon 13 resulted in the production of a shortened usherin protein, that localized normally in the photoceptor periciliary region. Exon-skipping levels of approximately 20 % were already able to restore the retinal dysfunction in the *ush2a* mutant larvae. RNA antisense oligonucleotides (AONs) were investigated for their potential to specifically induce human *USH2A* exon 13 skipping. Lead AON QR-421a was identified using cultured retinoblastoma cells, and further validated for exon skipping potential in iPSC-derived photoreceptor

precursor cells from homozygous *USH2A* c.2299delG patients and healthy donors, and the retina of non-human primates. Collectively, our data highlight exon skipping as a highly promising treatment for RP caused by mutations in exon 13 of the *USH2A* gene.

P 049: Genetic compensation prevents myopathy and heart failure in an in vivo model of Bag3 deficiency

TOPIC: DISEASE MODELS

Federica Diofano[1], *Karolina Weinmann*[1], *Isabelle Schneider*[1], *Wolfgang Rottbauer*[1], *Steffen Just*[1]

[1] *Ulm University Medical Center- Internal Medicine II, Molecular Cardiology, Ulm, Germany*

ABSTRACT TEXT

Mutations in the molecular co-chaperone Bcl2-associated athanogene 3 (BAG3) are found to cause dilated cardiomyopathy (DCM), resulting in cardiac systolic dysfunction and heart failure and myofibrillar myopathy (MFM), characterized by protein aggregation and myofibrillar disintegration in skeletal muscle cells. Here, we generated a CRISPR/Cas9-mediated Bag3 knockout zebrafish line and found, in contrast to Morpholino-modified antisense oligonucleotide-induced ablation of Bag3, the complete preservation of heart and skeletal muscle structure and function during embryonic development. Intriguingly, genetic compensation, a process of transcriptional adaptation which acts independent of protein feedback loops, was found to prevent heart and skeletal muscle damage in our model of CRISPR/Cas9-induced Bag3 deficiency. Proteomic profiling and quantitative real time PCR analyses identified Bag2, another member of the Bag protein family, significantly upregulated on a transcript and protein level, implying that the assured decay of Bag3 mutant mRNA in CRISPR-induced bag3-/- zebrafish caused the transcriptional upregulation of Bag2 expression. We further show here that Morpholino-mediated knock-down of Bag2 in homozygous bag3-/- mutant zebrafish embryos evoked severe functional and structural heart and skeletal muscle defects, similar to Morpholino-mediated Bag3 ablation, whereas Bag2 knock-down in bag3+/+ or bag3+/- embryos did not result in (cardio-)myopathy.Our findings provide for the first time evidence that genetic compensation might vitally influence the penetrance of disease-causing Bag3 mutations in model organisms but also human patients.

P 050: A zebrafish model for HAX1-associated congenital neutropenia

TOPIC: DISEASE MODELS

Larissa Doll[1], *Narges Aghaallaei*[1], *Advaita M. Dick*[1], *Baubak Bajoghli*[1]

[1] *University Hospital Tübingen, Division of Translational Oncology, Tübingen, Germany*

ABSTRACT TEXT

Severe congenital neutropenia (CN) is a rare heterogeneous group of diseases, characterized by a granulocytic maturation arrest. Autosomal recessive mutations in the HCLS1-associated protein X1 (HAX1) gene are frequently detected in affected individuals. However, the precise role of HAX1 during neutrophil differentiation is poorly understood. To date, no reliable animal model has been established to study HAX1-associated CN. In this study, we sought to determine the role of *hax1* in zebrafish hematopoiesis. We used morpholino-mediated knockdown and CRISPR-Cas9 approaches to perform loss-of-function analysis. We found that *hax1* knockdown reduced the number of neutrophils, without affecting the development of HSPCs and monocytes/macrophages.

Given that increased apoptosis of myeloid progenitors and decreased activity of the G-CSF signaling are two main observations associated with HAX1 deficiency in CN patients, we examined to what extent cellular viability and the G-CSF signaling were affected in the *hax1* deficient embryos. We used three approaches to assess apoptosis. Although cell death was increased, there was no correlation with neutrophils. In contrast, we found the expression level of downstream target genes of the G-CSF signaling pathway were significantly decreased. The reduced neutrophil numbers could also be reversed by G-CSF, which is also the main therapeutic intervention for patients who have CN. Hence, our results demonstrate that zebrafish is a suitable model for HAX1-associated neutropenia.

P 051: Acromegaly aging genetic signature

TOPIC: DISEASE MODELS

Abdalla Elbialy[1]

[1] *Faculty of veterinary medicine- Damanhour university, Fish diseases, Alexandria, Egypt*

ABSTRACT TEXT

Acromegaly is a pathological condition that is caused by over-secretion of growth hormone (GH) and develops mainly from pituitary adenoma. Excess GH exposure over a prolonged period of time leads to a wide range of systemic manifestations and comorbidities. Studying the effect of excess GH on the cellular level could help to understand the underlying causes of acromegaly health complications and comorbidities. In our previous publications, we have shown that excess GH reduces body side population (SP) stem cells and induces signs of premature aging in an acromegaly zebrafish model. Here we study acromegaly aging in greater depth at the level of gene expression. We investigated whether acromegaly induces aging signature in different organs. Using the GenAge database, our acromegaly model showed a significant and robust aging genetic signature in the muscle but not in other organs. Likewise, the hierarchical clustering of WT, acromegaly, and aged RNA seq data from various organs revealed aging only in acromegaly muscle. We, therefore, identified overlapping DEGs in different organs between the acromegaly and the aged zebrafish. Importantly, about half of the muscle, liver, and brain acromegaly DEGs overlapped with aged DEGs. Interestingly, overlapping was observed in the same way; acromegaly up DEGs overlapped with aged DEGs, not down DEGs, and vice versa. We then identified the biological functions of overlapping DEGs. Enrichment database analysis and gene ontology (GO) showed that most overlapping muscle genes were involved in the metabolism of aging. while overlapping liver DEGs were involved in metabolic pathways, response to hypoxia, and endoplasmic reticulum (ER) stress. Thus, this study provides a full aging genetic signature of acromegaly at the gene expression level.

P 052: Acromegaly stem cells

TOPIC: DISEASE MODELS

Abdalla Elbialy[1]

[1] *Faculty of veterinary medicine- Damanhour university, Fish diseases, Alexandria, Egypt*

ABSTRACT TEXT

Acromegaly is a hormone disorder pathological condition that develops as a result of growth hormone over-secretion from the pituitary gland. In early life, the blood level of growth hormone (GH) is high, corresponding to rapid somatic growth. Its level gradually declines during adult life and aging. This age-related decline in plasma GH level is termed "somatopause", which has been well documented in various mammalian species. For a long time, age-related symptoms such as muscle mass reduction have been associated with somatopause; thus, GH therapy has been used as an antiaging drug. On the other hand, somatotropic axis mutations have been associated with longevity in mice and humans. Genetic disruption of the GH receptor gene drastically increased longevity from 25 % to over 60 %.

In order to investigate whether excess GH (as in the case of acromegaly) could increase longevity or lead to premature aging, we produced a zebrafish acromegaly model and studied excess GH effects on stem cells and aging. Here we show that the acromegaly zebrafish model progressively reduced the number of side population (SP) stem cells in different organs and increased oxidative stress in stem cells. Importantly, the decline in the stem cells was even more apparent than in aged fish. The controversy and debate over the use of GH as an anti-aging therapy have been going on for several years. In this study, excess GH induced aging signs such as increased senescence-associated (SA)-β-galactosidase staining of abdominal skin and similarity of the pattern of gene expression between aged and acromegaly zebrafish. Thus, this study highlights the role of excess GH in acromegaly stem cells.

P 053: Pre-clinical identification of drugs targeting POLG disorders by using a zebrafish/yeast trans-species approach (ZIPPY)

TOPIC: DISEASE MODELS

Nicola Facchinello[1], Claudio Laquatra[2], Lisa Locatello[1], Giorgia Beffagna[3], Raquel Branas Casas[1], Chiara Fornetto[4], Laura Martorano[1], Andrea Vettori[5], Giovanni Risato[3], Rudy Celeghin[1], Alberto Dinarello[1], Agnes Delahodde[6], Francesco Vanzi[4], Andrea Rasola[2], Luisa Dalla Valle[1], Maria Berica Rasotto[1], Tiziana Lodi[7], Enrico Baruffini[7], Francesco Argenton[1], Natascia Tiso[1]

1 *University of Padova, Department of Biology, Padova, Italy*
2 *University of Padova, Department of Biomedical Sciences, Padova, Italy*
3 *University of Padova, Department of Cardio-Thoraco-Vascular Sciences and Public Health, Padova, Italy*
4 *University of Florence, European Laboratory for Non-Linear Spectroscopy, Florence, Italy*
5 *University of Verona, Department of Biotechnologies, Verona, Italy*
6 *Universite Paris-Sud Universite Paris-Saclay, Institute for Integrative Biology of the Cell I2BC, Gif-sur Yvette, France*
7 *University of Parma, Department of Chemistry Life Sciences and Environmental Sustainability, Parma, Italy*

ABSTRACT TEXT

In humans, the mitochondrial DNA (mtDNA) is replicated by the DNA polymerase gamma (POLG), encoded by a nuclear gene. Mutations in POLG cause a set of mitochondrial diseases with Mendelian inheritance, collectively named POLG-related disorders, characterized by mtDNA depletion or accumulation of multiple deletions. To date, more than 300 pathogenic mutations have been reported in the Human DNA POLG Mutation Database. The mutations are associated with a spectrum of clinical presentations, ranging from infantile-onset epilepsies, liver failure, polyneuropathy, ataxia, dilated/hypertrophic cardiomyopathy to late-onset ophthalmoplegia and muscle weakness. To a limited extent, clinical phenotypes correlate with the mtDNA phenotype. The therapeutic treatment of POLG diseases is currently limited to symptom management.

Taking advantage of simple and cost-effective models, represented by the unicellular yeast and the invertebrate *C. elegans*, we have already identified a panel of yeast/worm pre-screened drugs; in parallel, we have generated Polg mutants in the vertebrate zebrafish (zf), faithfully modelling the human condition.

The main goal of our study is the identification of drugs with therapeutic effects on POLG mitochondrial pathologies. We are adopting a multi-species approach, based on drug pre-screen in Polg-deficient yeast (mip1) and *C. elegans* (polg-1) strains, followed by drug validation in zf Polg mutants. The screen of drugs in yeast is performed by evaluating the rescue of respiratory growth defects due to mtDNA instability ("petite" phenotype) in mip1 mutants. More in-depth analysis includes quantitative evaluation of Mip1 expression, respiratory activity and mtDNA levels. Positive hits are then analyzed in zf Polg models, evaluating the rescue of pathological phenotypes, including mtDNA depletion, impaired respiratory activity and altered mt-nucleus retrograde signaling. Preliminary results of this screen will be illustrated in the presentation of the study.

P 054: A novel laser-induced lesion paradigm to image osteoblast - immune cell interactions in vivo

TOPIC: DISEASE MODELS

Karina Geurtzen[1], Isabell Weber[2], Franziska Knopf[1]

1 *Center for Regenerative Therapies Dresden CRTD and Center for Healthy Aging, TU Dresden, Dresden, Germany*
2 *Boston Consulting Group, Boston Consulting Group, München, Germany*

ABSTRACT TEXT

Crosstalk between bone and immune cells, in particular osteoblasts and macrophages, has the potential to trigger bone formation and regeneration but also to impair both processes, if disturbed. In vivo models to image such interactions in real time are sparse. Zebrafish have become a powerful model to study bone formation and regeneration, also in the context of inflammation and leukocyte recruitment. Zebrafish larvae develop bones comparably fast, which makes them ideally suited to perform live cell imaging of osteoblasts and leukocytes in developing skull bones. Here, we report on a novel laser-induced lesion paradigm to image osteoblast-immune cell interactions in a developing skull bone in zebrafish in vivo.

Using the knowledge that macrophages get attracted by cell debris, we established a sterile wounding assay in the larval opercular bone, in which approx. 10 % of osteoblasts are killed by laser-assisted cell ablation. Recovery of the osteoblast population occurs by proliferation of the remaining cells within a single day. Using spinning disc confocal microscopy, we tracked the immediate response of macrophages which migrated into the site of ablation within minutes. A significant proportion of the respective cells display an inflammatory phenotype, characterized by enhanced *tnf-a* and *irg1* expression. This recruitment of macrophages is potentially triggered by release of ROS at the injury site. Finally, we tested the recruitment of macrophages during pharmacological glucocorticoid exposure, which impairs bone formation and found reduced macrophage migration into the region of interest. Altogether, our novel lesion paradigm presents a valuable tool to further study the interaction between bone and immune cells, and can be used to elucidate the signals driving appropriate and disturbed macrophage recruitment in vivo.

P 055: Renoprotective role of prostaglandin reductase 2 (PTGR2)

TOPIC: DISEASE MODELS

Aikaterini Kourpa[1], *Reinhold Kreutz*[2,3], *Daniela Panáková*[1,3]

[1] *Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Cardiovascular and Metabolic Disease, Berlin, Germany*
[2] *Institute of Clinical Pharmacology and Toxicology, Berlin Institute of Health, Berlin, Germany*
[3] *DZHK German Centre for Cardiovascular Research, Partner site Berlin, Berlin, Germany*

ABSTRACT TEXT

Prostaglandins and particularly prostaglandin E_2 (PGE_2) play a crucial role in the initiation and progression of kidney inflammation and chronic kidney disease (CKD). Physiologically, PGE_2 increases glomerular filtration barrier (GFB) permeability leading to proteinuria and its upregulation in podocytes is linked to increased fluid flow shear stress (FFSS) as observed during glomerular hyperfiltration.

Our previous studies in Munich Wimstar Frömter (MWF) rat model suggest PTGR2 as a novel potential candidate for proteinuria development. PTGR2 is an important enzyme involved in the prostaglandin pathway as it terminally inactivates PGE_2 signaling. The mechanisms of how PTGR2 and particularly its substrate 15-keto-PGE_2 contribute to the initiation and/or progression of CKD are currently unclear. Moreover, whether this inactivation pathway can be involved in renal signaling remains unexplored.

Our preliminary studies in zebrafish embryos demonstrate the loss of *ptgr2* leading to a functional permeability defect in GFB, thus mimicking a proteinuria-like phenotype. In addition, reduced *ptgr2* levels cause profound morphological changes to the pronephros. Altogether, our data suggest a developmental role for PTGR2 in kidney morphogenesis and support the hypothesis PTGR2 may function renoprotectively in CKD. Thus, the PGE_2/15-keto-PGE_2/PTGR2 pathway may be a potential novel target for renoprotection.

P 056: Targeted knockout of GABA-A receptor gamma 2 subunit provokes transient light-induced reflex seizures in zebrafish larvae

TOPIC: DISEASE MODELS

Uday Kundap[1], *Meijiang Liao*[1], *Richard E. Rosch*[2], *Dominic R. W. Burrows*[2], *Martin P. Meyer*[2], *Bouchra Ouled Amar Bencheikh*[1], *Patrick Cossette*[1], *Eric Samarut*[1]

[1] *The University of Montreal Hospital Research Center CRCHUM, Department of Neuroscience, Montreal, Canada*
[2] *MRC Centre for Neurodevelopmental Disorders- Institute of Psychiatry- Psychology and Neuroscience- King's College London., Department for Developmental Neurobiology, London, United Kingdom*

ABSTRACT TEXT

Epilepsy is a common primary neurological disorder characterized by the chronic tendency of a patient to experience epileptic seizures, which are abnormal body movements or cognitive states that result from excessive, hypersynchronous brain activity. Epilepsy has been found to have numerous etiologies and, although about two-thirds of epilepsies were classically considered idiopathic, the majority of those are now believed to be of genetic origin. Mutations in genes involved in gamma-aminobutyric acid (GABA)-mediated inhibitory

neurotransmission have been associated with a broad range of epilepsy syndromes. Mutations in the GABA-A receptor gamma 2 subunit gene (*GABRG2*), for example, have been associated with absence epilepsy and febrile seizures in humans. Several rodent models of *GABRG2* loss of function depict clinical features of the disease; however, alternative genetic models more amenable for the study of ictogenesis and for high-throughput screening purposes are still needed. In this context, we generated a *gabrg2* knockout (KO) zebrafish model (which we called R23X) that displayed light/dark-induced reflex seizures. Through high-resolution *in vivo* calcium imaging of the brain, we showed that this phenotype is associated with widespread increases in neuronal activity that can be effectively alleviated by the anti-epileptic drug valproic acid. Moreover, these seizures only occur at the larval stages but disappear after 1 week of age. Interestingly, our whole-transcriptome analysis showed that *gabrg2* KO does not alter the expression of genes in the larval brain. As a result, the *gabrg2*$^{-/-}$ zebrafish is a novel *in vivo* genetic model of early epilepsies that opens new doors to investigate ictogenesis and for further drug-screening assays.

P 057: A role for Btbd9 in hepatic steatosis

TOPIC: DISEASE MODELS

So-Hyun Lee[1], *Seok-Yong Choi*[1], *Myeong-Kyu Kim*[2]

[1] *Chonnam National University Medical School, Department of Biomedical Sciences, Gwangju, Republic of Korea*
[2] *Chonnam National University Medical School, Department of Neurology, Gwangju, Republic of Korea*

ABSTRACT TEXT

Restless legs syndrome (RLS) affects approximately 10 % of the general population and causes neurological sleep disorder that makes a strong urge to move legs. Individuals predisposed to iron deficiency anemia, neuropathy, and renal insufficiency are more susceptible to RLS. However, there have been no report on the association between RLS and liver disease to date. BTB/POZ domain-containing protein 9 *(btbd9)* has been reported to be associated with RLS in human. We found that *btbd9* is expressed in the zebrafish liver. To explore a role for Btbd9 in zebrafish, we generated *btbd9* mutant by the CRISPR/Cas9 system. To our surprise, RNA-Seq analysis revealed a significant difference in the expression levels of genes associated with lipid metabolism between WT and *btbd9* mutant. Adult *btbd9*$^{-/-}$ zebrafish showed significantly enlarged liver when compared to WT, suggesting a direct link between *btbd9* and liver pathology. In addition, H&E staining showed severe hepatic steatosis in *btbd9*$^{-/-}$ liver. Taken together, these data suggests a novel role for Btbd9 in liver pathogenesis. We are currently exploring a molecular link between Btbd9 and liver disease.

P 058: Zebrafish as a model organism of human cardiovascular disease

TOPIC: DISEASE MODELS

Monika Maciąg[1], *Artur Wnorowski*[1], *Maciej Maj*[1], *Anita Płazińska*[1]

[1] *Medical University of Lublin, Department of Biopharmacy, Lublin, Poland*

ABSTRACT TEXT

Cardiovascular diseases are a leading cause of mortality globally. Therefore, searching efficacious new therapies is an important and intense area of research. Recently, the use of zebrafish model in preclinical studies has greatly increased. The time- and cost effective assays make larval zebrafish as a perfect platform for high-throughput *in vivo* study of complex processes.

So far, a few cardiotoxic drugs have been proposed as zebrafish-based heart failure models. Doxorubicin, β-adrenergic agonists, and terfenadine are characterized by well-documented cardiotoxicity. Therefore, the aim of our study was to uncover how zebrafish heart responds to cardiotoxic treatment, and then determine whether human medications are able to protect from heart abnormalities and may manage heart dysfunction in zebrafish.

Zebrafish were exposed to cardiotoxic drugs for 96 hours post fertilization. The compounds were compared in respect to the elicited mortality, changes in heart rate and morphologic alterations. All of tested compounds display concentration-dependent heart rate inhibition, however in different range of concentrations. Intriguingly, aristolochic acid-treatment was highly toxic and the most potent at suppressing heart rate.

Here, we try to answer one of the most fundamental question about whether zebrafish heart responds to cardiotoxic and cardioprotective drugs in the same as humans. And it may and should therefore be applied for investigation of novel therapeutic strategy to treat cardiac diseases.

The authors acknowledge financial support from the National Science Centre, Poland (Grant 2017/25/B/NZ7/02654).

P 059: Esco2 regulates migration of neural crest cells

TOPIC: DISEASE MODELS

Valeria Mastrodonato[1], *Ilaria Costa*[1], *Federica Zanardi*[1], *Fabio Iannelli*[1], *Gianluca Deflorian*[1], *Dana Branzei*[1]

[1] *IFOM, FIRC Institute of Molecular Oncology, Milan, Italy*

ABSTRACT TEXT

Cohesin is a multiprotein complex required for sister chromatid cohesion, chromatin organization and gene transcription regulation. Cohesin structural components include SMC1A, SMC3, RAD21 and SA1/SA2, which enclose DNA strands in a ring-like structure. During G1 phase, NIPBL and MAU-2 ensure cohesin loading onto chromatin, while during mitosis cohesin is removed by WAPL, to allow sister chromatids separation. A key role in cohesion establishment is played by ESCO1 and ESCO2 during S phase. In particular, ESCO2 is an acetyltransferase that promotes sister chromatin cohesion establishment via acetylation of SMC3 residues. Point mutations in human *ESCO2* gene lead to Roberts syndrome (RBS), a rare autosomal recessive disorder characterized by growth retardation, microcephaly, craniofacial malformations, limb development defects and low rate survival after birth. An *esco2* mutant generated by retroviral insertion in zebrafish (*Percival et al., 2015*) recapitulates phenotypic RBS traits, such as severe central nervous system and heart defects, craniofacial abnormalities and fin development impairment. Considering that neural crest give rise to diverse cellular derivatives such as neurons, cartilages, bones and cardiomyocytes, all affected by the depletion of Esco2, we asked whether Roberts syndrome could be considered also a neurocristopathy. RNAseq performed on 24 hpf *esco2* mutant embryos revealed a significant downregulation of neural crest related genes, such as *sox9a*, *sox11a*, *semaphorin6* and *sonic hedgehog* related genes. Moreover, time-lapse performed on transgenic line *sox10:mRFP* showed impaired neural crest migration in *esco2* mutant. In the future, we aim to identify novel interactors to shed light on the involvement of Esco2 in neural crest development and migration.

P 060: Development of zebrafish with a mutation in coasy gene as a possible model for COPAN, a rare neurodegenerative disorder

TOPIC: DISEASE MODELS

Luca Mignani[1], *Ram Basnet*[1], *Paola Agazzi*[1], *Giuseppe Borsani*[1], *Daniela Zizioli*[1], *Dario Finazzi*[1]

[1] *Università degli Studi di Brescia, Dipartimento di Medicina Molecolare e Traslazionale, Brescia, Italy*

ABSTRACT TEXT

CoASY gene encodes for COASY, a bifunctional enzyme that catalyses the last two steps in the biosynthesis of Coenzyme A (CoA) from pantothenic acid. These activities are performed by two separate domains, phosphopantetheine adenylyltransferase that coverts 4'-phosphopantetheine into dephospho-coenzyme A (dpCoA) and dephospho-CoA kinase that phosphorylates dpCoA to form CoA. Mutations in *CoASY* are associated with the COPAN disease that belongs to a cluster of disorders named Neurodegeneration with Brain Iron Accumulation (NBIA). These disorders are characterized by excessive iron accumulation in the brain, particularly affecting the basal ganglia.

Due to the high homology between human and zebrafish protein sequences, the lack of specific and fruitful animal models and the limited amount of molecular details describing the pathogenesis of the disease, we decided to generate a Knock Out animal model for *coasy*. We took advantage of the CRISPR-Cas9 technique to induce locus specific mutations on the gene. We managed to get chimaeric founders that passed to F1 generation two different mutations, a 10-bp mutation (Δ10), a 10- bp indel (deletion of 2 bp, insertion of 12 bp, IN10).

We incrossed IN10 fishes and we analysed two different pulls of circa 50 embryos, at 5 dpf, and surprisingly we were able to find homozygous embryos. This result was confirmed by different assays (HMA, restriction digestion) and by Sanger sequencing. The presence of homozygous embryos at 5 dpf suggests the possibility to obtain Knock Out fishes for *coasy*, which is in contrast with the expectation of embryonic lethality. We started a preliminary embryonic characterization from F1 incross. Furthermore, we are growing F2 fishes, for both the mutations, to evaluate the possibility of these KO embryos to survive and become adults.

Our findings suggest the possibility to obtain an animal model for COPAN that can highlight the pathogenesis and the mechanism at the basis of this still unclear disease.

P 061: The muscle glycogen phosphorylase (Pygm) knockdown leads to structural changes in zebrafish skeletal muscles

TOPIC: DISEASE MODELS

Marta Migocka-Patrzałek[1], Anna Lewicka[1], Magdalena Elias[1], Małgorzata Daczewska[1]

[1] *University of Wroclaw, Department of Animal Developmental Biology, Wroclaw, Poland*

ABSTRACT TEXT

The metabolic enzyme, muscle glycogen phosphorylase (PYGM), plays an important role in the glycogenolysis. Mutation in the PYGM gene leads to glycogen storage disease type V called also McArdle disease (#232600 in the OMIM database). The clinical symptoms include physical exercise intolerance (premature fatigue), muscle cramps, myalgia, a high level of creatine kinase in blood and transient myoglobinuria. Patient's muscle tissue is altered, with large vacuoles with glycogen inside. So far, no efficient treatment has been found.

Zebrafish is an effective animal model of several human muscle diseases (Plantié et al., 2015, Molecules 20(4):6237-53, doi: 10.3390/molecules20046237). The main goal of our studies was to investigate whether zebrafish could be also used to generate a model of McArdle disease. The two forms of the zebrafish (*Danio rerio*) enzyme, Pygma and Pygmb, share more than 80 % amino acid sequence identity with humans. We show that the Pygm mRNA and protein level are correlated with glycogen level in distinct stages of zebrafish development. The simultaneous *pygma* and *pygmb* morpholino knockdown resulted in a reduced Pygm level in zebrafish morphants, which exhibited altered, disintegrated muscle structure and accumulation of glycogen granules in the subsarcolemmal region. In our studies we show morphological, structural and biochemical abnormalities of animals with Pygm knockdown. Our data indicate that the zebrafish model of McArdle disease might contribute to further understanding of its molecular mechanisms and leads toward development of effective treatment (Migocka-Patrzalek et al. 2020, Int J Biochem Cell Biol, 118, doi.org/10.1016/j.biocel.2019.105658).

Acknowledgement: National Science Centre (grant no. 2017/01/X/NZ4/00093), Polish State Committee for Scientific Research, Project No. 1068/S/IBE/2018.

P 062: Deficiency of the glutamate transporter eaat2a leads to spontaneous epileptic seizures in larval zebrafish

TOPIC: DISEASE MODELS

Stephan Neuhauss[1], Hotz Adriana[1], Stephanie Niklaus[1], Sverre Myren-Svelstad[2], Laetitia Lalla[2], Jurisch-Yaksi Nathalie[2], Emre Yaksi[2]

[1] *University of Zurich, Department of Molecular Life Sciences, Zurich, Switzerland*

[2] *Norwegian Institute of Science and Technology, Kavli Institute for Systems Neuroscience- Department of Neuromedicine and Movement Science, Trondheim, Norway*

ABSTRACT TEXT

Epileptic seizures are the result of excessive synchronized neuronal activity across the central nervous system. Excessive excitatory signaling of glutamate may cause epilepsy. Under physiological conditions, glutamate is removed from the synaptic cleft primarily by the excitatory amino acid transporter 2 (EAAT2). Malfunctioning of this transporter could lead to glutamate accumulation and subsequent prolonged neuronal excitation. Here we show that knockout (KO) of the paralogue EAAT2a in zebrafish leads to distinct epileptic features in larvae.

First, homozygous mutant larvae display specific epileptic behavior, represented by bursts of rapid swimming and swirling around their own body axis. Moreover, by neuronal and glial calcium imaging we observed sudden episodes of excessive activity spreading over the whole brain, suggesting that EAAT2a-deficient animals suffer from generalized seizures. By studying cellular activity in-depth, we found

reduced neuronal baseline activity and a potential loss of glial function. Our findings therefore open up new possibilities of finding contributing factors and mechanisms of epilepsy, implicating glial function in epileptogenesis.

P 063: Dynamin-related GTPase, Drp1, is required for BNip1-mediated photoreceptor apoptosis in zebrafish

TOPIC: DISEASE MODELS

Yuko Nishiwaki[1], *Mamoru Fujiwara*[1], *Ichiro Masai*[1]

[1] *Developmental Neurobiology Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan*

ABSTRACT TEXT

In zebrafish, a BH3-only SNARE protein, BNip1, induces photoreceptor apoptosis in response to vesicular fusion defects (Nishiwaki et al. (2013) *Dev Cell* 25, 374-387). BNip1 functions as a t-SNARE component of syntaxin18 complex, is localized on ER membrane and regulates retrograde transport from Golgi to ER. BNip1 also has a BH3 domain, which releases pro-apoptotic protein Bax from Bcl2-mediated inhibition. We previously reported that retinal photoreceptors undergo BNip1-dependent apoptosis in zebrafish *β-SNAP1* mutants. BNip1 pro-apoptotic activity is activated only when BNip1 forms the syntaxin18 cis-SNARE complex. Since *β-SNAP1* promotes disassembly of this cis-SNARE complex during vesicular fusion process, the current our model is that BNip1 pro-apoptotic activity is activated on ER membrane. To confirm this, we overexpressed ER-targeted Bcl2 in *β-SNAP1* mutants. Consistently, overexpression of ER-targeted Bcl2 significantly inhibited photoreceptor apoptosis in zebrafish *β-SNAP1* mutants, suggesting that the interaction of BNip1 with Bcl2 on ER is the first step of apoptotic pathway. However, it remains to be elucidated how BNip1 on ER transfer the apoptotic signal to mitochondria. It was reported that formation of ER-mitochondria contact is required for apoptosis. Drp1 is a key molecule to form ER-mitochondria contact site as a ground for mitochondria fission and apoptosis. We found that knockdown of Drp1 significantly inhibits photoreceptor apoptosis in *β-SNAP1* mutants. These data suggest that Drp1 mediates BNip1-mediated apoptotic pathway probably through the formation of ER-mitochondria contact.

P 064: Deeply Conserved Cardiac Enhancers Guide Zebrafish Heart Regeneration

TOPIC: DISEASE MODELS

Chris Onderisin[1,2], *Xuefei Yuan*[1,2], *Rifat Sajid*[1], *Ian C. Scott*[1,2]

[1] *University of Toronto, Dept. of Molecular Genetics, Toronto, Canada*

[2] *The Hospital For Sick Children, Developmental & Stem Cell Biology, Toronto, Canada*

ABSTRACT TEXT

The cardiac ventricle is comprised of three cardiomyocyte (CM) layers: primordial, trabecular, and cortical. CM layers behave differently following ventricular apex resection, with replenishment of cortical CMs occurring prior to restoration of primordial CMs. The mechanisms that drive these CM-subtype specific behaviours remain elusive, though activities of specific cardiac enhancer elements may play a role in guiding such differences. Using ATAC-seq, we previously identified several evolutionarily conserved, accessible chromatin regions in cardiac progenitor cells isolated from late-gastrula stage embryos. Here, we report the activities of these Accessible Conserved Noncoding Elements (aCNEs), under homeostatic and post-injury conditions. aCNE:GFP reporters display spatially restricted domains of activity, marking specific myocardial and non-myocardial tissues. Three aCNEs show GFP expression proximal to the injury border-zone; whereas aCNEs 15 & 21 (*prox1*- and *hey2*- proximal enhancers, respectively) broadly mark multiple cardiac compartments pre- and post-injury, aCNE 1 (*hand2*-proximal) activity within the myocardium is specifically expanded from primordial-only to trabecular layers following injury. aCNE 1 activity is maintained at the wound from 7–60 days-post-injury, and loss of both copies of the aCNE 1 enhancer result in moderate to severe cardiac regeneration defects. Together with inducible lineage tracing data showing contribution of aCNE 1+ cells to all CM layers during development, these findings highlight the dynamic nature of cardiac enhancer elements during cardiac development & regeneration, giving further credence to the role of CM-regeneration Enhancer Elements (CREEs) in directing heart repair.

P 065: Modeling Spinocerebellar Ataxia-16 in zebrafish: U-box domain knockout of stub1 gene affects Purkinje neuron morphology and leads to behavioural changes

TOPIC: DISEASE MODELS

Yasaman Pakdaman[1], *Elsa Denker*[2], *Eirik Austad*[2], *Will Norton*[3], *Ingvild Aukrust*[1], *Stefan Johansson*[1], *Per Morten Knappskog*[4], *Ståle Ellingsen*[2]

[1] *University of Bergen, Department of Clinical Science, Bergen, Norway*
[2] *University of Bergen, Department of Biological Sciences, Bergen, Norway*
[3] *University of Leicester, Department of Neuroscience- Psychology and Behaviour, Leicester, United Kingdom*
[4] *Haukeland University Hospital, Department of Medical Genetics, Bergen, Norway*

ABSTRACT TEXT

Introduction: Spinocerebellar ataxia-16 (SCA16) is an autosomal recessive neurodegeneration disorder that develops due to deficiencies in the *STUB1* gene encoding the ubiquitin E3 ligase and co-chaperone CHIP. There is limited knowledge regarding the pathogenic role of mutant CHIP *in vivo*. This study aims to characterize *stub1* in wild-type zebrafish and generate a zebrafish model for SCA16 by CRISPR/Cas9-mediated mutagenesis of zebrafish *stub1*.

Material and Methods: The expression and localization patterns of wild-type Chip and its substrates were explored in the adult zebrafish brain by immunohistochemistry. Chip expression levels were also determined in various zebrafish tissues by droplet digital PCR. CRISPR/Cas9 was used to generate a stable mutant zebrafish line with a 28 amino acid deletion in the functional U-box domain of Chip. Homozygous mutant offspring were examined for development of ataxia-related physiological and behavioural phenotypes.

Results: Stub1 transcripts were found in a wide range of tissues, being predominantly expressed in eggs, testis, and brain. Immunohistochemistry studies demonstrated localization of Chip together with its interacting proteins in the Purkinje cells of the cerebellum. Initial characterization of homozygous mutant fish indicated irregular morphology and organization of the Purkinje cells as well as changes in the anxiety-oriented behaviours.

Conclusions: We have characterized wild-type Chip in adult zebrafish, and established a *stub1* mutant zebrafish line which provides a useful model system to investigate the role of the Chip U-box domain in SCA16.

P 066: Smarce1, a component of SWI/SNF complex controls cardiomyocyte proliferation in zebrafish

TOPIC: DISEASE MODELS

Deung-Dae Park[1], *Isabelle Schneider*[1], *Wolfgang Rottbauer*[1], *Steffen Just*[1]

[1] *Ulm University Medical Center- Internal Medicine II, Molecular Cardiology, Ulm, Germany*

ABSTRACT TEXT

In mammals, myocardial infarction leads to the formation of an irreversible fibrotic scar. In contrast to humans, the adult zebrafish heart can regenerate after myocardial infarction. The impressive regenerative capacity of the zebrafish heart is owing to the maintenance of a robust proliferative competence of adult cardiomyocytes (CMs). Regeneration is mediated by cell cycle re-entry of existing CMs rather than by the recruitment of cardiac stem cells for rapid regeneration. But the mechanisms controlling and inducing CMs dedifferentiation and re-entry of the cell cycle are still unknown.

Heart of stone (*hos*) is an ENU-induced mutant zebrafish line displaying defective embryonic heart growth. By positional cloning, we identified the causative gene mutation within the evolutionary highly conserved SWI/SNF-associated complex member Smarce1. SWI/SNF complex is a chromatin remodeling complex crucial for the proper development of all studied organisms. A major role of the complex is to enable the binding of transcription factors to genomic DNA by loosening condensed nucleosomes in an ATP-dependent manner. Homozygous *hos* mutants can be discriminated from their wild-type (wt) siblings by a severely thickened ventricular wall and an almost complete obliteration of the ventricular lumen at 96 hours post fertilization (hpf). We found significantly increased CM proliferation in *hos* mutant embryos compared to wt. Interestingly, an increased CM proliferation was first detected starting at 96 hpf. To define the underlying molecular mechanism in *hos* mutants, we next assessed transcriptional profiles by RNA-sequencing and analyzed DEGs from these data.

We found altered expression patterns of important cell cycle regulators in *hos* mutants compared to wt, and validated these results by cardiac-specific qRT-PCR and Western Blot. In summary, our findings suggest a crucial role of the SWI/SNF complex in the regulation of cardiomyocyte proliferation and potentially also cardiac regeneration.

P 067: Profiling the cis-regulatory landscapes of the zebrafish endocrine pancreas

TOPIC: DISEASE MODELS

Chiara Perrod[1]

[1] *i3S - Instituto de Investigação e Inovação em Saúde, Vertebrate Development and Regeneration, Porto, Portugal*

ABSTRACT TEXT

Within the pancreas, the endocrine compartment is responsible for glucose homeostasis, which is compromised in diabetes. Spatio-temporally regulation of gene expression is required for proper pancreas development and function. It has been shown that many disease-associated mutations are located in non-coding regions, many overlapping with cis-regulatory elements (CREs), highlighting the importance to investigate the role of CREs on transcriptional regulation and their implication in disease.

Here, we performed ChIP-seq to identify CREs in adult zebrafish endocrine pancreas by characterising the active chromatin regions (H3K27ac and H3K4me3). By comparing these data with available human profiles we will seek for functional orthologs CREs. Importantly, we observed high similarities in the epigenetic landscapes of genes crucial for endocrine pancreatic function.

To further fine-tune our screening, we will merge these results with *in vivo* binding of a cluster of key transcription factors (PDX1, FOXA2, NKX2.2, NKX6.1 and MAFB), known to cooperatively bind to CREs that control human endocrine pancreas genes. Due to the lack of specific antibodies for zebrafish proteins, we are generating knock-in lines tagging these transcription factors with an HA epitope using CRISPR-Cas9 driven homologous direct repair (HDR). Up to now, we successfully targeted the *nkx2.2a* locus.

Altogether, these results will lead to the identification of the zebrafish CREs in endocrine pancreas, thus providing a powerful and easy-to-edit platform to investigate the causative role of CREs mutations in diabetes by *in vivo* functional assays.

P 068: A new zebrafish model for Pseudoxanthoma elasticum (PXE)

TOPIC: DISEASE MODELS

Klaudia Porok[1], *Dávid Czimer*[1], *Viktória Lavró*[2], *Zsolt Gyüre*[1], *Krisztina Fülöp*[3], *Zelin Chen*[4], *Shawn M. Burgess*[4], *András Váradi*[3], *Máté Varga*[1]

[1] *ELTE Eötvös Loránd University, Department of Genetics, Budapest, Hungary*
[2] *University College London, Division of Biosciences, London, United Kingdom*
[3] *RCNS, Institute of Enzymology, Budapest, Hungary*
[4] *National Human Genome Research Institute, Translational and Functional Genomics Branch, Bethesda, USA*

ABSTRACT TEXT

Calcification of various tissues is a serious global issue, which has been associated with aging, cancer and autoimmune diseases. There are both environmental and genetic factors behind this phenomenon and exploring and understanding them is essential for the development of efficient therapeutic techniques. Pseudoxanthoma elasticum (PXE) is a rare genetic disease, a prototype of calcification disorders, resulting from the dysfunction of ABCC6, a transport protein found in the membranes of cells. It is characterized by calcification in various tissues (e.g. eyes, skin, arteries) and currently it has no cure, treatments target symptoms only. Preclinical studies of PXE have been successful in mice, proving the usefulness of animal models for the study of the disease.

Here we present a new zebrafish (*Danio rerio*) model for PXE. By resolving some ambiguous assemblies in the zebrafish genome, we show that there are two functional and one non-functional paralogs for *ABCC6* in zebrafish (*abcc6a*, *abcc6b.1* and *abcc6b.2*, respectively). We created single and double mutants for the functional paralogs and characterized their calcification defects with a combination of techniques. Our results support a role for *abcc6a* in zebrafish calcification and suggest that impairment of *abcc6b.1* does not affect this biological process. On the other hand, *abcc6b.1* loss-of-function results in considerable shorter lifespan.

P 069: Generating a CRISPR/Cas9 zebrafish model to unravel SMA pathogenesis and for drug discovery

TOPIC: DISEASE MODELS

Marilou Préville[1], *Charlotte Zaouter*[1], *Kessen Patten*[1]

[1] *INRS, Institut Armand-Frappier Santé Biotechnologie, Laval, Canada*

ABSTRACT TEXT

Spinal Muscular Atrophy (SMA) is a rare autosomal recessive neurodegenerative disease. It is the leading genetic cause of infant mortality for which there is currently no effective cure. SMA is characterized by a degeneration of lower alpha motoneurons of the spinal cord caused by the deletion of *SMN1* gene on chromosome 5q13 leading to muscle weakness and atrophy. Although SMN is ubiquitously expressed, the mechanism underlying the degeneration of specifically the motoneurons remains poorly understood. Morpholino oligonucleotides based knockdown are commonly used in zebrafish to study SMA. However, the specificity of this technique is still controversial. Thus, we aim to generate a more reliable and stable model to study SMA. Using the CRISPR/Cas9 system, we targeted the zebrafish smn gene to create a knockout (KO) model. We successful identified a zebrafish *smn* KO mutant. These fish showed a significant reduced locomotion compared to controls. SMN protein levels were also significantly reduced in our *smn* zebrafish mutants. We are currently characterizing further our CRISPR/Cas9 model. Moreover, this model will be used for high throughput drug screening. In the long-term, our study may help find new avenues for therapeutic strategies in SMA.

P 070: Glycine decarboxylase deficiency-induced motor dysfunction in zebrafish is rescued by counterbalancing glycine synaptic level

TOPIC: DISEASE MODELS

Riché Raphaëlle[1], *Meijiang Liao*[1], *Pena Izabella*[2], *Leung Kit-Yi*[3], *Lepage Nathalie*[2], *Greene Nicolas*[3], *Sarafoglou Kyriakie*[4], *Drapeau Pierre*[1], *Schimmenti Lisa*[5], *Eric Samarut*[1]

[1] *CRCHUM, Neurosciences, Montreal, Canada*
[2] *CHEO, Pediatrics, Ottawa, Canada*
[3] *UCL, Developmental Biology, London, United Kingdom*
[4] *UMN, University of Minnesota Masonic Children's Hospital, Minneapolis, USA*
[5] *Mayo Clinic, School of Medicine, Rochester, USA*

ABSTRACT TEXT

Glycine encephalopathy (GE), or nonketotic hyperglycinemia (NKH), is a rare recessive genetic disease caused by defective glycine cleavage and characterized by increased accumulation of glycine in all tissues. Here, based on new case reports of GLDC loss-of-function mutations in GE patients, we aimed to generate a zebrafish model of severe GE in order to unravel the molecular mechanism of the disease. Using CRISPR/Cas9, we knocked out the gldc gene and showed that gldc–/– fish recapitulate GE on a molecular level and present a motor phenotype reminiscent of severe GE symptoms. The molecular characterization of gldc–/– mutants showed a broad metabolic disturbance affecting amino acids and neurotransmitters other than glycine, with lactic acidosis at stages preceding death. Although a transient imbalance was found in cell proliferation in the brain of gldc–/– zebrafish, the main brain networks were not affected, thus suggesting that GE pathogenicity is mainly due to metabolic defects. We confirmed that the gldc–/– hypotonic phenotype is due to NMDA and glycine receptor overactivation, and demonstrated that gldc–/– larvae depict exacerbated hyperglycinemia at these synapses. Remarkably, we were able to rescue the motor dysfunction of gldc–/– larvae by counterbalancing pharmacologically or genetically the level of glycine at the synapse.

P 071: Zebrafish models for Arrhythmogenic Cardiomyopathy: Opportunities for the identification of early pathogenetic events and new therapeutic targets

TOPIC: DISEASE MODELS

Giovanni Risato[1], Rudy Celeghin[2], Mila Della Barbera[1], Marco Cason[1], Maria Bueno Marinas[1], Kalliopi Pilichou[1], Francesco Argenton[2], Gaetano Thiene[1], Cristina Basso[1], Natascia Tiso[2], Giorgia Beffagna[1]

[1] *University of Padua, Department of Cardiac-Thoracic-Vascular Sciences and Public Health, Padua, Italy*

[2] *University of Padua, Department of Biology, Padua, Italy*

ABSTRACT TEXT

Background: Arrhythmogenic Cardiomyopathy (AC) is an inherited heart disease characterized by progressive fibro-fatty substitution of the myocardium, leading to ventricular arrhythmias and high risk of sudden cardiac death in young and athletes. Despite the discovery of causative genes, early molecular events leading to tissue damage and arrhythmias remain elusive. The AC form linked to the junctional protein Desmoplakin (Dsp) is the most challenging AC type, being less easily identifiable using classical ECG and echocardiographic tools.

Purpose: The aim of our study is the characterization of stable KO zebrafish (zf) AC models to identify *in vivo* early pathogenic events leading to the onset of the disease due to Dsp dysfunction. The final goal is the assessment of our zf AC models as suitable tools to evaluate the role of the physical exercise and test the efficacy of pathway-directed drugs.

Methods: Our zf models include a Dspa mutant line obtained by ENU mutagenesis, a Dspb mutant line, generated by CRISPR/Cas9 strategy, and Dspa/Dspb double mutants. All AC models were characterized at embryonic, larval and adult stage by confocal and electron microscopy (TEM), heart rhythm measurement, q-PCR and WB.

Results: At embryonic and larval stages, our models display altered heart rate and pericardial effusion. Moreover, the analysis of signaling pathways detects a cardiac-specific reduction (80 %) of Wnt/Beta-catenin signaling. Adult fish exhibit an irregular heart morphology, mild bradycardia, cardiomegaly and pericardial effusion; about 1 % of the mutants dies suddenly starting from 3 months of age. H&E staining and TEM detected "pale" desmosomes, age-related reduced thickness and non-compact myocardium. Physical stress tests induced a 1.5-fold increase of the mortality in mutant larvae.

Conclusions: Our zf models recapitulate AC features pointing to zf as a suitable system for the *in vivo* screening of molecularly-targeted drugs under normal and stress-induced conditions.

P 072: Functional Studies of Undiagnosed Diseases Network (UDN) human gene variants

TOPIC: DISEASE MODELS

Jimann Shin[1], Ryan McAdow[1], Wyndham Ferris[1], Paul Gontarz[1], Andrzej Krezel[2], Julian A. Martinez-Agosto[3], Jennifer B. Phillips[4], Jeremy Wegner[4], Judy Peirce[4], John Postlethwait[4], Monte Westerfield[4], Lilianna Solnica-Krezel[1]

[1] *Washington University in St. Louis- School of Medicine, Department of Developmental Biology, Saint Louis, USA*

[2] *Washington University in St. Louis- School of Medicine, Department of Biochemistry and Molecular Biophysics, Saint Louis, USA*

[3] *Semel Institute for Neuroscience and Human Behavior David Geffen School of Medicine at UCLA, Department of Psychiatry, Los Angeles, USA*

[4] *University of Oregon, Institute of Neuroscience, Eugene, USA*

ABSTRACT TEXT

Combining technologies for genome editing and next-generation sequencing allow us to generate animal models to test candidate human disease variants with increasing efficiency. Conceptually, known or newly identified human genetic variants can be tested in model organisms by introducing same mutations into their genomes using genome editing tools. The clinical sites of the UDN identify candidate disease sequence variants in undiagnosed patients, submit the information to the UDN gateway database, and the multi-institutional Model Organism Screening Center (MOSC) tests the human variants in relevant model organisms. The Washington University Zebrafish-MOSC is currently modeling 9 UDN cases, including *BCL6 COREPRESSOR* (*BCOR)* and *Down Syndrome Cell Adhesion Molecule Like 1* (*DSCAML1*), submitted by the UCLA Clinical Site. Here, we present two ongoing cases of human variants modeled in zebrafish. 1) A novel 18 bp in-frame deletion p.G1464_R1469del in *BCOR*. Using CRISPR/Cas9 genome editing, we generated two frame-shift mutations and

a 21bp in-frame deletion mutation, which is highly similar to the human p.G1464_R1469del mutation. Surprisingly, we detected loss of dorsal and/or pelvic fins in two frame-shift mutants, and morphological abnormalities of dorsal and pelvic fins in fish homozygous for the in-frame deletion mutation. This supports a hypomorphic nature of the human p.G1464_R1469del mutation. 2) A *de novo* heterozygous mutation of p.V1237L in *DSCAML1* on X Chromosome. The genetic locus of p.V1237L variant is highly conserved in the zebrafish genome. Using CRISPR/Cas9 and prime editing technology, we generated p.V1237L knock-in as well as frame-shift and large deletion alleles. These zebrafish allelic mutants will be useful to understand the function of the human p.V1237L variant. Taken together, our data show that allelic series including humanized variants in zebrafish can help to test pathogenicity and understand the function of human genetic variants.

P 073: Practical applications of CRISPR knock in: generation of cacna1c mutant zebrafish

TOPIC: DISEASE MODELS

Ewa Sieliwonczyk[1], *Bert Vandendriessche*[1], *Maaike Alaerts*[1], *Dries Knapen*[2], *Lucia Vergauwen*[2], *Carine Moers*[2], *Dirk Snyders*[3], *Bart Loeys*[1], *Dorien Schepers*[1]

[1] *University of Antwerp, Center for Medical Genetics, Antwerp, Belgium*
[2] *University of Antwerp, Zebrafishlab, Antwerp, Belgium*
[3] *University of Antwerp, Laboratory for Molecular- Cellular and Network Excitability, Antwerp, Belgium*

ABSTRACT TEXT

Introduction: The similarity between the human and the zebrafish cardiac action potentials makes zebrafish an attractive model for cardiogenetic disorders. However, introducing precise base pair substitutions (knock in, KI) by the CRISPR/Cas9 technique in zebrafish remains challenging. In our efforts to generate a KI zebrafish model of the human *CACNA1c* c.2570C>G long QT syndrome mutation, we have combined early genotyping with next-generation sequencing (NGS) to improve the KI efficiency.

Methods: Zebrafish eggs were injected with Cas9 mRNA or protein, 1–2 gRNA and four different conformations of ssODN: identical ("target") or complementary ("non-target") to the gRNA binding sequence, symmetric or asymmetric. The detection of KI events was performed by NGS on a MiSeq instrument (Illumina). To select embryos for breeding, DNA was extracted from live embryos at 3 days post fertilization with the Zebrafish Embryo Genotyping device.

Results: Variable KI rates were observed, ranging from 0.1 to 1.58 %. The highest KI rate was observed with Cas9 mRNA with 1 gRNA and the right asymmetric target ssODN. Embryos from the Cas9 mRNA injection group containing >0.2 % KI reads were raised to maturity and crossed out to assess germline transmission. One out of 10 fish passed the edited allele on to 25 % of its offspring.

Conclusion: KI experiments in zebrafish show a high degree of variability. The KI efficiency tends to be lower in disease modeling than in methodological studies as the experiments are more restrictive. We intend to overcome these limitations by combining early genotyping with an NGS based detection technique.

P 074: Novel targets for atrial fibrillation disease mechanism using zebrafish model

TOPIC: DISEASE MODELS

Ilcim Thestrup[1], *Pia Rengtved Lundegaard*[1], *Morten Salling Olesen*[1]

[1] *University of Copenhagen, Biomedical Sciences, Copenhagen, Denmark*

ABSTRACT TEXT

Our primary goal is to improve the prospects of patients suffering from Atrial Fibrillation (AF), by dissecting its pathophysiological processes using zebrafish disease model. AF is one of the most common type of supraventricular tachycardia, associated with various risk factors such as stroke, heart failure and increased mortality. While diagnostic tools are improving, current available treatments remain to be limited and inefficient, as mechanisms underlying both the onset and progression of AF remain poorly understood.

Our laboratory focuses on the genetic causes of AF and has recently identified proto-oncogene ErbB2 (Epidermal Growth Factor Receptor 2), which appears to be associated with AF, by Genome-Wide Association Studies (GWAS). Interestingly, mice depleted from ErbB2, display cardiac hemorrhage and disrupted atrial function. As a consequence, these mice develop conduction block specifically in the atrium, leaving ventricular conduction unaffected. Similarly, in AF, due to rapid and disorganized atrial activation, atrial conduction is impaired which can be detected on electrocardiogram (ECG) by lack of a P-wave and irregular QRS complexes. These findings further suggest a potential role of ErbB2 in development of AF. Thus, by using the genetic zebrafish mutants as tool, we aim to gain better insights into the human AF etiology and disease mechanism. Our aims are to; 1) Characterize cardiac function in adult *erbb2a* zebrafish genetic mutants and search for hallmarks of AF, 2) Identify key molecular and cellular processes of AF during onset and progression, 3) Perform phenotype-based small molecule screening to identify the signaling pathway in which ErbB2 is involved. We hypothesize our approach will allow us to gain better understanding of disease onset and progression in a molecular and cellular level and provide novel targets for AF pathophysiology.

P 075: Loss of Slc39a14 causes simultaneous manganese deficiency and hypersensitivity in zebrafish

TOPIC: DISEASE MODELS

Karin Tuschl[1,2], Richard J White[3], Leonardo E Valdivia[1,4], Stephanie Niklaus[5], Isaac H Bianco[1], Ian M Sealy[3], Stephan CF Neuhauss[5], Corinne Houart[2], Stephen W Wilson[1], Elisabeth Busch-Nentwich[3]

1 *University College London, Department of Cell and Developmental Biology, London, United Kingdom*
2 *Kings College London, Department of Developmental Neurobiology, London, United Kingdom*
3 *University of Cambridge, Department of Medicine, Cambridge, United Kingdom*
4 *Universidad Mayor, Facultad de Ciencias, Santiago, Chile*
5 *University of Zurich, Institute of Molecular Life Sciences, Zurich, Switzerland*

ABSTRACT TEXT

Hypermanganesaemia with Dystonia 2 (HMNDYT2) is a neurodegenerative disorder presenting with accumulation of manganese (Mn) in the brain and progressive dystonia-parkinsonism. It is caused by mutations in SLC39A14, a Mn uptake transporter. In HMNDYT1, a second Mn transporter defect, chelation therapy significantly improves neurological symptoms and stabilises disease progression. In contrast, response to chelation therapy in individuals with HMNDYT2 is variable and some patients' neurology deteriorates upon chelation treatment. The reasons for this discrepancy are unknown. A zebrafish model of HMNDYT2, the mutant *slc39a14*^{U801}, shows Mn accumulation in the brain and abnormal swimming, recapitulating the patient phenotypes. Here we use this mutant to study the gene regulatory response to loss of *slc39a14* in conjunction with Mn exposure and uncover targets of Mn toxicity. We performed 3'tag RNA sequencing on wild-type and mutant *slc39a14*^{U801} zebrafish larvae at five days post fertilisation unexposed and exposed to $MnCl_2$ from 48 hours post fertilisation. Anatomical and GO enrichment analyses show that genes affected by Mn exposure in wild types are primarily expressed in the brain and eye. We find an enrichment of genes involved in calcium homeostasis and the unfolded protein response, linking Mn toxicity to endoplasmic reticulum stress. As expected, homozygous *slc39a14*^{U801} individuals have increased sensitivity to elevated Mn levels as demonstrated by a much stronger transcriptional response to the exposure. However, surprisingly, we also find that most genes that are de-regulated in unexposed mutants are rescued by Mn treatment. This suggests that the mutants have Mn deficiency in addition to Mn-driven neurotoxicity. This has important implications for chelation therapy in HMNDYT2 patients and might help to explain the observed adverse effects. Together our work demonstrates the combined power of the zebrafish model and whole organism gene expression analysis.

P 076: DTYMK is essential for genome integrity and neuronal survival

TOPIC: DISEASE MODELS

Jo Vanoevelen[1], *Jörgen Bierau*[1], *Erik-Jan Kamsteeg*[2], *Levinus Bok*[3], *Ron A Wevers*[4], *Marjo van der Knaap*[5], *Marianna Bugiani*[6], *Jun Mei Frisk*[7], *Mark O' Driscoll*[8], *Han Brunner*[1], *Arthur van den Wijngaard*[1], *Ghada Abdel-Salam*[9], *Liya Wang*[7], *Constance Stumpel*[1]

1 *Maastricht University Medical Centre+, Clinical Genetics, Maastricht, Netherlands*
2 *Radboud UMC, Human Genetics, Nijmegen, Netherlands*
3 *Màxima Medical Center, Pediatrics, Veldhoven, Netherlands*
4 *Radboud University Medical Centre, Translational Metabolic Laboratory, Nijmegen, Netherlands*
5 *VUMC, Child Neurology, Amsterdam, Netherlands*
6 *VUMC, Neuropathology, Amsterdam, Netherlands*
7 *Swedish University of Agricultural Sciences, Anatomy- Physiology and Biochemistry, Uppsala, Sweden*
8 *University of Sussex, Genome Damage and Stability Centre, Brighton, United Kingdom*
9 *National Research Centre, Clinical Genetics, Cairo, Egypt*

ABSTRACT TEXT

Human nucleotide metabolism is a complex, tightly controlled pathway regulating numerous cellular processes such as nucleic acid synthesis and repair. Here, we describe *DTYMK* deficiency as the cause of a severe neurodegenerative disease in two unrelated families.

DTYMK encodes the dTMPK (deoxythymidylate monophosphate kinase) enzyme which catalyzes the penultimate step in the biosynthesis of dTTP. We describe two children showing severe postnatal microcephaly, lack of neurodevelopment and overall growth retardation with extensive atrophy of the cerebral cortex. Exome sequencing identified two variants in *DTYMK*.

No significant dTMPK enzyme activity could be detected in the patients' fibroblasts, indicating a loss-of-function effect of the variants. Additionally, EdU labelling in fibroblasts confirmed a marked proliferation defect. We generated a *dtymk* loss-of-function allele in zebrafish. Homozygous *dtymk* mutant zebrafish are not viable beyond 5dpf and show microcephaly, small eyes, developmental delay, cardiac edema and massive edema of the brain. Biochemical analysis of dTMPK activity in mutant zebrafish larvae confirmed that the allele represents a loss-of-function allele leading to undetectable enzyme activity. Furthermore, impairment of proliferation was detected in the brain of mutant zebrafish larvae. as well as increased apoptosis. Further molecular analysis revealed genome instability due to ribonucleotide incorporation and defects in the DNA-damage response repair mechanism. The striking similarities between the human and zebrafish phenotype strongly suggest a causal link between dTMPK deficiency and the neurodegenerative phenotype, observed in both patients.

In summary, by combining genetic and biochemical approaches in different models, we identified loss-of-function in *DTYMK* as the cause of a severe pediatric neurodegenerative disease. This study highlights the essential nature of dTTP synthesis in maintaining genome integrity and neuronal survival.

P 077: Drug screening for angiogenesis modulators in zebrafish

TOPIC: DISEASE MODELS

Viviana Vedder[1,2,3], *Zouhair Aherrahrou*[1,2,3], *Jeanette Erdmann*[1,2,3]

1 *Universität zu Lübeck, Institute for Cardiogenetics, Lübeck, Germany*
2 *University Heart Centre, Lübeck, Lübeck, Germany*
3 *DZHK, German Centre for Cardiovascular Research, Hamburg/Kiel/Lübeck, Germany*

ABSTRACT TEXT

Angiogenesis, a process that forms new blood vessels from existing ones, is associated with a variety of diseases. Irregularities in the formation of secondary blood vessels can lead to atherosclerosis, diabetes and hypertension, among others, which are among the major risk factors for cardiovascular disease (CVD), the leading cause of death worldwide. Zebrafish provide a well-established platform for high-content screening and can be used to investigate the effect of drugs on angiogenesis *in vivo*. We established the angiogenesis assay using sunitinib malate, which is a known kinase inhibitor, that has been previously tested in zebrafish. With a small molecule library of nearly 1300 compounds we are currently performing phenotype screening to identify and investigate drugs regulating angiogenesis for their clinical applications. Our drug library covers the main anatomic therapeutic chemical (ATC) groups: nervous system, cardiovascular system and anti-infectives for systemic use. To examine our phenotypes we evaluate data from fluorescent images of Tg(*fli1a:eGFP*)y1 and

brightfield movies of the heart and the caudal aorta above the urogenital pore. This data will be used to measure vessel length, thickness and lumen formation as well as pulse, heart phenotype, erythrocyte shape and count and blood flow.

P 078: A CRISPR/Cas9 vector system for neutrophil-specific gene disruption in zebrafish

TOPIC: DISEASE MODELS

Yueyang Wang[1], Alan Hsu Y.[1], Eric Walton[2], Ramizah Syahirah[3], Tianqi Wang[1], Wenqing Zhou[1], Qing Deng[4]

1 *Purdue University, Biological Sciences, West Lafayette, USA*
2 *Duke University, Molecular Genetics and Microbiology, Durham, USA*
3 *Purdue Univeristy, Biological Sciences, West Lafayette, USA*
4 *Purdue Univerisity, Biological Sciences, West Lafayette, USA*

ABSTRACT TEXT

Tissue-specific knockout techniques are essential for probing the tissue-specific functions of specific genes in development and disease. Here, we report a robust system for neutrophil-specific gene inactivation in zebrafish, with ubiquitous expression of sgRNAs and neutrophil-restricted expression of Cas9 protein. Loss of the *rac2* or *cdk2* gene in neutrophils results in significantly decreased cell motility, which is restored by re-expressing Rac2 or Cdk2 in the corresponding knockout background. The subcellular location of Rac activation, actin structure and stress are determined in both the wild-type and *rac2* knockout neutrophils randomly migrating in tissue. In addition, neutrophil speed is also significantly reduced using an alternative system where the Cas9 protein is ubiquitously expressed and the *rac2*-targeting sgRNA is expressed only in neutrophils. Together, here we introduce a potent tool that can be used to advance the utility of zebrafish in identifying and characterizing gene functions in neutrophils.

P 079: Resemblance of skeletal phenotypes between germline and somatic lrp5 mutants illustrates the feasibility of the zebrafish for CRISPR-based G0 reverse genetic screening of osteoporosis candidate genes

TOPIC: DISEASE MODELS

Andy Willaert[1], Jan Willem Bek[1], Chen Shochat Carvalho[2], Hanna De Saffel[1], Frans Rodenburg[3], Adelbert De Clercq[1], Annekatrien Boel[1], Juriaan Metz[4], David Karasik[2], Paul Coucke[1]

1 *Centre for Medical Genetics- Ghent University, Department of Biomolecular Medicine, Ghent, Belgium*
2 *Bar Ilan University, Faculty of Medicine in the Galilee, Safed, Israel*
3 *Tokyo Institute of Technology, School of Life Science and Technology, Tokyo, Japan*
4 *Radboud University, Department of Animal Ecology and Physiology, Nijmegen, Netherlands*

ABSTRACT TEXT

Genome-wide association studies (GWAS) have strengthened our understanding of the genetic architecture of common, complex diseases such as osteoporosis. Nevertheless, to attribute functional skeletal contributions of candidate genes to osteoporosis-related traits there is a need for efficient and cost-effective in vivo functional testing. This can be achieved through CRISPR-based reverse genetic screens, where phenotyping is traditionally performed in stable germline knockout (KO) mutants. However, recently it was shown that first generation (G0) mosaic mutant zebrafish (so-called crispants) recapitulate the phenotype of germline KO's. To deliver a proof-of-concept we compared a stable KO and crispant zebrafish model for the lrp5 gene. In human, recessive loss-of-function mutations in LRP5, a co-receptor in the WNT signaling pathway, cause Osteoporosis-pseudoglioma syndrome. In addition, several GWAS studies identified LRP5 as a major risk locus for osteoporosis-related phenotypes. In this study, we showed that early stage lrp5 KO larvae display decreased notochord ossification and malformations of the head cartilage. Quantitative µCT and mass-spec element analysis of the adult skeleton revealed decreased vertebral bone volume and bone mineralization, which are hallmark features of osteoporosis. Regenerating adult fin tissue showed a decreased response of the wnt-signaling pathway in lrp5 KO mutants. On the other hand, lrp5 crispants were generated

by micro-injecting one-cell stage embryos with CRISPR RNP complexes containing Cas9 and a two-part gRNA (tracrRNA:crRNA duplex). These crispants generally showed a milder, but nonetheless highly similar skeletal phenotype and a similarly reduced wnt pathway response compared to lrp5 KO mutants. In conclusion, we present the first genetic osteoporosis zebrafish model and we show that crispant screening in zebrafish is a promising approach for rapid functional screening of osteoporosis candidate genes.

P 080: Age-Related Hearing Loss in the Zebrafish model: investigating the mechanisms underlying aging sensory loss, synaptopathy and morphological correlates

TOPIC: DISEASE MODELS

Man Ieng Wong[1], *Flora Gordillo-Martínez*[1], *Ieng Hou Lau*[1], *Paulo J. Fonseca*[2], *Raquel O. Vasconcelos*[1]

[1] *University of Saint Joseph, Institute of Science and Environment, Macao, China*
[2] *Universidade de Lisboa, Departamento de Biologia Animal and cE3c, Lisbon, Portugal*

ABSTRACT TEXT

Age-related hearing loss (ARHL) has been recognized as the most common sensorineural impairment associated with aging and the most prevalent form of hearing loss. Over the last century an increasing amount of research on inner ear pathologies related to ARHL has been reported, however, the underlying molecular pathway and physiological control mechanism of this senescent impairment is still far from fully understood.

The zebrafish (*Danio rerio*), which share homologous inner ear structures to mammals, is a well-established vertebrate model in hearing research providing unmatched technical advantages to investigate the molecular and physiological mechanisms of ARHL.

In the present study, the hearing sensitivity of zebrafish (AB line) was determined based on Auditory Evoked Potential recording technique from both healthy reproductive adults (7 months old, 27–30 mm total length) and aged specimens (24–30 months, 36–40 mm). We found auditory sensitivity differences of up to 30 dB within the species best hearing range (600–1000 Hz). Results also revealed significant increase in auditory response latencies of 0.8–1.4 ms.

Ongoing research focuses on changes in the sensory hair cells of the inner ear saccule (the main auditory end organ) and the synaptic function with primary auditory neurons (based on the quantity of presynaptic Ribeye b and postsynaptic MAGUK proteins). We are also investigating changes in saccular gene expression and its interaction with noise-induced hearing loss in the zebrafish model.

Education and Outreach

P 081: Zebrafish: More than an experimental model, a powerful educational tool

TOPIC: EDUCATION AND OUTREACH

Geonildo Disner[1], Maria Alice Pimentel Falcao[1], Adolfo Luis Almeida Maleski[1,2], Joao Batista-Filho[1,2], Ana Carolina Seni-Silva[1,2], Milena Marcolino-Souza[1], Amanda Beatriz Silva Soares[1], Leticia Balan-Lima[1], Aline Ingrid Andrade-Barros[1], Natalia Vitorino Leite-Santos[1], Jefferson Thiago Gonçalves Bernardo[1], Bruno Santos[1], Kamila Gomes[1], Lucas Hipolito[1], Viviane Tomazia Cunha[1], Wilton Queiroz Souza[1], Adriana Mortara Almeida[1], Carla Lima[1], Monica Lopes-Ferreira[1]

[1] *Immunoregulation Unit of the Laboratory of Applied Toxinology CeTICs/FAPESP, Butantan Institute, São Paulo, Brazil*

[2] *Post-Graduation Program of Toxinology, Butantan Institute, São Paulo, Brazil*

ABSTRACT TEXT

In the Zebrafish Platform of Butantan Institute we use *Danio rerio* as our main model for understanding the immune response regulation and pre-drug molecules therapeutic properties. As part of an Innovation and Dissemination Research Center (CeTICS), our purposes is to use this charismatic species to connect society and science through research dissemination. The zebrafish ease of creation, rapid external development and the transparency of the embryo allow the complete growth to be accompanied. Some educational activities highlighted here include: 1) "*Paulistinha Chega às Escolas*"(i.e., zebrafish gets to schools), whose goal is to share knowledge about the work to students from schools. The teachers were guided and the participants receive a book with activities about zebrafish, its history and applications. They host a speaker for a lecture and later visit the laboratory, interview researchers and get to know the vivarium. Next, they participate in a short experiment, collect the data and present the results. 2) "*Zebrafish Platform open doors*"-this activity happened on July 30 and 31, 2019, we presented the laboratory facilities through guided tours along with examples of researches. Didactic activities like puzzles, coloring books, and other interactive actions were held during the event to captivate the youngest guests, 800 visitors showed up. 3) *"Workshop for teachers"*, for this activity a book was prepared to present zebrafish model to school teachers based on the laboratory expertise, addressing similarities between the development of zebrafish and humans, anatomy, genetics and environmental toxicology in order to train teachers so that they are disseminators of knowledge. This set of activities makes it possible to reach the community and bring them closer to the universe of scientific research through zebrafish, favoring the science popularization and diffusion, and enabling the connection between researchers, students, and community. Support FAPESP and CAPES.

P 082: Overview of AK KAB and next target: Recommendations and Guidelines for cleaning aquatic housing systems

TOPIC: EDUCATION AND OUTREACH

Hans-Peter Popp[1]

[1] *Arbeitskreis Käfigaufbereitung AK KAB c/o Tecniplast Deutschland GmbH, Working Group Aquatics, Hohenpeißenberg, Germany*

ABSTRACT TEXT

For more than 12 years the German Working Group for Cage Processing „AK KAB".

(Arbeitskreis Käfigaufbereitung) has been working on summarizing and publishing state of the art recommendations and guidelines for effective cleaning of rodent cages. AK KAB has already published 5 editions of their so called „orange brochure" in both German and English, both either available in a printed or digital format (available from the FELASA-website).

Apart from rodents as the main species in many laboratory animal facilities also aquatics species like zebrafish, xenopus and axolotls to name just a few are being used for research as well. To address the specific needs of users of aquatic systems, the AK KAB founded a new working group in 2018, consisting of experts for cleaning aquatic equipment. This group inside the AK KAB has been active, putting together important specific aspects of aquatic housing systems inside biomedical research laboratories.

The ultimate goal is to provide recommendations and guidelines for processing aquatic housing systems in a proper and sustained way.

At FELASA 2019 in Prague the working group already presented a first structure and initial contents of this new planned brochure as „ Guideline for cleaning aquatics housing systems". The follow-on online survey with the specific title „Aquatic organisms husbandry: the tank cleaning process" provided over 160 individual responses. Experts from many countries around the world using various aquatic species in their research facilities contributed their feedback. The analysis confirmed to the working group that their activities are on target and lead to some aspects being updated already in time for the 1st edition of the brochure.

The presentation will introduce the 1st edition of the brochure „Guidelines for cleaning aquatics housing systems", covering main topics and the present day situation. Furthermore an outlook is given on specific topics under preparation for the next edition of this brochure.

Emerging Techologies

P 083: Multi-sample light-sheet imaging of embryonic zebrafish

TOPIC: EMERGING TECHOLOGIES

Stephan Daetwyler[1], *Carl Modes*[2], *Kyle Harrington*[3], *Jan Huisken*[4], *Reto Fiolka*[1]

[1] *UT Southwestern Medical Center, Cell Biology, Dallas, USA*
[2] *Max Planck Institute of Molecular Cell Biology and Genetics MPI-CBG, Center for Systems Biology Dresden, Dresden, Germany*
[3] *University of Idaho, Virtual Technology & Design, Moscow, USA*
[4] *Morgridge Institute for Research, Medical Engineering, Madison, USA*

ABSTRACT TEXT

To quantitatively understand biological processes that occur over long time periods, it is desirable to image multiple samples simultaneously, and automatically process and analyze the resulting datasets. Here, we present a complete multi-sample preparation, imaging, processing and analysis workflow to quantify the development of the vascular volume in zebrafish over several days [1].

Up to five live zebrafish embryos were mounted and imaged simultaneously over several days using selective plane illumination microscopy (SPIM, [2]). The resulting large image dataset of several terabytes was processed in an automated manner on a high-performance computer cluster and segmented using a novel segmentation approach that uses images of red blood cells as training data. This analysis yielded a precise quantification of growth characteristics of the whole vascular network, head vasculature and tail vasculature over embryo development. Interestingly, we found that the best growth models to explain the data all include an effective, logarithmic rescaling of time.

Our multi-sample imaging pipeline demonstrates effective upgrades to conventional single-sample imaging platforms and paves the way for diverse quantitative long-term imaging studies such as xenotransplantation experiments, studies of rare cell behavior, or small-scale screens. It advocates a holistic approach based on multi-sample imaging using SPIM with integrated data processing and analysis to reveal and understand biological processes that occur over long time periods.

1 Daetwyler, S. et al. (2019) "Multi-Sample SPIM Image Acquisition, Processing and Analysis of Vascular Growth in Zebrafish." *Development*
2 Daetwyler, S, and Huisken, J. (2016). "Fast Fluorescence Microscopy with Light Sheets." *The Biological Bulletin*.

P 084: Small organism dispensing, orienting and imaging robot

TOPIC: EMERGING TECHOLOGIES

Jan De Sonneville[1], *Kees-Jan van der Kolk*[2]

[1] *Life Science Methods BV, R&D, Leiden, Netherlands*
[2] *Life Science Methods BV, Research and Development, Leiden, Netherlands*

ABSTRACT TEXT

A robot was invented that can dispense, orient and image small aquatic organisms.

This robot features a robotic arm that can move the head in three dimensions (XYZ).

The head has a camera to locate organisms and to verify if operation is working as planned.

Furthermore, the head contains a tube and motor to rotate the tube. The tube is connected to a syringe pump.

The complete process of dispensing and imaging is automated as follows:

1. Using deep-learning, a first organism is located in a petri dish multiwell plate.
2. A FEP tube with a bent tip is turned around the Z-axis towards the head of the organism, and is placed in front of the head.
3. The organism is sucked up in a volume of 6 microliter in the tube.
4. For dispensing, the tube is moved to the destination well, and the organism is pumped out of the tube.
4. For imaging, gently the water under the organism is pumped out until the organism is visible in the tip of the tube.
5. A small droplet of gel is sucked up to fix the tail in the tube, and subsequently a droplet of air is sucked up to keep the organism from sinking.
6. The tube is lowered into a cuvette from where an integrated epi-fluorescent microscope can view the organism from the side.
7. Using deep-learning, the organism or region of interest is positioned in the field of view of the microscope.
8. Using another deep learning model, the organism is rotated to reproduce the viewing orientation.
9. Microscopy can be done from multiple rotations and multiple planes, to produce one or more image stacks
10. After imaging the organism is dispensed into a destination plate.

This robot enables the following applications:

1. Automated dispensing with a fixed amount of added liquid per dispensed organism.
2. Automated egg sorting to select fertilised eggs.
3. Automated brightfield whole organism imaging for eg toxicity studies.
4. Automated fluorescent imaging of a region of interest, eg the zebrafish heart, liver, head or tail.

P 085: Characterising vascular heterogeneity during zebrafish development

TOPIC: EMERGING TECHOLOGIES

Marleen Gloger[1], *Kaska Koltowska*[1]

[1] *Uppsala University, Immunology Genetics and Pathology, Uppsala, Sweden*

ABSTRACT TEXT

The blood and lymphatic vasculature are important for nutrient and oxygen supply as well as maintaining the fluid homeostasis. In mammals, the global heterogeneity and hierarchy of blood and lymphatic vasculature have been precisely described. They are marked by cellular and morphological characteristics of several vessel subtypes and their perivascular cell environments. In zebrafish, morphological identification of the vascular hierarchy and heterogeneity has not been systematically defined. This raises the question if this heterogeneity exists in zebrafish. Here we aim to uncover the vast vessel network diversity across vascular beds.

To generate a comprehensive characterisation of the different vascular networks, we used the strengths of zebrafish high-resolution imaging across the development up to early adult stages. We have optimized the light sheet imaging of whole zebrafish expressing vascular transgenic lines, from 2 days post-fertilization (dpf) embryos to 17 dpf larvae. The rapid imaging using light sheet microscopy (Leica SP8 DSL) allows us to simultaneously illuminate developing blood and lymphatic vasculature and image a 16 dpf old zebrafish in less than 25 min. This fast-high-resolution imaging set up, in conjunction with vascular and perivascular transgenic lines, enable us to visualize and define small variations within key vascular beds in a whole organism. Thus, identifying similarities and differences to mammalian vascular hierarchy and heterogeneity. This advanced high-resolution light sheet imaging technique can be used in putative projects for analysis of different organ development.

P 086: In Vivo Biomolecular Imaging of Zebrafish Embryos using Confocal Raman Spectroscopy

TOPIC: EMERGING TECHOLOGIES

Hakon Hogset[1], *Conor C. Horgan*[1], *Mads S. Bergholt*[1], *James P. K. Armstrong*[1], *Vincenzo Torraca*[2], *Qu Chen*[1], *Timothy J. Keane*[1], *Laurence Bugeon*[3], *Margaret J. Dallman*[3], *Serge Mostowy*[2], *Molly M. Stevens*[1]

[1] *Imperial College London, Department of Materials, London, United Kingdom*
[2] *London School of Hygiene & Tropical Medicine, Department of Infection Biology, London, United Kingdom*
[3] *Imperial College London, Department of Life Sciences, London, United Kingdom*

ABSTRACT TEXT

Zebrafish embryos are widely used for microscopic analysis of complex biological processes, typically using fluorescence microscopy. Here, we demonstrate that confocal Raman spectroscopic imaging can be used as a complementary approach for biomolecular imaging and analysis of zebrafish embryos. Raman spectroscopic imaging can be used to directly obtain hyperspectral datasets without the use of labelling, and can be used in combination with multivariate component analysis to visualize biomolecular features in biological samples (Kallepitis et al., 2017). We outline a workflow of sample preparation, imaging and analysis and validate this method by collecting three-dimensional biomolecular images of whole zebrafish embryos and resolving fine anatomical features at subcellular spatial resolution. We also apply confocal Raman spectroscopic imaging for the biomolecular profiling and discrimination of wild-type and ΔRD1 mutant mycobacteria in a zebrafish embryo model of tuberculosis. Finally, we demonstrate the use of confocal Raman spectroscopic imaging for *in vivo* temporal monitoring of the wound response in living zebrafish embryos. Overall, confocal Raman spectroscopic imaging constitutes a new imaging modality for zebrafish research, enabling the first comprehensive biomolecular analysis in fully intact and living zebrafish embryos.

P 087: Fluorescently tagged knock-in allele allows live imaging and degradation-mediated manipulation of planar cell polarity in zebrafish

TOPIC: EMERGING TECHOLOGIES

Maria Jussila[1], *Curtis Boswell*[1,2], *Brian Ciruna*[1,2]

[1] *The Hospital for Sick Children, Developmental & Stem Cell Biology Research Program, Toronto, Canada*
[2] *University of Toronto, Department of Molecular Genetics, Toronto, Canada*

ABSTRACT TEXT

Planar cell polarity signalling (PCP) coordinates the uniform orientation, structure and movement of cells within a plane of a tissue during development. PCP activity is based on the asymmetric localization of its core components on cell membranes. In order to understand how asymmetric component localization is translated into polarized cell behaviour, and to study endogenous PCP activity in real time in zebrafish embryos, we have used CRISPR/Cas9 gene editing to target a superfolder GFP linker cassette (sfGFP-l) onto the N-terminus of Vangl2 protein, a core and specific PCP regulator. Fish homozygous for this *vangl2* knock-in allele, and fish trans-heterozygous for *vangl2* knock-in and *vangl2* loss-of-function alleles are viable and fertile, demonstrating that the sfGFP-l-Vangl2 fusion protein is functional. Our analysis of sfGFP-l-Vangl2 localization has revealed robust plasma membrane localization prior to the onset of gastrulation, which is earlier than in previous immunohistochemical studies. On single-cell level in the neuroepithelium Vangl2 shows a polarized localization to anterior cell membranes as well as to cell protrusions, and it becomes apically polarized as the neural tube midline forms. We also show that manipulation of PCP signaling changes endogenous Vangl2 localization in the developing neural tube. These observations validate the functionality of our endogenously labeled *vangl2* allele and highlight its sensitivity and utility. To manipulate Vangl2 protein levels in the early embryo, we have combined our sfGFP-l-Vangl2 line with zGrad, a GFP-specific protein degradation system. Strikingly, zGrad-dependent modulation of sfGFP-l-Vangl2 protein levels phenocopies *vangl2* mutant phenotypes and reveals an unexpected phenotype in heterozygote animals. In the future, conditional zGrad strategies will permit temporal and tissue-specific analysis of PCP function across diverse embryonic, juvenile and adult contexts.

P 088: Primed Conversion for advanced in vivo precision imaging

TOPIC: EMERGING TECHOLOGIES

Konstantinos Kalyviotis[1], *Maaike Welling*[1,2], *Periklis Pantazis*[1]

[1] *Imperial College London, Bioengineering, London, United Kingdom*
[2] *ETH Zurich, Biosystems Science and Engineering, Basel, Switzerland*

ABSTRACT TEXT

Labelling strategies using green-to-red photoconvertible fluorescent proteins allow for visualisation of both the entire population of cells in green and a selected population in red. This combination of global and sparse labelling yields great potential for facilitating lineage tracing and fate assignments after photoconversion. Spatially confined green-to-red photoconversion can however neither be accomplished with near-UV nor with high-power, pulsed laser illumination due to lack of axial confinement and negligible conversion [1]. Our recent report of a novel photochemical mechanism called *primed conversion* overcomes this long-standing problem by using dual-wavelength illumination with blue (488nm) and red to far-red (600–800nm) laser light [2]. Importantly, primed conversion allows for confined photoconversion of small volumes in three dimensions by selectively intersecting the two laser beams in a common focal spot, yielding axial confinement unachievable by conventional photoconversion [3]. The discovery of the mechanism responsible for primed conversion enabled us to rationally engineer primed convertible variants of most photoconvertible fluorescent proteins with improved brightness and photostability, essential properties for long-term *in vivo* imaging [4]. To elucidate complex cellular and molecular dynamics underlying embryonic development and disease progression, we combined primed conversion with light-sheet imaging and bioimage informatics [5]. Further, we introduce a novel design of an optical lineage-tracing system, termed PhOTO-Bow, that will greatly facilitate high-fidelity segmentation, classification, and long-term tracking of individual cells *in vivo*.

1 Pantazis and Supatto. Nat. Rev. Mol. Cell Biol. 15, 327–339 (2014).
2 Dempsey et al. Nat Meth 12, 645–648 (2015).
3 Mohr et al. Nat Protoc 11, 2419–2431 (2016).
4 Mohr et al. Angew. Chem. Int. Ed. 56:11628–11633 (2017).
5 Welling et al. Elife 21;8. pii: e44491 (2019).

P 089: In vitro fertilization as a method for increasing breeding efficiency in Giant Danio

TOPIC: EMERGING TECHOLOGIES

Olivia Knight[1], *Mariana Sampaio*[1], *Joana Monteiro*[1], *Ana Catarina Certal*[2]

[1] *Champalimaud Foundation, Fish Platform, Lisbon, Portugal*
[2] *Champalimaud Foundation, Fish Platform and Molecular Tools and Transgenic Platform, Lisbon, Portugal*

ABSTRACT TEXT

Giant Danio (*Devarius aequipinnatus*) is as an emerging model for neuroscience comparative studies with Zebrafish. Therefore, we have been optimizing its housing and husbandry protocols. Breeding Giant Danio in a routine manner has been challenging in several ways. They appear to be monogamous and because of that it is necessary to perform time consuming breeding trials to find matches. Monogamy also represents a downside for infrastructure investment because a large amount of space is required to grow colonies from which only a small percentage of fish become breeding pairs. The low number of breeding couples per se, it's an additional constraint. In addition, Giant Danio do not respond well to time-controlled breeding, which is required for experiments that depend on synchronized embryos and for generation of stable transgenics based on constructs delivery into one cell stage embryos.

Here, we present *in vitro* fertilization (IVF) as an alternative method for breeding Giant Danio. This method will allow us to overcome all the constrains described above and expand the usage of this species as a comparative model organism in neuroscience, by opening way to experimental protocols that were not possible so far.

P 090: A simple and effective F0 knockout method for rapid screening of behaviour and other complex phenotypes

TOPIC: EMERGING TECHOLOGIES

Francois Kroll[1], Gareth Powell[1], Marcus Ghosh[1], Paride Antinucci[2], Timothy Hearn[1], Hande Tunbak[3], Sumi Lim[1], Harvey Dennis[4], Joseph Fernandez[5], David Whitmore[1], Elena Dreosti[3], Stephen Wilson[1], Ellen Hoffman[5], Jason Rihel[1]

1 *University College London, Department of Cell and Developmental Biology, London, United Kingdom*
2 *University College London, Department of Neuroscience- Physiology & Pharmacology, London, United Kingdom*
3 *University College London, Wolfson Institute for Biomedical Research, London, United Kingdom*
4 *University of Bristol, Faculty of Science, Bristol, United Kingdom*
5 *Yale School of Medicine, Child Study Center, New Haven- Connecticut, USA*

ABSTRACT TEXT

Hundreds of human genes are associated with neurological diseases, but translation into tractable biological mechanisms is lagging. Larval zebrafish are an attractive model to investigate genetic contributions to neurological diseases. However, current CRISPR-Cas9 methods are difficult to apply to large genetic screens studying behavioural phenotypes. To facilitate rapid genetic screening, we developed a simple sequencing-free tool to validate gRNAs and a highly effective CRISPR-Cas9 method capable of converting >90 % of injected embryos directly into F0 biallelic knockouts. We demonstrate that F0 knockouts reliably recapitulate complex mutant phenotypes, such as altered molecular rhythms of the circadian clock, escape responses to irritants, and multi-parameter day-night locomotor behaviours. The technique is sufficiently robust to knockout multiple genes in the same animal, for example to create the transparent triple knockout crystal fish for imaging. Our F0 knockout method cuts the experimental time from gene to behavioural phenotype in zebrafish from months to one week.

P 091: 1H-NMR lipidomics in embryonic zebrafish

TOPIC: EMERGING TECHOLOGIES

Rhiannon Morgan[1], Marie Phelan M[2], Richard Barrett-Jolley[1], Mandy Peffers[1], Gemma Walmsley[1]

1 *University of Liverpool, Department of Musculoskeletal Biology- Institute of Ageing and Chronic Disease, Liverpool, United Kingdom*
2 *University of Liverpool, Institute of Integrative Biology, Liverpool, United Kingdom*

ABSTRACT TEXT

Disrupted lipid metabolism is a pathogenetic mechanism in several mammalian diseases that affect muscle including lipid storage myopathies and centronuclear myopathies. The embryonic zebrafish is an ideal system to model such disorders as they undergo rapid myogenesis and develop externally. Research in this area has been hampered by difficulties in extracting, identifying and quantifying lipids. In this study we aimed to carry out ^{1}H-NMR lipid profiling in developing zebrafish embryos and focused on the protruding mouth stage (72 hours post fertilisation (hpf)), which has mature muscle structure.

Lipids were extracted using chloroform from groups of pooled AB wildtype embryos at various developmental stages with ^{1}H-NMR spectra acquired at high-field (700 MHz). We first evaluated the optimum number of 72 hpf embryos per sample and determined that 10 embryos gave good signal: noise. The inclusion of chorions in the sample had no significant difference on the lipidome of 24 hpf embryos, in fact dechorionation increased in group variability. Embryos at 24 hpf, 48 hpf and 72 hpf stages were analysed and samples from embryos of different developmental age clustered separately upon supervised multivariate analysis. We then quantified lipids from several classes and saturation levels of fatty acids at these developmental stages by NMR. As the yolk is known to be lipid-rich, we also bisected 72 hpf embryos into samples containing predominantly tail muscle for comparison with those containing head and yolk sac; as expected, head and yolk sac samples clustered closely to the whole embryo samples and separate to the tail samples.

In conclusion, we have optimised and validated lipidomic evaluation of zebrafish embryos using ^{1}H-NMR and confirmed our ability to detect lipidomic differences in embryos as they develop using this technique. The removal of head and yolk sacs prior to lipid extraction may be required for evaluation of low abundance lipids in tail muscle.

P 092: Sequence features of efficient CRISPR-Cas9 crRNAs for zebrafish genome editing using crRNA-tracrRNA-Cas9 ribonucleoprotein complex

TOPIC: EMERGING TECHOLOGIES

Keita Okada[1], Takuya Takada[1], Kanae Aoki[1], Teruyuki Tabei[1], Katunori Imai[1], Yuki Bonkohara[1], Deshani C. Ranawakage[1], Kamachi Yusuke[1]

[1] *Kochi University of Technology, School of Environmental Science and Engineering, Kami, Japan*

ABSTRACT TEXT

The CRISPR-Cas9 system has been widely used in genome editing experiments; however, their success depends on the performance of crRNAs/gRNAs, particularly regarding their on-target activity. It has been indicated that the nucleotide composition of target site sequences complementary to crRNAs/gRNAs is critical for efficient cleavage by CRISPR-Cas9, but current knowledge is not sufficient to accurately predict on-target activity. Various crRNAs/gRNAs design tools are available to assist with the selection of guide sequences; however, these are based on particular methods of CRISPR-Cas9 and thus suggested to be applicable only to similar experimental conditions. Notably, the current tools are based on experiments performed on specific model organisms and/or use of a single gRNA rather than a more native RNA complex composed of crRNA and tracrRNA, which is increasingly used in recent CRISPR-Cas9 applications. In this study, we evaluated on-target cleavage activities of 35 crRNAs in the form of the crRNA-tracrRNA-Cas9 ribonucleoprotein (RNP) complex both in a zebrafish embryo assay and an in vitro cleavage reaction. For the embryo assay, the RNP complexes were first injected into zebrafish embryos, and then their genomic DNAs were examined for the occurrence of indels by using Sanger sequence-based methods called TIDE and ICE to determine cleavage efficiency of each crRNA. These results showed that the crRNAs were categorized into high, moderate and low activity groups, each of which has characteristic sequence features. The current gRNA design tools were found to partly predict on-target cleavage activities measured in our embryo assay setting. Interestingly, the cleavage activities of the tested crRNAs obtained by the in vitro cleavage reaction were little correlated with those by the embryo assay. Our findings on the sequence features of efficient and inefficient CRISPR-Cas9 crRNA will be useful for designing crRNAs in future genome editing experiments.

P 093: Computational modeling of motile cilia motion provides clues about the generated cerebrospinal flow in zebrafish embryo

TOPIC: EMERGING TECHOLOGIES

Huseyin Enes Salman[1], Nathalie Jurisch-Yaksi[2], Huseyin Cagatay Yalcin[1]

[1] *Qatar University, Biomedical Research Center, Doha, Qatar*

[2] *Norwegian University of Science and Technology, Faculty of Medicine and Health Sciences, Trondheim, Norway*

ABSTRACT TEXT

Background: Motile cilia are hair-like microscopic structures which move the fluids along the epithelial surfaces. Cilia cover a wide range of regions in the nervous system, such as the nasal cavity, spinal cord central canal, and brain ventricles. Motile cilia-driven cerebrospinal fluid (CSF) flow in the brain ventricles has an important role in the brain development. Embryos lacking motile cilia develop neurological defects due to altered CSF flow.

Aim: The aim of our study is to investigate the effect of motile-cilia motion on the altered CSF flow, and to understand the role of CSF flow in the brain development and physiology.

Methods: The dynamics of motile-cilia driven flow is analyzed employing computational fluid dynamics (CFD) modeling. A 2D model is generated using the time-lapse microscopic movies showing movements of a fluorescently labeled motile-cilia in a zebrafish embryo (48-hour post-fertilization). The effects on the generated flow are elucidated by investigating the cilia beating angle, multiple cilia formations, and the phase difference between different ciliary beats.

Results: Ciliary beating generated a directional flow in the form of a circulating vortex. The angle of ciliary beating significantly affected the flow velocity. As the angle between the wall and cilia decreases, the flow becomes more efficient by achieving higher velocities. Multiple

cilia formations increased the flow velocity but the significance of multiple cilia is not as critical as the beating angle. Interestingly, phase difference between the multiple cilia beats increased the directional flow velocity.

Conclusion: Motile-cilia generated flow dynamics are investigated, and it is concluded that out-of-phase multiple ciliary beating is the optimum form of beating in order to generate a directional flow. We continue to study the effect of different frequencies (in-phase and out-of-phase) on CSF flow for multiple simultaneous cilia beats.

P 094: High throughput imaging screening for zebrafish mutants using Vertebrate Automated Screening Technology (VAST)

TOPIC: EMERGING TECHOLOGIES

Renae Skoczylas[1], João Campos Costa[2], Beata Filipek Gorniok[2], Kaska Koltowska[1]

[1] *Uppsala University, Immunology- Genetics and Pathology, Uppsala, Sweden*
[2] *Uppsala University, Organismal Biology, Uppsala, Sweden*

ABSTRACT TEXT

Large scale screening of CRISPRs generated zebrafish involves considerable time. Embryo handling and observation under fluorescent microscopes requires a high level of technical skills. To avoid tedious manual steps of loading, positioning and rotating zebrafish larvae for high-throughput imaging screens we, together with the Genome Engineering Zebrafish, SciLifeLab national facility, established a pipeline to facilitate faster screening and identification of zebrafish mutants. Here we describe the application of this pipeline to screen 10 CRISPR generated mutants.

Using Vertebrate Automated Screening Technology (VAST, from Union Biometrica) multiple screening steps can be automatised. VAST is designed to handle and image, large numbers of 2–7–day post fertilisation (dpf) zebrafish larvae using an automated platform. It combines automated sample handling, with bright-field and fluorescence microscopy. By utilising the Large Particle (LP) sampler, embryos are dispensed in an automated manner before and after imaging. After image acquisition, the LP dispenser deposits each larva into the multi-well plate for downstream application, such as genotyping or further screening. We have applied VAST to efficiently screen 5 dpf zebrafish embryos, expressing *Tg(lyve1b:DsRed;fli1a:nEGFP)* to identify mutants with lymphatic defects. We have screened 10 mutant lines, acquired around 80 good quality images for each line. The success rate for screening of a 96 well plate was 80 % on average where good images were acquired and embryos were successfully genotyped after. This screening platform provide efficient and accurate screening allowing the identification of mutants with higher precision than standard manual fluorescent microscopy screens.

P 095: ZFBONE: an ImageJ toolset for semi-automatic analysis of bone structures

TOPIC: EMERGING TECHOLOGIES

Marco Tarasco[1], Fabrice P. Cordelières[2], M. Leonor Cancela[1,3], Vincent Laizé[1]

[1] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *Bordeaux Imaging Center, UMS 3420 CNRS - University of Bordeaux - US4 INSERM, Bordeaux, France*
[3] *Department of Biomedical Sciences and Medicine - Algarve Biomedical Centre - Centre for Biomedical Research, University of Algarve, Faro, Portugal*

ABSTRACT TEXT

The last decade has seen an increased interest in the discovery of new compounds with bone anabolic activity that could increase the well-being of patients affected by skeletal disorders such as osteoporosis. Because it brings several technical advantages over classical rodent systems, zebrafish (*Danio rerio*) has been increasingly used in screening pipelines aiming at identifying compounds with pharmacological potential. In this regard, several *in vivo* assays[1-3] have been developed and successfully applied to assess the effects of pro/anti-osteogenic molecules during zebrafish bone development and regeneration. A major bottleneck common to all these procedures is the time-consuming image analysis due to a lack of dedicated automatic/semi-automatic tools enabling this highly specialized analysis. The aim of this work was to develop open-source, freely available, user-friendly, rapid and reliable tools to assess zebrafish bone structures,

saving time during morphometric analysis and reducing operator-related bias. ZFBONE is a toolset gathering macros that can be used to assess the effects of osteogenic/osteotoxic compounds from 2D images of bone structures such as developing operculum, regenerating caudal fin and scales. This toolset was developed using the open source ImageJ software, it is easy to use and modify. These tools will not only standardize the available screening protocols but will certainly be useful to the whole zebrafish community, in particular to labs working in tissue engineering, regenerative medicine and skeletal biology.

1 Tarasco et al. (2017) COMP BIOCHEM PHYS C 197:45-52
2 de Vrieze et al. (2014) OSTEOPOROS INT 25:567-578
3 Cardeira et al. (2016) SCI REP 6:39191

This study was funded by the Portuguese Foundation for Science and Technology (FCT) through the projects UID/Multi/04326/2019 and UIDB/04326/2020. M. Tarasco was supported by the FCT through the PhD grant SFRH/BD/128634/2017 and by the COST action CA15124-NEUBIAS.

P 096: An in vivo proteomic approach identifies Bin1 as a novel Cavin4 interactor

TOPIC: EMERGING TECHOLOGIES

Zherui Xiong[1], Thomas E. Hall[1], Harriet Lo[1], Kerrie-Ann McMahon[1], Nick Martell[1], Robert G. Parton[1,2]

[1] *The University of Queensland, Institute for Molecular Bioscience, St Lucia, Australia*
[2] *the University of Queensland, Centre for Microscopy and Microanalysis, St Lucia, Australia*

ABSTRACT TEXT

Proximity-dependent biotin labelling (BioID) is a new approach that has recently gained momentum for discovery proteomics and for spatial mapping of protein-protein interactions in living cells. However, the reductionist *in vitro* applications described to date, while powerful in their own right, lack the complexity and context to address phenomena that can only be modelled *in vivo*, for example during the differentiation of specialised cell types. Here, we describe an approach which applies the BioID method in a living vertebrate model, the zebrafish. We used our new technique to screen for interactors of Cavin4, a membrane protein that is involved in the development of the T-tubule system, an intricate membrane system critical for effective muscle contraction which cannot be meaningfully modelled *in vitro*. In addition to known interactors, we identified Bin1b, a bar domain protein essential for muscle T-tubule formation, as a novel Cavin4-associated protein. Bin1b binds directly to Cavin4b, as shown using a cell-free expression system. Exogenous expression of zebrafish Bin1b in a model cell culture system causes formation of extensive plasma membrane tubules that specifically recruit Cavin4b. We propose a novel role for Cavin4b-Bin1b interactions in the formation and/or maturation of the muscle T-tubule system.

Epigenetics, Gene Regulation and Genomic Resources

P 097: A Conserved Notochord Enhancer Controls Pancreas Development in Vertebrates

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

João Amorim[1,2], Ana Gali-Macedo[1,2], Hugo Marcelino[1,2], Renata Bordeira-Carriço[1,2], Silvia Naranjo[3], Solangel Rivero-Gil[3], Joana Teixeira[1,2], Mafalda Galhardo[1,4], Joana Marques[1,2]

[1] *Universidade do Porto, Instituto de Investigação e Inovação em Saúde, Porto, Portugal*
[2] *Universidade do Porto, Instituto de Biologia Molecular e Celular, Porto, Portugal*
[3] *Universidad Pablo de Olavide, Centro Andaluz de Biología del Desarrollo, Seville, Spain*
[4] *Universidade do Porto, Centro de Investigação em Biodiversidade e Recursos Genéticos, Porto, Portugal*

ABSTRACT TEXT

The notochord is an evolutionary novelty in vertebrates that functions as an important signaling center during development. Notochord ablation in chicken has demonstrated that it is crucial for pancreas development; however, the molecular mechanism has not been fully described. Here, we show that in zebrafish, the loss of function of nog2, a Bmp antagonist expressed in the notochord, impairs β cell differentiation, compatible with the antagonistic role of Bmp in β cell differentiation. In addition, we show that nog2 expression in the notochord is induced by at least one notochord enhancer and its loss of function reduces the number of pancreatic progenitors and impairs β cell differentiation. Tracing Nog2 diffusion, we show that Nog2 emanates from the notochord to the pancreas progenitor domain. Finally, we find a notochord enhancer in human and mice Nog genomic landscapes, suggesting that the acquisition of a Nog notochord enhancer occurred early in the vertebrate phylogeny and contributes to the development of complex organs like the pancreas.

P 098: Analysing the chromatin landscape during zebrafish endoderm formation

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Randa Elsayed[1], Sascha Ott[1], Andrew Nelson[2]

[1] *University of Warwick, Warwick Medical School, Coventry, United Kingdom*
[2] *University of Warwick, School of Life Sciences, Coventry, United Kingdom*

ABSTRACT TEXT

Precise spatial and temporal regulation of gene expression is critical to embryonic development. This is achieved in part via transcription factor binding to *cis*-regulatory modules (CRMs). The ability of transcription factors to access CRMs, however, is controlled by nucleosome positioning and chromatin accessibility. Thus, changes in gene expression are likely to be regulated by changes in the chromatin landscape. My project aims to identify and characterise such changes during endoderm development from specification to the onset of organogenesis using both RNA-seq andAssay for Transposase-Accessible Chromatin with sequencing (ATAC-seq) to identify endoderm-specific CRMs. The endoderm makes major contributions to liver, pancreas, thymus, thyroid and respiratory and gastrointestinal tract. Understanding the gene regulatory mechanisms governing endoderm development is essential to understanding endoderm organogenesis and developmental defects.

Since endoderm is a minor cell population during embryogenesis and therefore challenging to study at the level of the whole embryo, I have employed two distinct approaches to enrich for endoderm: 1) to capture early endoderm-specific gene expression and chromatin accessibility I have performed overexpression of the master regulator of endoderm specification, *sox32*; 2) to capture endoderm-specific gene expression and chromatin accessibility during early organogenesis I have generated a three-colour transgenic line to specifically FACS isolate and study endoderm at 28 hours post-fertilization (h.p.f.). My analyses reveal numerous putative CRMs functioning within developing endoderm and will provide a clearer understanding of how correct gene regulation is achieved during endoderm formation.

P 099: dre-miR-26a-1 has essential role in the anti-inflammatory effect of TnP (Thalassophryne nattereri peptide) on zebrafish-induced neutrophilia model

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Maria Alice Falcao[1,2], *Amanda Beatriz Silva Soares*[1], *Geonildo Rodrigo Disner*[1], *Joao Batista-Filho*[1,3], *Leticia Balan-Lima*[1], *Cristiani Isabel Banderó Walker*[2], *Carla Lima*[1], *Monica Lopes-Ferreira*[1]

[1] *Instituto Butantan, Unit of Imunorregulation Zebrafish Platform, São Paulo, Brazil*
[2] *Federal University of Sergipe, Post-Graduation Program of Pharmaceutical Science, Sao Cristovao, Brazil*
[3] *Instituto Butantan, Post-Graduation Program of Toxinology, São Paulo, Brazil*

ABSTRACT TEXT

The peptide *Tn*P presents as valuable potential to design a new drug to demyelinating conditions as multiple sclerosis due to its ability to control the traffic of inflammatory leukocytes to central nervous system. We hypothesize that the reduction in the translation of target mRNAs that encode inflammatory genes promoted by microRNAs could be one of the molecular basis of the therapeutic effect of *Tn*P. In this work our aim was to investigate the role of dre-miR-26a-1, a member of the highly conserved miR-26 family as one of the mechanisms of *Tn*P for regulation of neutrophilic inflammation using zebrafish as a model. The anti-inflammatory effect of *Tn*P at 0.1 µM applied in prophylactic or therapeutic regimens was investigated by evaluation of neutrophil recruitment to the wound in the tailfins of 72 hpf zebrafish larvae stimulated with *Salmonella abortus* LPS from wildtype, dre-mir-26a-1 morpholino (MO) or mimic dre-mir-26a-1 rescue groups. We demonstrate that neutrophilia in the injured tail of zebrafish larvae can be amplified by additional LPS stimulation; *Tn*P per se does not amplify this basal recruitment and, on the contrary, in this model exploiting the optical clarity of the zebrafish embryos and larvae we confirmed the therapeutic effect of *Tn*P removing neutrophils from the wound in a dose-dependent manner. During dre-miR-26a-1 knockdown, the *Tn*P effectiveness is impaired, in contrast when we overexpressed the dre-miR-26a-1 using miRNA mimic, the anti-inflammatory effect of *Tn*P is improved, reducing the neutrophils recruitment to the wound. Taken together, these results highlight that dre-miR-26a-1 is one of the essential mechanisms for therapeutic effect of *Tn*P and could be used or its targets as promising therapeutic candidates for enhance the resolution of inflammation. This work was supported by FAPESP and in part by the Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior – Brazil (CAPES) – Finance Code 001.

P 100: Defective pseudouridylation of rRNAs results in altered translational control in zebrafish

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Renáta Hamar[1], *Noémi Borbély*[1], *Mirjam Penc*[1], *Eszter Balogh*[2,3], *Regina Légrádi*[2,3], *Dóra K. Menyhárd*[4], *Kálmán Tory*[2,3], *Máté Varga*[1]

[1] *Eötvös Loránd University, Department of Genetics, Budapest, Hungary*
[2] *Semmelweis University, 1st Department of Paediatrics, Budapest, Hungary*
[3] *MTA-SE, "Lendület" Nephrogenetic Research Laboratory, Budapest, Hungary*
[4] *MTA-ELTE, Protein Model. Res. Group and Laboratory of Structural Chemistry and Biology, Budapest, Hungary*

ABSTRACT TEXT

The emergence of epitranscriptomics as a fast growing new field highlighted that post-transcriptional epigenetic modifications provide a new layer of complexity in the control of gene expression. The most abundant type of epitranscriptomic RNA modification is pseudouridylation, the isomerization of uridine into pseudouridine, catalyzed by specific pseudouridine synthase (PUS) enzymes. Although, there is a growing body of evidence that pseudouridylation may affect the structure, stability and function of RNAs, overall the biological consequences of this modification remain poorly understood.

Unlike most other known PUS enzymes, which recognize a specific sequence context, the function of dyskerin is guided by H/ACA box small nucleolar RNAs (snoRNAs). A growing body of evidence suggests that functionally distinct H/ACA box snoRNAs have tissue-specific expression profiles, suggesting differential pseudouridylation in different tissues. Considering that rRNA is one of the major targets of dyskerin-function and its decreased pseudouridylation may result in malfunctioning ribosomes, dysfunction of tissue-specific snoRNAs could be a driving force behind some ribosomopathies.

Recently we created an allelic series targeting the zebrafish *dkc1* gene, which encodes dyskerin. These novel null and hypomorphic lines enable us to characterize the consequences of dyskerin impairment and explain how translational dysfunction through ribosome deficiency leads to disease.

We were able to show that the disruption in a major ribosomal biogenesis factor has global effects on a developing organism, particularly during cell fate decisions. We confirmed that *dkc1* null mutants are unable to process the zygotic pre-rRNA and the maternal rRNA is not degraded in the expected timeframe. Hypomorphic *dkc1* mutant fish show significant growth defects, abnormal pigmentation, and reduced lifespan. These mutants are also prone to malignant transformations, especially melanomas.

P 101: Hematopoieitic lineage specific transgenic reporter lines

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Jovana Jovičić[1], *Petr Bartůněk*[1]

[1] *Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of Cell Differentiation, Prague, Czech Republic*

ABSTRACT TEXT

The main objective of the project is to generate zebrafish transgenic system in order to characterize regulators of erythroid and myeloid lineages in early stages of zebrafish development and in adult hematopoiesis.

As a model organism Zebrafish (*Danio rerio*) has many advantages compared to traditional murine models; high numbers of progeny, rapid, external development, cost-effective maintenance are some of the recognized attributes. Through the last decades zebrafish has been superior model in developmental genetics. Also, zebrafish has been proven to be valuable in hematopoiesis research, sharing with mammals conserved genetic material. Distal genetic elements that control efficiency and rate of transcription from a specific promoter are recognized as enhancers.

Our aim is to generate erythroid and myeloid progenitor cell-specific transgenic reporters in zebrafish. In our study we intend to characterize hematopoietic regulators in early stages of zebrafish development and in adults. Using ATAC sequencing analysis of adult kidney marrow progenitor cells and 72 hours post fertilization (hpf) embryo, we chose DNA sequences of a genomic region suspected of having enhancer activity, responsible for regulation of myeloid and erythroid specific genes. Selected sequences were cloned in front of β-globin minimal promoter which requires enhancer activity in order to express reporter gene. Reporter constructs for many putative erythroid and myeloid enhancers were examined in zebrafish embryos transiently. The use of enhancer sequences in combination with minimal promoter is a novel, alternative approach to generate transgenic line with stronger expression patterns in zebrafish. This transgene zebrafish lines could be utilized to study early stages of blood development as well as leukemia in adults. In addition, this approach could be used to express gene(s) of interest in transient manner as well.

P 102: CRISPR/Cas9 genome depletion reveals the crucial role of natterin-like for the early stages of embryonic zebrafish (Danio rerio) development

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Adolfo Luis Almeida Maleski[1,2], *Ana Carolina Seni-Silva*[1,2], *Milena Marcolino-Souza*[1], *Mônica Lopes-Ferreira*[1], *Carla Lima*[1]

[1] *Butantan Institute, Immunoregulation Unit of the Laboratory of Applied Toxinology CeTICs/FAPESP, São Paulo, Brazil*
[2] *Butantan Institute, Post-Graduation Program of Toxinology, São Paulo, Brazil*

ABSTRACT TEXT

Natterin is a family of proteins first found in the venom of *T. nattereri* containing in C-terminal region a pore-forming toxin with an aerolysin core important for membrane penetration and activation of inflammasome. Several Natterin-like protein sequences have been found in venomous and non-venomous fish species including zebrafish. In order to investigate the function of Natterin-like in the activation of inflammasome and innate immune response to infections in zebrafish, we used CRISPR/Cas9 depletion to create *natterin-like* zebrafish knockout larvae. To this end, we identified a functional gRNA targeting at the first exon of the *natterin-like* gene of the cromossome 7 (ID: 795232, XM_017356964, 966 bp) to synthesize the crRNA:TRAcrRNA *natterin-like* form that was co-injected (2–3 nL) with synthetic

Cas9 protein using a range of sgRNA:Cas9 doses (50:250; 50:125; 25:125, and 12.5 µg/nL:125 µg/nL) at 0 hpf embryos. Larvae were analyzed after 24, 48, 72 and 96 h by microscopy. First, we observed that while the highest doses kept 80 to 90 % of the larvae alive until 96 hpf compared with control larvae, the lowest doses led to 50 % mortality of morphants. All doses led to anatomical abnormalities such as development delay, yolk and heart edema, scoliosis, pigmentation and swimming bladder loss, and the increment of the doses promoted an increase in the incidence of the reported anomalies. The lowest dose caused heart edema, and the highest dose induced both sub-lethal or teratogenic defects. The dose-dependent morphant phenotypes persisted at 96 hpf with a decrease of 12.5 % in body length, 74 % in swimming bladder area, and 10 % in eye and head measurements, and alterations in heart rate. Finally, locomotory activity alterations were observed after injection of the 25:125 dose, indicative of neural effect. Together, our data suggested that *natterin-like* gene is crucial for the early stages of embryonic zebrafish development. This work was supported by FAPESP and by CAPES.

P 103: The NIPBL role in gene expression during development and pathogenic conditions

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Mara Mazzola[1], *Alex Pezzotta*[1], *Marco Spreafico*[1], *Anna Marozzi*[1], *Anna Pistocchi*[1]

[1] *University of Milan, Medical Biotechnology and Translational Medicine, Milan, Italy*

ABSTRACT TEXT

One of the main function of cohesin genes is the regulation of gene expression by chromatin remodelling and recognition of specific sites on the genome alone or in combination with different proteins. *NIPBL*, a cohesin-loading factor, is one of the five cohesin genes mutated in association with the Cornelia de Lange Syndrome, a rare multisystemic disorder. We generated a zebrafish model for *nipblb* knock-down and demonstrated a significant downregulation of *ccnd1*, a direct target of the canonical Wnt pathway. *CCND1* is also downregulated in the Cornelia de Lange syndrome patients with mutation in NIPBL, suggesting that a reduction of the Wnt signalling likely contributes to the disease. More recently, mutations in cohesion genes have been identified also in patients with Acute Myeloid Leukemia (AML), although they are usually secondary events that contribute to clonal expansion rather than driving oncogenic mutations. In particular, we demonstrated a correlation between mutations in *Nucleophosmin 1* (*NPM1*), one of the most frequently mutated gene in AML, and *NIPBL* both in human patients and zebrafish. In this model, we showed that *NPM1* mutations and nipblb downregulation, by modulating the canonical Wnt pathway, increased the number of myeloid precursors, a frequent features of myeloid diseases. Furthermore, we demonstrated that NIPBL exerted positive regulation of *RUNX1*, a master gene in haematopoiesis, in healthy donors, AML patients and zebrafish. To further address the role of NIPBL in gene expression regulation during haematopoiesis, we performed RNAseq analyses on zebrafish embryos following nipblb downregulation. We identified several genes differently expressed, including those related to the Cornelia de Lange syndrome, canonical Wnt pathway and master regulators of haematopoietic lineages, confirming the role of NIPBL during normal development but also highlighting its involvement in the insurgence of haematological diseases.

P 104: DANIO-CODE: Compendium and analysis of enhancers and promoters during zebrafish development

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Ferenc Mueller[1], *Damir Baranasic*[2], *Kadir Abdul Murrakam*[3], *Matthias Hoertenhuber*[3], *Piotr Balwierz*[2], *Ada Jimenez Gonzalez*[1], *Fabio d'Orazio*[2], *Tobias Zehnder*[4], *Ben Brown*[5], *Fiona Wardle*[6], *Monte Westerfield*[7], *Carsten Daub*[8], *Boris Lenhard*[2]

1 *University of Birmingham, Institute of Cancer and Genomic Sciences, Birmingham, United Kingdom*
2 *Imperial College London, Medical Research Council- London Institute of Medical Sciences, London, United Kingdom*
3 *Karolinska Institutet, Biosciences and Nutrition, Stockholm, Sweden*
4 *zehnder@molgen.mpg.de Max Planck Institute, Molecular Genetics, Berlin, Germany*
5 *Lawrence Berkeley National Laboratory, Molecular Ecosystems Biology, Berkeley, USA*
6 *King's College London, Randall Centre for Cell and Molecular Biophysics, London, United Kingdom*
7 *University of Oregon, Institute of Neuroscience, Eugene, USA*
8 *Karolinska Institutet, Biosciences, Stockholm, Sweden*

ABSTRACT TEXT

DANIO-CODE is an international effort to annotate the functional elements of the zebrafish genome. (https://www.birmingham.ac.uk/generic/danio-code/index.aspx). Published and unpublished genomics data of 37 labs was annotated and reanalyzed by standardised pipelines to create a track hub, which is currently available to upload to UCSC or Ensembl genome browsers at https://danio-code.zfin.org. Its current database contains over 1300 genome-wide datasets generated by 17 assay types (e.g. ChIP-seq, RNA-seq, ATC-seq, CAGE-seq etc.) from 38 stages of development (https://www.birmingham.ac.uk/generic/danio-code/index.aspx). As a first output from this community resource we comprehensively identify cis-regulatory elements by integrating a range of transcriptome, chromatin modification, topology and chromatin accessibility datasets during embryo development. We classify promoters into distinct classes based on their architecture and developmental usage characteristics and demonstrate dynamic use of alternative promoters, which need to be considered in genome editing designs aiming to block transcription. By using machine learning, we successfully obtain characteristic footprints strongly separating active promoters and enhancers from other accessible regions, and to more accurately delineate and classify enhancers into active and poised states. Finally, we explore the apparent conservation of epigenetic marks and chromatin topology organization on the level of TADs and predictability of sub-TAD topologies between fish and mammals.

P 105: Identifying tissue-specific enhancers of Prox1a in zebrafish

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Virginia Panara[1], *Tatjana Haitina*[2], *Kaska Koltowska*[3]

1 *Uppsala Univeristy, Igp, Uppsala, Sweden*
2 *Uppsala University, Ebc, Uppsala, Sweden*
3 *Uppsala University, Igp, Uppsala, Sweden*

ABSTRACT TEXT

Spatiotemporal control of gene expression is dependent on complex upstream regulation and specific transcription factor binding. It allows expression of a gene in specific tissues and can regulate processes such as cell-type specification. Identifying the enhancers driving gene expression is one of the methods to uncover upstream regulators and allow detailed characterization of their function. The zebrafish model is an excellent tool for rapid enhancer screens *in vivo*, and for generating reporter lines.

Little is known about the enhancer elements regulating *prospero-related Homeobox 1a* (*prox1a)*. This gene has a complex expression pattern in tissues originating from different germ layers, such as the retina (ectodermal), liver and pancreas (endodermal) and muscles and lymphatic vessels (mesodermal). Here, I describe a pipeline to identify enhancer elements regulating this expression.

To identify candidate sequences, we have performed *Mvista* analysis and uncovered conservation peaks in non-coding DNA of the Prox1 gene region spanning 379 mb. Those sequences were verified with histone methylations associated with open regions of chromatin. We have identified 10 candidate sequences and cloned them into the ZED vector (Bessa et al., 2009). For each sequence, the plasmid was injected into one-cell stage zebrafish and embryos were screened at 48 hpf for positive GFP and RFP signal. The F0 adults were screened for founders by fluorescence microscopy and expression was confirmed with confocal microscopy. F1 zebrafish generated

from the founders were raised to further characterize the expression in stable lines. Of the generated transgenic reporter lines, 7 showed spatially and temporally specific expression, coherent with published data of Prox1a.

This method constitutes a fast, efficient way of testing candidate enhancers to identify spatiotemporal expression drivers, which led us to generate a comprehensive resource for studying the expression and regulation of Prox1a.

P 106: DNA methylation signatures of Vitamin K-related genes as Cancer Biomarkers: lessons from the Zebrafish

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Vânia Roberto[1], *Gil Martins*[1], *Michael Viegas*[2], *Paulo J. Gavaia*[1]

[1] *Center of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *SPAROS, Lda, Olhão, Portugal*

ABSTRACT TEXT

Vitamin K (VK)-related proteins participate in vital extra-hepatic processes, including bone metabolism, calcification, inflammation and carcinogenesis, but information on regulation by epigenetic factors is scarce. DNA methylation is an epigenetic modification known to regulate gene expression and to be influenced by nutrition, drugs and lifestyle, being frequently deregulated in disease.

Here we investigated (i) DNA methylation impact on VK-related genes expression, (ii) altered methylation patterns by VK1 dietary supplementation and (iii) methylation signatures of VK-related genes as potential cancer biomarkers.

Zebrafish juveniles were fed for 90 days with VK1 enriched diets (0 and 400 mg VK1/kg). Gene expression and DNA methylation of VK-dependent proteins (*f2, bglap*), VK cycle enzymes (*vkorc1, vkorc1l1, ggcx*) and VK receptor (*nr1i2*) were analyzed in specific tissues.

HCT116 DNMT3B/DNMT1 double knockout cells (demethylation *in vitro* model) were used to confirm the impact of DNA methylation on the expression of F2, BGLAP and NR1I2, the most promising genes identified in zebrafish.

Finally, F2, BGLAP and NR1I2 methylation status were compared in normal and tumor tissues from 6 cohorts at The Cancer Genome Atlas. F2 and NR1I2 had at least one probe differentially methylated in 5 cohorts and BGLAP in 3 cohorts. The vast majority of identified probes had an Area under the ROC curve >0.8 indicative of good diagnostic biomarkers. Kaplan-Meier curves evidenced NR1I2 methylation as good overall-survival (OS) and Recurrence-Free Survival predictor in pancreatic cancer (p-value=0.006 and 0.0125, respectively). BGLAP probe was an OS prognostic biomarker in colorectal cancer (p-value=0.0062). All probes presented higher sensitivity and specificity than the biomarkers currently used in the clinic.

Financial support from the FCT (UIBD/04326/2020) and European Maritime and Fisheries Fund (EMFF/FEAMP) through the National Operational Programme MAR2020 (ALGASOLE-16-02-01-FMP-0058).

P 107: Elucidating the genetic regulatory network underlying heart pacemakers using DamID and ATAC-seq

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Agata Sulej[1], *Maciej Łapiński*[1], *Karim Abu Nahia*[1], *Cecilia Winata*[1,2]

[1] *International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Zebrafish Developmental Genomics, Warsaw, Poland*
[2] *Max-Planck Institute of Heart and Lung Research, IIMCB Research Group, Bad Nauheim, Germany*

ABSTRACT TEXT

Rhythmical contractions of the heart are regulated by the electrical impulses generated by the pacemakers- sinoatrial node (SAN) and atrioventricular node (AVN). The molecular mechanism of pacemaker development and function is still insufficiently determined. The goal of the project is to elucidate the gene regulatory network downstream of the transcription factors Tbx3, Tbx2, and Isl1, which have been shown to be responsible for suppression of the cardiomyocyte genetic program and promoting development of the pacemaker cells. As the first step towards this, we are attempting to establish the DamID-seq method to identify their genome-wide binding sites in whole embryos .In combination with ATAC-seq profiling of the chromatin landscape in specific populations of cells isolated from the pacemaking regions expressing EGFP in transgenic lines ex. ET33-mi59B, we hope to identify target genes regulated by these transcription factors

and determine the mechanism for pacemaker development and function. This in turn will facilitate the generation of zebrafish models for cardiac arrhythmia and biological pacemakers.

The project First TEAM/2016 -1/8 is carried out within the First Team program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

P 108: Functional analysis of isl1 regulatory regions in zebrafish embryos

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Eugeniusz Tralle[1,2], *Cecilia Winata*[1,3]

[1] *International Institute of Molecular and Cell Biology, Laboratory of Zebrafish Developmental Genomics, Warsaw, Poland*
[2] *Postgraduate School of Molecular Medicine, Postgraduate School of Molecular Medicine, Warsaw, Poland*
[3] *Max Planck Institute for Heart and Lung Research, IIMCB Research Group, Bad Nauheim, Germany*

ABSTRACT TEXT

Early steps of zebrafish heart organogenesis rely on migration and differentiation of cells derived from two lateral heart fields. Whereas the first heart field (FHF)-associated cells migrate to the midline and fuse into a linear heart tube relatively early in embryonic development, subsequent processes rely on continuous addition of second heart field (SHF)-derived cardiac progenitors (CPs) to the venous and arterial poles of the growing heart. We aim to delineate the contribution of the SHF-derived progenitors and trace the process leading to the establishment of various structures of the heart. To this end, we are attempting to generate a transgenic line which labels a specific population of SHF progenitors. The *isl1* transcription factor is expressed in a variety of embryonic tissues including the SHF progenitors population. In order to find regions that could drive tissue-specific *isl1* expression in the SHF, we cross-referenced ChIP-seq and ATAC-seq datasets deposited in public repositories. We then performed a transient enhancer assay using the e1b-GFP-Tol2 vector, followed by establishing stable transgenic lines with GFP expression patterns corresponding to the SHF. Out of the 11 candidate enhancer regions tested, we identified 7 with tissue-specific GFP expression, of which 3 express GFP in the heart or associated regions. Our results suggest that the enhancer regions may regulate different aspects of *isl1* expression pattern.

P 109: Identification of common ovarian and testicular miRNAs among cultured fish species

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Tosca Van Gelderen[1], *Jose Antonio Alvarez-Dios*[2], *Jerome Montfort*[3], *Violette Thermes*[3], *Francesc Piferrer*[1], *Julien Bobe*[3], *Laia Ribas*[1]

[1] *Institute of Marine Sciences, Group of Biology of Reproduction, Barcelona, Spain*
[2] *University of Santiago de Compostela, Department of Applied Mathematics, Santiago de Compostela, Spain*
[3] *French National Institute for Agriculture- Food and Environment, Laboratory of Physiology and Genomics of Fish, Rennes, France*

ABSTRACT TEXT

MicroRNAs (miRNAs) are important post-transcriptional regulators of gene expression in a wide variety of physiological processes. In cultured fish, it has been shown that miRNAs play a role in the reproductive system, where gonad-specific miRNAs are involved. In order to identify common aquacultured markers for early sex-development and to improve reproduction of farmed fish, we compared our zebrafish data with published data of sex-specific gonadal miRNAs to four farmed fish species (i.e., Atlantic cod, catfish, Nile tilapia and European sea bass) together with available data for transgenic zebrafish. In our fish facilities, AB zebrafish were kept at standard husbandry conditions until adulthood when ovaries and testes were dissected. miRNAs were isolated and specific RNA libraries were prepared and sequenced by Illumina technology (50 bp 1 x 50 bp, v4, HiSeq). About 16 million clean reads were obtained from gonadal samples. After determining differentially expressed miRNAs in ovary versus testis, we found 18 significantly upregulated miRNAs specific for ovaries and 25 for testes. Comparing our data with transgenic zebrafish miRNAs, we found one common differentially expressed miRNA specific for each sex: dre-miR-146b-5p in the ovary and dre-miR-212-5p in the testis. FISH analysis revealed the expression of miR146 in the germ cells but not in supporting cells of the ovaries. Significantly expressed miRNAs (normalized reads >100) from the five teleost fish species showed 31 common miRNAs in the gonads, in which five miRNAs were specific for ovaries and other five for testes. Although further

research is needed, these common and sex-specific miRNAs in several cultured fish species reveal potential miRNA biomarkers for early development and sex differentiation. Functional studies will demonstrate the molecular mechanism behind these miRNAs and their role in sex differentiation.

P 110: Mechanisms of hematopoietic cell reprogramming

TOPIC: EPIGENETICS, GENE REGULATION AND GENOMIC RESOURCES

Zuzana Vondráková[1], *Jana Oltová*[1], *Ondřej Svoboda*[1], *Olga Machoňová*[1], *Petr Bartůněk*[1]

[1] *Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of Cell Differentiation, Prague, Czech Republic*

ABSTRACT TEXT

Hematopoiesis is the process in which mature hematopoietic cells are generated by proliferation and differentiation of hematopoietic stem cells. According to the hierarchical model of hematopoiesis, multipotent hematopoietic stem cells can undergo self-renewal and differentiation into erythroid, myeloid and lymphoid lineages. However, in contrast to this hierarchical model, the hematopoietic cells can follow alternative paths during their differentiation. Moreover, it has been shown that, under certain conditions, already committed cells can be reprogrammed or can transdifferentiate into other cell type.

Here, we are using unique model of immortalized zebrafish erythroid progenitor cell line (ZEB) to reprogram erythroid cells into myeloid lineage. The ZEB cells were originally obtained from the whole kidney marrow culture of adult gata1:dsRed transgenic zebrafish. Their proliferation and survival are dependent on erythropoietin (Epo). However, the gradual replacement of Epo by granulocyte-colony stimulating factor (Gcsf) – important cytokine for granulocytes and stem cell production, the cells undergo erythro-myeloid switch. After 14 days of reprogramming, the cells grown in the presence of Gcsf lose erythroid markers and change their morphology. Based on qPCR, we observed that during time the expression of myeloid marker genes increases on the expanse of erythroid markers.

To understand the molecular mechanisms of this reprogramming switch, we focused on the analysis of open chromatin (ATAC-seq) and epigenetic signatures (ChIP-seq). Changes at the chromatin level showed specifically activated and silenced genes that might be important for this reprogramming process and might serve as regulators of downstream erythroid/myeloid signaling pathways. In summary, the ZEB cell line can be used as a model to better understand molecular mechanisms involved during cell reprogramming as well as a model to study malignancies (leukemia).

Evolution

P 111: Haematopoiesis in sea lamprey

TOPIC: EVOLUTION

M. Kovář[1], *P. Bartůněk*[1]

[1] *Czech Academy of Sciences, Institute of Molecular Genetics, Prague, Czech Republic*

Hematopoiesis is specific process in vertebrates during which terminally differentiated blood cells with specific functions arise from a hematopoietic stem cells (HSC) through different progenitors. Now, we have a deep knowledge about the hematopoiesis in standard vertebrate models, but in the field of the evolution of hematopoiesis our knowledge is limited. The main reason is that process of the hematopoiesis is well conserved. In all standard vertebrate models, we can identify the same key regulatory genes/proteins involved in the regulation of hematopoiesis as well we can observe the same blood cell types. Therefore, in order to get to the roots of hematopoietic cell lineage evolution, we need to employ an unconventional animal model(s) such as sea lamprey, which is the representative of a jawless taxon, also being the most ancestral vertebrate living up to date. The aim of this project is to map the hematopoiesis in sea lamprey on cellular and genetic level with special attention to the embryonic development.

P 112: Evolution of heterotypic cell interactions underlies colour pattern diversification in Danio species

TOPIC: EVOLUTION

Marco Podobnik[1], *Hans-Georg Frohnhöfer*[1], *Christopher Dooley*[1], *Anastasia Eskova*[1,2], *Christiane Nüsslein-Volhard*[1], *Uwe Irion*[1]

[1] *Max Planck Institute for Developmental Biology, eCNV, Tübingen, Germany*
[2] *IBM Deutschland Research und Development GmbH, IBM Systems, Böblingen, Germany*

ABSTRACT TEXT

The genetic basis of morphological variation provides a major topic in evolutionary biology. The genus *Danio* displays a striking variation of colour patterns ranging from horizontal stripes, to vertical bars or spots. In zebrafish, *Danio rerio*, the strict horizontal orientation of the stripes depends upon the horizontal myoseptum as anatomical landmark, otherwise stripe formation is a self-organizing process based on cell-contact–mediated interactions among three different types of chromatophores. Patterning mutants from *D. rerio* provide candidate genes that might have evolved to contribute to the differences observed in *Danio* species. Here, we show that stripe or bar formation differentially depend on the horizontal myoseptum, but self-organization is a common mechanism of pattern formation. Several mutations in the barred sibling species, *Danio aesculapii*, lead to phenotypes that indicate species-specific differences in the interactions between chromatophores. Using a reciprocal heterozygosity test in interspecific hybrids, we identify an evolved gene, which regulates heterotypic cell interactions in both species but contributes to patterning differences between species. Our results highlight the power of in vivo genetic tests, which are now possible in non-model organisms by CRISPR/Cas9–mediated reverse genetics, to identify loci that underlie morphological variation of complex traits in vertebrates.

Genomic Resources

P 113: Genotype-dependent differences in gonadal transcriptomes for spontaneous and temperature-induced neomales in wild zebrafish populations

TOPIC: GENOMIC RESOURCES

Alejandro Valdivieso[1], *Catherine Wilson*[2], *Angel Amores*[2], *Maira da Silva Rodrigues*[3], *Rafael Henrique Nóbrega*[3], *Peter Bazel*[2], *Laia Ribas*[1], *John H. Postlethwait*[2], *Francesc Piferrer*[1]

[1] *Institut de Ciències del Mar- Consejo Superior de Investigaciones Científicas CSIC, Department of Renewable Marine Resources, Barcelona, Spain*
[2] *Institute of Neuroscience, University of Oregon, Eugene, USA*
[3] *Institute of Biosciences, Department of Structural and Functional Biology, São Paulo, Brazil*

ABSTRACT TEXT

Sex determination in fish is a labile trait easily influenced by environmental factors, often temperature. A mismatch between genotypic and phenotypic sex usually tends towards genotypic females developing as males referred to as neomales. Neomales are being discovered in wild populations of several fish species. Thus, understanding the genetic basis leading toward neomales is of great interest in a global climate change scenario. Wild zebrafish strains possess a ZZ/ZW chromosomal sex determination system, in contrast to laboratory strains, which lack a Z chromosome and assume a polygenic sex determination mechanism. Whether temperature alters the sex ratio in wild strains resulting in neomales and whether the gonadal transcriptome differs between genetic males (ZZm) and neomales (ZWm) is unknown. We assessed the rate of sex reversal in Nadia and EkkWill wild strains using different families exposed to 28 °C (control) and 34–36 °C (masculinizing) temperatures during sex. In adults, histomorphometrics and transcriptomic analyses by RNA sequencing provided insights into gonadal morphology and function. Results showed, surprisingly, that both wild strains were at least as susceptible

to the masculinizing effects of elevated temperature as the laboratory strain, with abundant spontaneous and temperature-induced neomales, although the two natural strains had different genotype-by-environment interactions. Histologically, Nadia ZWm but not ZZm showed a higher number of spermatozoa after exposure to elevated temperature. Transcriptomic results had a strong family influence. Further, ZWm testes had a transcriptome profile indistinguishable from ZZm testes. Taken together, these results suggest a genetic basis underlying both the production of neomales and their response to temperature with potential functional consequences in reproductive capacity. Identifying the genes involved will improve our understanding of the mechanisms of zebrafish sex determination.

Germ Line, Early Development and Patterning

P 114: Wt1 contributes to blood-cerebrospinal fluid barrier function in zebrafish

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Vera Hopfenmüller[1], *Birgit Perner*[1], *Christoph Englert*[1]

[1] *Leibniz Institute on Aging - Fritz Lipmann Institute FLI, Molecular Genetics Lab, Jena, Germany*

ABSTRACT TEXT

The Wilms tumor suppressor gene *WT1* encodes a zinc finger transcription factor, which is highly conserved among vertebrates. It is a key regulator of urogenital development and plays multiple roles during embryonic development. Inactivation of *WT1* in humans leads to Wilms tumor, a pediatric kidney cancer, as well as to other genitourinary disorders. In contrast to mammals, bony fish possess two paralogous *Wt1* genes, namely *wt1a* and *wt1b*. Using zebrafish as an animal model, a substantial amount of work has been directed to elucidate the function of the *wt1* genes for kidney and heart development, maintenance as well as regeneration. After performing detailed expression studies via whole mount *in situ* hybridization we have discovered a new expression domain of *wt1a* in the dorsal hindbrain of zebrafish larvae. Based on marker analyses we identified the *wt1a* expressing cells to be ependymal cells of the fourth ventricle choroid plexus. The choroid plexus is a component of the blood-cerebrospinal fluid barrier, which plays a role in protecting the brain but also produces cerebrospinal fluid and regulates its composition. By injecting fluorescent tracers into respective mutants, we demonstrate that *wt1a* contributes to the barrier properties of the choroid plexus. This work is to the best of our knowledge the first to identify Wt1 in the zebrafish brain and to characterize its function.

P 115: C-ECi: A CUBIC-ECi combined clearing method for 3D imaging of the fish ovary and follicular content analysis

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Manon Lesage[1], *Manon Thomas*[1], *Jérôme Bugeon*[1], *Adèle Branthonne*[1], *Stéphanie Gay*[1], *Emilie Cardona*[1], *Marie Haghebaert*[2], *Fabrice Mahé*[2], *Julien Bobe*[1], *Violette Thermes*[1]

[1] *INRAE, Fish Physiology and Genomics Institute LPGP, Rennes, France*

[2] *Université de Rennes- CNRS, Institut de recherche mathématique de Rennes IRMAR, Rennes, France*

ABSTRACT TEXT

Oogenesis is a complex cellular process, during which germ cells (or oocytes) proliferate, differentiate and become progressively surrounded by somatic cells to form ovarian follicles that grow until egg maturity. For several decades, many efforts have been made to improve the understanding of these mechanisms and their regulation in fish. However, in terms of timing, a comprehensive overview of the dynamics of oocyte growth and recruitment is still lacking at the level of the entire organ. Follicular content analyses have indeed been mainly based on 2D histological sections data, which remain challenging to extrapolate to the whole ovary. More recently, numerous tissue-clearing

methods have been developed for 3D imaging of entire organs, but no attempts have been reported in fish ovary that provides specific challenges, due to the high lipid content of growing follicles. Here, we established a novel clearing protocol combining solvent-based (ECi) and aqueous-based (CUBIC) clearing methods, which allows optimizing fluorescent staining and 3D imaging of ovarian follicles. Using this new protocol (called C-ECi), we succeeded in describing and analyzing the follicular content in both medaka and trout ovaries after 3D segmentation analyses. Thus, we provide for the first time exhaustive and quantitative data on follicles enclosed in fish ovary. Combined with immunostaining techniques, this promising procedure will be useful in subsequent functional studies to improve the knowledge of ovarian fish biology. This novel method now opens new perspectives for further understanding the biology of the fish ovary and should provide interesting new insights on follicular dynamics.

P 116: Analyzing the role of miR-202 in granulosa cells proliferation in the medaka (Oryzias latipes) ovary using 3D imaging

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Manon Lesage[1], *Jerôme Bugeon*[1], *Stéphanie Gay*[1], *Manon Thomas*[1], *Julien Bobe*[1], *Violette Thermes*[1]

[1] *INRAE, Fish Physiology and Genomics Institute LPGP, Rennes, France*

ABSTRACT TEXT

Female gamete production relies on well-coordinated molecular and cellular processes controlling the oocyte development (oogenesis). Growth and maturation of these germ cells are supported by somatic cells (granulosa cells) that progressively surround them, thus forming follicles. In fish, as in other vertebrates, these complex mechanisms have been extensively studied both in terms of endocrine/paracrine regulation, secreted factors and intrinsic signaling pathways. The role of small non-coding RNAs in this regulation remains however largely unknown and poorly investigated in fish. The microRNA 202 (miR-202) is one of the most highly expressed miRNAs in the fish ovary and is specifically expressed in granulosa cells of developing follicles in the medaka ovary. The mutant CRISPR medaka line, depleted of miR-202, exhibits low fecundity, low egg fertilization rate and impaired follicular growth. Here, we used advanced 3D imaging techniques (ECi clearing and confocal microscopy) combined with immunofluorescent staining (anti-phospho-Histone-3) to analyze granulosa cells proliferation in the mutant ovary. The total number of dividing cells (pH3+ cells) in whole ovaries was determined using a computational semi-automatic 3D segmentation procedure. Results showed that the proliferation rate tends to increase in mutant ovaries compared to wild type ovaries, suggesting that mir-202 plays a critical role in female gametes formation through the regulation of granulosa cells proliferation/differentiation.

P 117: Activation of Matrix Metalloproteinase 13 in Danio rerio and its role in collagen remodeling in vivo

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Matthew Mabey[1], *Bryan Crawford*[1]

[1] *University of New Brunswick, Biology, Fredericton, Canada*

ABSTRACT TEXT

Living tissues are made not only of cells but also a surrounding extracellular matrix (ECM) which is continuously secreted and modified. Cells modify the ECM using enzymes called Matrix Metalloproteinases (MMPs) which are essential for normal tissue remodeling that occurs during development and wound healing. These enzymes are also activated in many diseases that involve abnormal tissue remodeling like arthritis, heart disease, cancer and diabetes. Collagens are abundant proteins in the ECM, and the MMPs that degrade them are called collagenases. Unfortunately, we know very little about how collagenases are regulated in living tissues. Zebrafish have only one type of collagenase – called Mmp13 – and I have developed molecular tools that allow me to observe and measure its activation using immunoblots of embryo homogenates, as well as in situ in intact zebrafish embryos using the Epitope Mediated MMP Activation (EMMA)-assay. Furthermore, using a collagen hybridizing peptide (CHP) that binds denatured collagen, I have correlated this data with Mmp13 activation patterns detected using the EMMA assay. Using the results of my project I plan on analyzing the effects of pharmacological

inhibitors of specific classes of proteases, I hope to identify the molecules activating Mmp13 in living tissues. Finally, it has recently been shown that excess Mmp13 activity is responsible for peripheral diabetic neuropathy, so I plan to apply EMMA assay to determine if high glucose concentrations affect the post-translational activation of Mmp13 in vivo.

P 118: Uracil in the DNA during the early development of zebrafish

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Kinga Nagy[1,2], Latifa Kazzazi[3], Éva Surányi[1,2], Dorottya Magyari[3], Kornélia Kulcsár[1], Viktória Perey-Simon[1], Máté Varga[3], Beáta Vértessy[1,2]

[1] *Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Budapest, Hungary*
[2] *RCNS, Institute of Enzymology, Budapest, Hungary*
[3] *ELTE Eötvös Loránd University, Department of Genetics, Budapest, Hungary*

ABSTRACT TEXT

The presence of uracil in DNA is usually the sign of erroneous polymerase activity (dUTP being incorporated instead of dTTP) or cytosine deamination. As the latter results in mutagenic U:G mismatches in the double helix, cells have evolved error repair mechanisms to ensure that such mismatches are repaired promptly, and the freely available dUTP pool is kept at relatively low steady-state levels, to prevent uracil incorporation into the DNA. Recent data suggests that the impairment or absence of the two enzymes that guard the cell from permanent uracil accumulation, uracil–DNA glycosylase (UNG) and dUTPase, can have profound morphological consequences on the development of animals.

Using zebrafish (*Danio rerio*) as a model we explore the dynamics of uracil incorporation into the DNA during development, and show that prior to the activation of the zygotic genome (MZT) uracil levels are significantly increased and only later reduced. This suggests the presence of an active regulatory mechanism that functions only in the post-MZT stages. We also show that the dNTP pool undergoes dynamic changes during early stages of development, indicating a specific regulatory mechanism for dNTP levels in the zebrafish embryo.

P 119: The dynamic life of the thyroid gland: Environmental cues regulating development and function of the thyroid follicular cells

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Macarena Pozo Morales[1], Ines Garteizgogeascoa Suner[1], Pierre Gillotay[1], Meghna Shankar[1], Sema Elif Eski[1], Valérie Wittamer[1], Sabine Costagliola[1], Sumeet Singh[1]

[1] *IRIBHM, Université libre de Bruxelles, Brussels, Belgium*

ABSTRACT TEXT

Cells do not exist in a vacuum. They are constantly communicating and interacting with their environment. Development and homeostasis requires various local cell-cell interactions at the molecular and mechanical levels. To develop an integrated model of organ formation and function, we utilize the zebrafish thyroid gland as it provides a simple and tractable model. To understand the various interactions occurring in the zebrafish thyroid gland, we developed an *in silico* molecular connectome, and focused on the role of immune and stromal cells on the physiology of the endocrine organ.

For this, we first developed the single-cell mRNA atlas of the thyroid gland, which yielded the profile of 6249 cells. The profiling provided the molecular diversity within the thyroid follicular cells, the functional unit of the endocrine gland, along with transcriptional signature of endothelial, immune and stromal cells. Notably, the atlas highlighted unique molecular differences between blood and lymphatic vessels. Moreover, we observed six unique patterns of gene signature covering the mesenchymal fibroblast of the stromal compartment. GO analysis revealed exclusive functionality to each gene signature (ECM deposition, immune modulation, amino acid metabolism), thereby suggesting that the fibroblast population employs division of labor to fulfil the various tasks that it is required to accomplish. Additionally, we identified fibroblast secreted *decorin* as a potential regulator of thyroid gland's growth. Finally, we observed the existence

of macrophages in the thyroid gland, potentially regulated by CXCL12- CXCR4 signaling axis. Live imaging demonstrated macrophage presence in the gland from 2 dpf, raising an exciting possibility on the role of immune cells in thyroid morphogenesis.

Our results identify the complex choreography undertaken by different cell-types to build and organize an organ, and shed lights on mechanisms that can lead to organ dysfunction and failure.

P 120: Ctgfa plays a positive role in skeletal muscle development of zebrafish (Danio rerio)

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Amey Rayrikar[1], *Chinmoy Patra*[1]

[1] *MACS-Agharkar Research Institute, Developmental Biology Group, Pune, India*

ABSTRACT TEXT

Cellular communication network factor 2 (CCN2), also known as connective tissue growth factor (CTGF) is a highly conserved modular protein of vertebrate origin. It has four domains *viz.* IGFB, vWC, TSP1, CT. It plays role in cell migration, cell-cell adhesion, angiogenesis, wound healing, chondrogenesis, skeletogenesis etc. *Ctgf* knockout mouse die immediately after birth due to lung collapse and also display hyperchondroplasia. Thus, it is not possible to study the role of Ctgf in maintenance and aging using *Ctgf* global knockout mouse. We used Zebrafish (*Danio rerio*) to study role of Ctgf in development and tissue homeostasis. Zebrafish has two paralogs of *ctgf viz. ctgfa* and *ctgfb*. In situ hybridization based spatio-temporal mRNA expression analysis suggests that *ctgfa* expresses in floor plate, notochord, adaxial cells, somites and heart. To dig into the role of Ctgf in embryonic development and tissue maintenance, we used deletion mutants of *ctgfa*. Seven base pair deletion in *ctgfa*$^{-/-}$ allele led to frame shift followed by insertion of a stop codon. Unlike global mouse knockout, zebrafish *ctgfa*$^{-/-}$ are viable and survive to adulthood. *ctgfa*$^{-/-}$ showed curved body phenotype at 2 days post fertilization (dpf) but recovered by 3 dpf. As *ctgfa* expresses in adaxial cells and somites; curving of the body might be related to abnormal muscle formation or development. To check this we performed immunostaining on skeletal muscles. Immunostaining reveals that *ctgfa*$^{-/-}$ embryos have defective skeletal muscle development at 30 hpf. To identify the reason behind the recovery of curved body phenotype, we analyzed *ctgfb* expression level at 33 hpf. Our quantitative expression analysis suggests, *ctgfb* transcripts get up-regulated in *ctgfa*$^{-/-}$ by ~1.5 fold. This suggests that Ctgfb might take over the role of Ctgfa in *ctgfa*$^{-/-}$ embryos, which needs to be explored. Collectively our data suggests that Ctgfa plays a role in development of skeletal muscles in zebrafish.

P 121: Jag1a-her6/her9 lateral inhibition patterns the zebrafish notochord

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Hector Sanchez-Iranzo[1], *Jonas Hartmann*[2], *Alba Diz-Muñoz*[1]

[1] *EMBL, Cell Biology and Biophysics, Heidelberg, Germany*
[2] *EMBL, Cell Biology and Biophysics / Developmental Biology, Heidelberg, Germany*

ABSTRACT TEXT

Organ development requires tight regulation for precursors cells to differentiate into the various cell types in the correct proportion and location. During development, this occurs thanks to complex gene regulatory networks that generate diverse patterns of gene expression which in turn determine fate. One of such signaling pathways is Notch, which generates both homogeneous (lateral induction) or salt-and-pepper alternating patterns (lateral inhibition) by signaling at cell-cell contacts. While the generation of these patterns has been extensively studied from the theoretical point of view, their connection to experimental studies remains limited. To quantitatively understand the Notch pathway, we use the zebrafish notochord. This organ constitutes the structural axis of the embryo, and is initially formed by unidimensionally arranged coin-shaped precursors, that will give rise to two different cell types: vacuolated and sheath cells. This cell fate decision is based on Notch signaling. Using mathematical modeling and newly generated fluorescent transgenic reporters, we show that contrary to currently thought the Notch ligand *jag1a* generates a lateral inhibition pattern. Moreover, we identify the notch target genes *her6* and *her9* as key downstream players, that repress *jag1a* transcription. In addition, we find that overexpression of *her6*

or *her9* is sufficient to determine sheath cell fate. With this work we describe a new gene regulatory network that generates a lateral inhibition pattern and establish the notochord as a new model system to study Notch signaling.

P 122: In vivo live imaging of signalling dynamics and pattern formation during mesendoderm induction

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Scott Wilcockson[1], *Martin Distel*[2], *Jacques Bothma*[3], *Caroline Hill*[1]

[1] *Developmental Signalling Lab, The Francis Crick Institute, London, United Kingdom*
[2] *Innovative Cancer Models, Children's Cancer Research Institute, Vienna, Austria*
[3] *Hubrecht Institute, Royal Netherlands Academy of Arts and Sciences and UMC Utrecht, Utrecht, Netherlands*

ABSTRACT TEXT

During embryonic development, the primordial germ layers undergo continuous subdivision through pattern induction and refinement coupled with morphogenesis. This concerted specification of cell fates can be monitored through the up- or down-regulation of key fate markers, in particular the transcription factors that drive these fate changes. In zebrafish, the mesendoderm is specified around the embryonic margin by Nodal and FGF signalling. Endodermal cells are specified within the first two cell tiers from the yolk syncytial layer, marked by the expression of the Nodal target gene *sox32,* and are intermingled with mesoderm which generates a salt-and-pepper pattern. However, it remains unclear how this heterogenous pattern is induced when the cells at the margin seemingly receive the same mesendoderm-inducing signals. Our understanding of the induction and resolution of the endodermal and mesodermal lineages has to date been limited to 'snap-shot' views of development and current live reporters are hampered by the lag-time in fluorescent protein folding. We therefore aim to utilise novel technologies to achieve the high-temporal resolution necessary to monitor embryonic patterning *in vivo*. The work described here shows how we are translating the LlamaTag system, previously described in *Drosophila,* as well as using the MS2 system to monitor the emergence of endodermal cells at the transcriptional and protein level downstream of Nodal and FGF signalling. Here we present data describing the LlamaTagging of the endodermal markers *sox32* and *sox17* and test the functionality of these constructs *in vivo*. In addition, we are modifying the DREKA (Dynamic Reporter for Erk Activity) signalling reporter to reduce off target Cdk1 activity and increase Erk specificity. Together these tools, along with other available reporters of signal pathway activity, will enable us to investigate the role of signalling dynamics and cellular heterogeneity in mesendodermal patterning.

P 123: A link between changes in germ cell divisions and germ plasm segregation in early zebrafish embryos

TOPIC: GERM LINE, EARLY DEVELOPMENT AND PATTERNING

Andreas Zaucker[1], *Pooja Kumari*[2], *Roland Dosch*[3], *Karuna Sampath*[1]

[1] *University of Warwick, Warwick Medical School- Division of Biomedical Sciences, Coventry, United Kingdom*
[2] *University of Oslo, Department of Biosciences- Section for Biochemistry and Molecular Biology, Oslo, Norway*
[3] *University of Göttingen, University Medical Center- Institute of Human Genetics, Göttingen, Germany*

ABSTRACT TEXT

The germline gives rise to the sperm and eggs, and ensures continuity of sexually reproducing species. In zebrafish, the germline progenitors, primordial germ cells (PGCs), are specified by "germ plasm", a phase separating condensate of RNAs and proteins that protects PGCs from acquiring somatic fates. How germ plasm is segregated to ensure correct PGC numbers is not well understood. We find that maternal mutants affecting the RNA-binding protein Y-box binding protein 1 (Ybx1) exhibit male bias, and *ybx1* mutant embryos have reduced germ plasm and PGCs. To investigate the underlying basis for the germline defects, we examined germ plasm dynamics in wild type and *ybx1* mutant embryos by live-imaging of reporters using lattice light sheet microscopy and spinning disk confocal microscopy. In wild type embryos, during cleavage stages, germ granules form large aggregates that segregate asymmetrically between daughter cells. At mid-blastula stages, germ plasm segregation is symmetric, with large germ granules positioned first at the cortex during mitosis, and then at the division site for a long duration, before the aggregates are sliced medially by the cytokinetic apparatus. During gastrulation, PGCs switch between asymmetric and symmetric modes. Germ granules are fragmented in the gastrula, and the residence time of the largest germ granule at the division site is very short, with asymmetric segregation to one daughter cell. Asymmetric germ

plasm segregation in dividing PGCs during gastrulation is associated with continued rotation of the mitotic spindle during metaphase and anaphase. By contrast, during symmetric cell divisions, spindle rotation is observed during metaphase, but not at anaphase. In *ybx1* mutant embryos, germ granules are significantly smaller and fragmented even at blastula stages, and the frequency of asymmetric cell divisions seems to be increased. Our findings suggest a link between changes in PGC cell divisions and germ plasm segregation mode.

Husbandry and Aquaculture

P 124: Male broodstock fed rotifers encapsulated with different microalgae: gamete quality and transgenerational osteological alterations

TOPIC: HUSBANDRY AND AQUACULTURE

Daniela Castro[1], Gil Martins[1,2], Tamára Santos[1], Patrícia Diogo[1,3], Marco Tarasco[1], Alexandre Rodrigues[3], Victória del Pino[3], João Navalho[3], João Varela[1,4], Elsa Cabrita[1,4], Hugo Pereira[1], Paulo J. Gavaia[1,2]

[1] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal*
[3] *Necton SA, Research and Development Unit, Olhão, Portugal*
[4] *Faculty of Sciences and Technology, University of Algarve, Faro, Portugal*

ABSTRACT TEXT

Broodstock diet is known to influence fish gonad maturation, gamete quality and larval development. Several studies have shown the important paternal contribution to the offspring quality. In the last decade, microalgae potential for aquafeeds has increased due to their nutritional content. Microalgae encapsulation in rotifers is commonly used in zebrafish larviculture. However, little is known on their role in adult breeding performance and larval quality, particularly male contribution to the offspring. Thus, we have investigated the dietary effects on zebrafish males fed with rotifers enriched with *Nannochloropsis* sp., *Haematococcus pluvialis*, *Tisochrysis lutea*, *Dunaliella salina*, and a blend thereof (25 % each). Reproductive success was evaluated by the number of laid eggs, fertilization and viability. Offspring growth performance was evaluated according to larval survival, total length and operculum mineralization area at 6 days post-fertilization. Results revealed a transgenerational effect, where males fed with rotifers encapsulated with *a* mixture of all microalgae, generated larvae with significantly higher operculum mineralization suggesting an improvement on bone development. Males fed rotifers encapsulated with *H. pluvialis* resulted in offspring with the lowest operculum mineralization, which indicates that this microalga holds an inadequate nutritional profile for zebrafish. No differences were observed on the number of eggs laid and larval survival between the different treatments. This work contributes to further understand the reproductive and transgenerational effects caused by paternal diets, by using encapsulated microalgae in rotifers. The broodstock nutritional modulation with microalgae is a valuable tool to improve offspring quality.

Work funded by ZEBRABLOOM-ALG-01-0247-FEDER-039896, Foundation for Science and Technology through UIDB/04326/2020 and INTERREG-Portugal-Spain project 0055 ALGARED+ 5E.

P 125: Adverse effects of naturally occurring infections on zebrafish research

TOPIC: HUSBANDRY AND AQUACULTURE

Marcus Crim[1]

[1] *IDEXX BioAnalytics, IDEXX BioAnalytics, Columbia, USA*

ABSTRACT TEXT

It is well established that both clinical and subclinical infections introduce confounding variability that can adversely impact research using animal models. Potential risks include an increased risk of type I and type II errors, loss of animals resulting in an unbalanced experiment, loss of an animal model, an inability to replicate animal studies, and use of more animals in order to obtain adequate statistical power. Undetected infections can impact development research by reducing fecundity, increasing morbidity and mortality of embryos and larvae, and altering the expression of cytokines and other signaling molecules that play important roles in both immunity and development. At least one common zebrafish pathogen, *Pseudoloma neurophilia*, is vertically transmitted (intraovum). Other infectious agents are shed during spawning alongside the eggs or sperm, or in feces, which can result in pseudo-vertical transmission to zebrafish larvae. As the use of zebrafish has expanded from its origin as a model organism for developmental genetics into an organism used for a wide variety of research areas, other potential adverse effects on biomedical research have emerged. A growing amount of experimental evidence demonstrates that infection with *P. neurophilia*, the most prevalent pathogen, alters behavior in adult and larval zebrafish. Infections caused by other common zebrafish pathogens including *Pseudocapillaria tomentosa* and *Mycobacterium* spp. can act as tumor promoters, potentially confounding carcinogenesis studies. While not well studied in zebrafish, the co-infection literature for commercially important aquaculture species shows that unrecognized subclinical viral infections can profoundly influence the severity of future infections caused by either unrelated viruses or pathogenic bacteria. Thus, detection, exclusion, and control of zebrafish pathogens are important for protecting the validity of experimental data in a wide range of biomedical research areas.

P 126: Archiving and phenotyping of zebrafish lines at the European Zebrafish Resource Center (EZRC)

TOPIC: HUSBANDRY AND AQUACULTURE

Robert Geisler[1], *Nadine Borel*[1], *Nathalie Decker*[1], *Sabine Kaba*[1], *Ravindra Peravali*[1], *Uwe Straehle*[1]

[1] *Karlsruhe Institute of Technology KIT, Institute of Biological and Chemical Systems - Biological Information Processing IBCS-BIP, Eggenstein-Leopoldshafen, Germany*

ABSTRACT TEXT

Centralized archiving of genetically modified zebrafish strains is essential to avoid redundant animal experiment and improves reproducibility. However no archiving facility existed in Europe before 2012. We have therefore established the European Zebrafish Resource Center (EZRC) at the KIT which supports zebrafish researchers by providing embryos and adult fish for 27,000 knock-out mutations from the Sanger Institute (representing nearly half of all protein-coding genes) as well as 2,000 mutations from ENU mutagenesis screens and 300 transgenic and wildtype lines. Moreover we offer plasmids, training in cryopreservation methods, a bioinformatics pipeline for efficient mapping of mutations and high-throughput behavioral and morphological screening.

Future directions include the archiving of characterized CRISPR lines, production and characterization of inbred wild-type lines, standardization of husbandry and improvements in cryopreservation methods.

P 127: Are anaesthetics affecting zebrafish behaviour? – a post-recovery study

TOPIC: HUSBANDRY AND AQUACULTURE

Sara Jorge[1,2], Jorge M Ferreira[2], I Anna S Olsson[2], Ana Valentim[2]

[1] *UTAD- Universidade de Trás-os-Montes e Alto Douro, CITAB - Centre for the Research and Technology of Agro-Environmental and Biological Sciences, Vila Real, Portugal*

[2] *i3S - Instituto de Investigação e Inovação em Saúde, Laboratory Animal Science group, Porto, Portugal*

ABSTRACT TEXT

Anaesthesia is routinely used in zebrafish research to avoid distress or pain caused by certain procedures (fin clipping, substance injection, imaging). Anaesthesia is characterized as a reversible intoxication of the CNS, but several concerns have been raised regarding how reversible it is and how long these alterations last. This has scientific and welfare implications in research by inducing unexpected behavioural changes capable of interfering with an experiment and normal zebrafish behaviour. Thus, we aim to study zebrafish full recovery from different anaesthetic protocols and how these protocols may influence anxiety-like behaviours.

Mixed-sex AB zebrafish were randomly assigned to 175 mg/L MS222 (n = 13), 45 mg/L clove oil (n = 15), 2 mg/L etomidate (n = 11) and 5/150 mg/L propofol/lidocaine (n = 12) treatment and control (clean water; n = 12). Anaesthetics were administered in a water bath and animals recovered in clean water 7 minutes after the loss of equilibrium. 1, 6 and 24 h after anaesthesia, animals' recovery was recorded. 28 h post-anaesthesia, animals were placed in the novel tank and exploration was recorded for 6 minutes. For analysis, the tank was divided in two by a virtual horizontal line to study space occupation. All videos were analysed with tracking software (Any-maze™).

There were no differences between the anaesthetics and the control animals at 1, 6 or 24 h post-anaesthesia regarding distance travelled, speed or immobility. At 24 h the control animals showed a decrease in the maximum speed compared with the treatment groups ($p < 0.001$). In the novel tank test, all the groups spent more time, swam more, and had higher maximum speed in the bottom of the tank than in the upper zone ($p < 0.05$), as expected in normal healthy zebrafish.

These different anaesthetic protocols seem suitable to be used in zebrafish anaesthesia research as they showed full recovery at 1 h, and they did not interfere with a validated and often used test of anxiety, the novel tank.

P 128: Targeting promising microalgae species for rotifers (Brachionus plicatilis) enrichment to improve zebrafish growth and osteological development

TOPIC: HUSBANDRY AND AQUACULTURE

Gil Martins[1,2], Matthew Castaldi[1], Hugo Pereira[1], Tamára Santos[1], Patrícia Diogo[1,3], Alexandre Rodrigues[3], Victória del Pino[3], João Navalho[3], João Varela[1,4], Paulo J. Gavaia[1,2]

[1] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*

[2] *Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal*

[3] *Necton SA, Research and Development Unit, Olhão, Portugal*

[4] *Faculty of Science and Technology, University of Algarve, Faro, Portugal*

The use of microalgae-enriched rotifers has shown improved larval growth, being therefore applied to zebrafish larviculture. However, its impact on zebrafish skeletal development is still poorly understood. Previous studies showed that high protein content contributes to increased larval survival and growth, while high dietary lipids and minerals have been associated with improved growth and reduction of severe skeletal anomalies. This work focused on investigating the skeletal development of zebrafish fed with microalgae-enriched rotifers (*Brachionus plicatilis*). The microalgal species were selected according to their nutritional profile: *Nannochloropsis* sp., *Tisochrysis* sp., *Tetraselmis* sp., *Spirulina* sp. and *Skeletonema* sp. Larvae were fed with each treatment for 30 days post-fertilization and survival, length, weight and severity of skeletal anomalies were evaluated. Results revealed that *Nannochloropsis* sp. improved zebrafish growth and weight, with significantly lower severe skeletal anomalies. *Tisochrysis* sp. and *Tetraselmis* sp. resulted in larval length and weight similar to *Nannochloropsis sp.*, with significantly higher severe skeletal anomalies. *Spirulina* sp. showed lower larval length compared to *Nannochloropsis* sp. and *Tisochrysis* sp. and increased severe skeletal anomalies. *Skeletonema* sp. yielded a length similar to *Spirulina sp.*, but showed reduced number of severe skeletal anomalies. *Nannochloropsis* sp. and *Skeletonema* sp. showed abundant Ca2+ and Sr2+

in their composition. Our previous research revealed that higher dietary Sr2+ reduced zebrafish skeletal anomalies; therefore, feeding *Nannochloropsis sp.* and *Skeletonema* sp. enriched rotifers benefit zebrafish development. Overall, results contribute to improving rotifer-enrichment methodologies towards better zebrafish growth and development.

Work funded by ZEBRABLOOM-ALG-01-0247-FEDER-039896, Foundation for Science and Technology through UIDB/04326/2020 and INTERREG-Portugal-Spain project 0055 ALGARED+ 5E.

P 129: The application of microplate reader-based colorimetric methods to measure environmental factors at biobide zebrafish facility

TOPIC: HUSBANDRY AND AQUACULTURE

Visila Moiche[1], *Ana Puertas*[1], *Juan Carlos Solis*[1], *Arantza Muriana*[1], *Iñaki Iturria*[1]

[1] *Biobide, R&D Department, San Sebastian, Spain*

ABSTRACT TEXT

Measurement of water quality parameters is a daily routine practice in zebrafish facilities across the world. Different available testing methods might be used depending on the facility design (colorimetric kits and/or automated monitoring systems). Here we describe the set-up of a simple and rapid method to measure water quality parameters such as pH or nitrogen levels by using a microplate reader. We design a specific protocol to read the absorbance of multiple water samples in a microplate according to the parameter needed to be measured from different systems.

The monitoring of environmental factors using colorimetric water chemistry kits provides punctual measures and it might be generally considered one of the most monotonous and unremarkable duties carried out in a fish facility on a routine basis. Hence, we implement an appealing method to asses several water quality parameters. Once the calibration curves are established for each of the measured parameters, the microplate is loaded with the number of samples corresponding to each system and the specific reagent solutions. We found that the use of this method clearly brought some benefits to our facility: the lower cost and the higher speed of the absorbance reading as multiple samples were set up in a 96- well microplate compared to the traditional use of manual colorimetric test kits which for individual samples are required. Therefore, this method might be considered as a robust and cost-effective method to monitor the health status of the aquatic environments from the different systems where our zebrafish colonies are allocated.

P 130: Zebrabase 3 – a major update of the intuitive fish tracking database

TOPIC: HUSBANDRY AND AQUACULTURE

Jana Oltová[1], *Jindrich Jindrich*[1], *Martin Hykl*[1], *Ctibor Skuta*[1], *Petr Bartunek*[1]

[1] *Institute of Molecular Genetics, Laboratory of Cell Differentiation, Prague, Czech Republic*

ABSTRACT TEXT

Zebrabase is a scalable, cross-platform fish tracking database developed especially for research fish facilities. It is built on three main cornerstones - comprehensive animal tracking, interactive breeding history and advanced management features. Moreover, an integrated QR code reader helps to streamline the workflow for entering new records into the database. Zebrabase has been developed at the Institute of Molecular Genetics in Prague (Czech Republic) by an interdisciplinary team of zebrafish researchers, programmers and web designers. Although initially designed as a solution for our own zebrafish facility, we aim to provide this tracking system to fish facilities worldwide to fulfill the needs of both small and large research facilities. After five years of continuous development, we are now releasing an internally completely redesigned version, Zebrabase 3, which will include significant functional improvements, like drag and drop support, position sharing in the facility, substock merging, as well as improved configuration and reporting options and enhanced communication features, which have been often requested by Zebrabase users. Whether your facility consists of a single rack or several independent rooms, Zebrabase may be the right solution for you.

P 131: Identification of individual zebrafish (Danio rerio): A refined protocol for VIE tagging whilst considering animal welfare and the Three R principles

TOPIC: HUSBANDRY AND AQUACULTURE

Anita Rácz[1], *Brooke Alan*[2], *Toni Dwyer*[2], *Shaun Killen*[2]

[1] *Eötvös Loránd University - ELTE, Genetics, Budapest, Hungary*
[2] *Institute of Biodiversity- Animal Health and Comparative Medicine- University of Glasgow, College of Medical- Veterinary and Life Sciences, Glasgow, United Kingdom*

ABSTRACT TEXT

Zebrafish are an important model system for scientific and medical research.

Despite this, marking zebrafish for individual identification purposes is not commonplace.

In other fish species, visible implant elastomer (VIE) tagging is used as a successful identification method but lacks important details regarding fish welfare.

We highlight previously unconsidered animal welfare issues through long-term observations of survival rate, tag retention, and tag colour on different populations and age-groups of zebrafish; and introduce an improved VIE tagging protocol. This improved protocol was developed and compared with original tagging procedures and associated negative effects, following animal welfare concepts described in the Three Rs principles, focusing on Refinement.We describe a novel protocol using lidocaine solution as an analgesic and post-tagging treatments with two healing agent to improve the wound healing.

The information from this study will be beneficial for the zebrafish research community as a guideline for implementing VIE tagging as a successful identification tool when differentiating between genetic lines, families, or individuals. And will also be beneficial for the whole fish biology community when considering important animal welfare questions in the future if they are using identification techniques which could be considered as potentially noxious stimuli for fish.

P 132: Zebrafish dietary supplementation as a screening tool for Aquaculture Nutrition and Human disease: Insights from macroalgae ethanolic extracts as a natural source of Vitamin K1

TOPIC: HUSBANDRY AND AQUACULTURE

Vânia Roberto[1], *Gil Martins*[1], *Leonardo Mata*[1], *Michael Viegas*[2], *Graça Miguel*[3], *Paulo J. Gavaia*[1]

[1] *Center of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *SPAROS, Lda, Olhão, Portugal*
[3] *Faculty of Sciences and Technology, University of Algarve, Faro, Portugal*

ABSTRACT TEXT

Functional foods have gained much attention in the last years since they can benefit physiological responses and/or lessen the risk of disease. A crucial contributing factor is a balanced dietary profile of consumed vitamins. In particular, vitamin K (VK) and related proteins are important for several metabolic processes, including bone metabolism, calcification, blood coagulation and also known to impact on stress, inflammation and carcinogenesis. Moreover, recent researches revealed that macroalgae contain potent bioactive compounds with antioxidant, antibacterial and/or antitumoral activities, evidencing their nutraceutical and pharmaceutical values.

Here we investigated 20 marine macroalgae as a natural source of VK1. Biological activities (*i.e.* antioxidant, anti-diabetic, anti-inflammatory) were then evaluated in ethanolic extracts from 7 macroalgae species. The most promising extract, from *Plocamium cartilagineum,* was screened for the complete set of vitamins content and later used to supplement the feed of 2 different fish species: the zebrafish (*Danio rerio*) and the Senegalese sole (*Solea senegalensis)*. Fish fed with control and supplemented diets were analyzed for growth and expression of genes involved in VK cycle, bone and lipid metabolisms, stress and inflammation responses. Results were compared to identify common outputs between the 2 species. We could validate the zebrafish as a model for aquaculture nutrition screenings and narrow down this time and money consuming process for the industry.

Finally, we investigated the genes identified in the fish models for their potential as biomarkers for human disease, using The Cancer Genome Atlas. Notably, different genes had diagnostic and/or prognostic value in liver, bile, prostate and colorectal human cancers.

Financial support from the FCT (grant UID/Multi/04326/2020) and European Maritime and Fisheries Fund (EMFF/FEAMP) through the National Operational Programme MAR2020 (grant ALGASOLE-16-02-01-FMP-0058).

P 133: Zebrafish embryo cryopreservation: a promising tool for alternative methods

TOPIC: HUSBANDRY AND AQUACULTURE

Maria Sampieri[1], Erika Molica Colella[1], Silvia Dotti[1]

[1] *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna-IZSLER, Centro di Referenza Nazionale per i Metodi Alternativi- Benessere e Cura degli Animali da Laboratorio, Brescia, Italy*

ABSTRACT TEXT

Zebrafish is a valid model already widely used thanks to its particular features (ease of maintenance, small size, high fecundity, rapid development, optical transparency of the embryo). According to the 3Rs principle (*Replacement, Reduction and Refinement*), fish embryos are considered as replacement methods to animal experimentation, since these developmental stages are likely to experience less or no pain, suffering, distress or lasting harm. The aim of the present study concerns the development of tests that allow preserving zebrafish embryos in order to facilitate their use in research and enhance the possibility of exchanging them between researchers, promoting the dissemination of the experimental model and reducing the number of animals used for scientific purposes. Viable fertilised eggs correctly developed were exposed to cryoprotectant agents (CPA) and Embryo Medium. Different developmental stages (24 hpf, 27 hpf, 48 hpf) of the embryos, which were analysed both with the chorion and treated with Pronase in order to remove it, were exposed to cryoprotectants like Methanol and DMSO at increasing dilutions (1M, 0,1M, 0,05M). After 24 h with CPA at 4 °C, embryos were brought back to room temperature and transferred in Embryo Medium at the target development temperature of 28 °C. Embryonic viability and correct embryonic development were assessed daily by optical microscopic observation to verify specific developmental endpoints (e.g. heartbeat, blood circulation, yolk sac and pericardial oedema, scoliosis). From preliminary results the use of CPA seems to be promising, however the dilution of use must be defined, because elevated concentrations have shown high mortality. The study was carried out to identify the ideal temperature to arrest or slow down embryo development, the useful cryoprotectant agent and the maximum incubation period in order to find the most applicable protocol that allows preserving the embryonic viability.

P 134: ZEB316 housing system to study microplastics in zebrafish

TOPIC: HUSBANDRY AND AQUACULTURE

Marco Tarasco[1], Gil Martins[1], Paulo J. Gavaia[1,2], Maria J. Bebianno[3], M. Leonor Cancela[1,2,4], Vincent Laizé[1]

[1] *Centre of Marine Sciences, University of Algarve, Faro, Portugal*
[2] *Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal*
[3] *Centre for Marine and Environmental Research, University of Algarve, Faro, Portugal*
[4] *Algarve Biomedical Centre and Centre for Biomedical Research, University of Algarve, Faro, Portugal*

ABSTRACT TEXT

Microplastics (MPs) are massively present in the aquatic environment and represent a major concern for the whole ecosystems but also for human health as they may enter the food web upon ingestion by aquatic organisms. The zebrafish (*Danio rerio*) offers significant advantages over classical animal models to assess environmental risks associated with aquatic pollutants such as MPs. However commercial housing systems are not appropriate to assess the toxic effect of microplastics as they contain components made of plastic polymers that may release micrometric plastic particles, manufacturing compounds, or adsorb chemicals. The ZEB316 stand-alone housing system presented here is a cost-effective and easy-to-built solution to perform state-of-the-art toxicological studies. It is built with inert and corrosion-resistant materials (i.e. stainless steel 316 and glass) and provides good housing conditions through efficient recirculation and filtration systems. The assessment of daily/weekly water parameters and fish growth performance showed that the ZEB316 provides

housing conditions comparable to those available from commercial housing systems. The efficacy of the ZEB316 mechanical filter for retaining recirculating MP particles was assessed for 24 h using 20–27 µm polyethylene fluorescent particles and paper filters with a 12–14 µm pore size. Fluorescence image analysis revealed that the mechanical filter efficiently retained more than 95 % of the total particles fed in the system, confirming the suitability of the ZEB316 to perform toxicological experiments with MPs[1].

1 Tarasco et al. (2020) Zebrafish 17:18-26. doi: 10.1089/zeb.2019.1801.

This work was funded by Portuguese national funds from the Foundation for Science and Technology (FCT) through projects UID/Multi/04326/2019, UIDB/04326/2020 and UID/MAR/00350/2019. Marco Tarasco was supported by the FCT through the PhD grant SFRH/BD/128634/2017.

P 135: Refinement of Water Quality in Zebrafish Pair Breeding

TOPIC: HUSBANDRY AND AQUACULTURE

Carole Wilson[1], Paul Barwood[2]

[1] *UCL, Fish Facility - Division of Biosciences, London, United Kingdom*
[2] *Fish Facility - Division of Biosciences, London, United Kingdom*

ABSTRACT TEXT

Pair breeding of zebrafish (*Danio rerio*) enables the generation of progeny with known genetic lineage and is essential for *in vivo* research. This practice is routinely carried out in small transparent breeding boxes with no water flow or filtration, containing less than a litre of water. This small volume amplifies water quality fluctuation and absence of filtration allows toxic waste products accumulation. This unstable environment has the potential to impact breeding performance, cause harm to the fish and affect the quality of science.

Factors such as timing of feeding before breeding and degradation of water quality are poorly investigated. Recommendations for best practice are scant, variable and predominantly reliant on anecdotal information.

In this investigation, AB:TL hybrid strain zebrafish were used at two different age points 4 and 12 months. The older cohort was used to compare water quality in the tanks – fasted, fed normally and fed extra prior to being transferred to the breeding boxes, the water was then tested the following day. A second trial compared static breeding boxes to boxes with flowing water and examined embryo production. This trial was also repeated using the younger cohort.

These trials show that although the timing of feeding prior to use of the breeding boxes does not influence breeding success, deterioration of water quality, such that it became harmful to the fish was affected by feeding times. The adjustment of feeding times and provision of flowing water ameliorates water quality issues without impacting breeding performance and can be seen as a major refinement to breeding practice.

Immunity and Infection

P 136: Evaluation of Zebrafish innate response to infection by different pathogens

TOPIC: IMMUNITY AND INFECTION

Ana Carolina De Seni[1,2], *Milena Marcolino-Souza*[1], *Adolfo Luis Almeida Maleski*[1,2], *Mônica Lopes-Ferreira*[1], *Carla Lima*[1]

[1] *Butantan Institute, Immunoregulation Unit of the Laboratory of Applied Toxinology CeTICs/FAPESP, São Paulo, Brazil*

[2] *Butantan Institute, Post-Graduation Program of Toxinology, São Paulo, Brazil*

ABSTRACT TEXT

The temporal separation present in zebrafish larvae provides a suitable system to study the vertebrate innate immune response *in vivo*, independently from the adaptive response. Zebrafish present ample conservation of signaling pathways, particularly those known for infection responses as *caspy*, *caspy2*, zASC, and IL-1β. Our aim in this work was to investigate the response of zebrafish larvae against a variety of stimuli including canonical and non-canonical pathogens. Zebrafish larvae at 1, 2, 3 or 5 days post fertilization (dpf) were exposed for 2 hours to the canonical antigens or pathogen Flagellin (TLRL-EPSTFLA 0.01 µg/ml), DMXAA (tlrl-dmx 1 µg/ml), *S. aureus* (HKSA 10^6 cell/ml) or to non-canonical antigens or pathogen as *E. coli* O55B (L2630 1 µg/ml), *S. abortus* (L5886 1 µg/ml), *S. typhimurium* (tlrl-hkst2 10^6 cell/ml). After 0, 6, 24, 48, and 72 hours post infection (hpi) larvae were investigated by the presence of abnormalities, mortality and locomotory activity. The evaluation of the inflammasome activation and caspase-a maturation was performed in the lysate of larvae from different groups by western blot using antibody anti-caspase-1 subunit p-10 (M20). The stimuli exposition did not induce increased mortality or abnormalities during the development, but 1 dpf larvae stimulated with *S. aureus* and *S. typhimurium* presented a decreased number of movements when compared to control, indicative of infection. The expression of pro-caspase-a was determined in the lysates of 2 dpf larvae stimulated with the STING ligand DMXAA and *S. abortus*, but casp-a in a both pro and mature forms was produced when 1 dpf larvae were only infected with *S. aureus* and *S. typhimurium*. These results indicate that larvae at 1 or 2 dpf of development are more susceptible to response against pathogens; canonical and non-canonical stimuli induce the production of caspase-a in an inactive form and the active form of caspase-a was only induced in infected larvae. Supported by FAPESP/CAPES.

P 137: Introduction of a Step-by-Step Protocol for the Eradication of Mycobacterium haemophilum in Zebrafish System

TOPIC: IMMUNITY AND INFECTION

Anita Rácz[1], *Toni Dwyer*[2], *Shaun Killen*[2]

[1] *Eötvös Loránd University - ELTE, Genetics, Budapest, Hungary*

[2] *Institute of Biodiversity- Animal Health and Comparative Medicine- University of Glasgow, College of Medical- Veterinary and Life Sciences, Glasgow, United Kingdom*

ABSTRACT TEXT

In 2017, the zebrafish unit at University of Glasgow experienced a detrimental outbreak of pathogenic bacterium, *Mycobacterium haemophilum*.

The presence of other bacterial species was also confirmed by bacteriology growth. The affected individuals composed of a wild-origin parental population sourced from India and their F1 offspring generation (in house). Bacteria were diagnostically confirmed to be present systemically in fish and within the water and biofilm of the recirculating zebrafish systems. In the absence of a publicly accessible step-by-step disinfectant protocol for these difficult-to-eliminate pathogens, we devised a successful procedure to eradicate *M. haemophilum* and Aeromonas species after colony removal using chlorine tablets (active ingredient Sodium dichloroisocyanurate) and Virkon Aquatic. Postdisinfection diagnostics did not detect pathogens in the system or in the new fish inhabiting the system that were tested. Newly established fish colonies have not shown similar clinical signs or disease-induced mortality in the 1-year period following system disinfection and repopulation.

Our aim is to provide a detailed disinfection procedure for the effective elimination of *M. haemophilum* and *A. hydrophila* from research-standard zebrafish units.

The simplicity of this disinfection protocol allows for simple adjustment to be used in different settings, such as flow-through or recirculation systems (both glass and polycarbonate designs), and can be adapted to cater to smaller or larger scales for other aquatic facilities as well.

It is a cost and time effective method to use for facility or quarantine unit disinfection before the introduction of new colonies of fish.

P 138: The effect of miR-99/RORa pathway on regulating neutrophil motility and inflammatory recruitment

TOPIC: IMMUNITY AND INFECTION

Tianqi Wang[1], *Alan Hsu*[1], *Jiao Wang*[2], *Sheng Liu*[3], *Jun Wan*[3], *Sandro Matosevic*[2], *Qing Deng*[1]

[1] *Purdue University, Department of Biological Sciences, West Lafayette, USA*
[2] *Purdue University, Department of Industrial and Physical Pharmacy, West Lafayette, USA*
[3] *Indiana University, School of Medicine, Indianapolis, USA*

ABSTRACT TEXT

Neutrophils form the front line in pathogen defense and plays significant roles in immune modulation. Over-amplified neutrophilic inflammation drives the immunopathology underlying a broad range of human diseases. The mechanism of how neutrophils respond and move to the inflamed sited is not fully elucidated. Here we report that *miR-99* is a novel modulator of neutrophil motility and recruitment. Neutrophil motility and recruitment to injury or infection sites are significantly reduced upon neutrophil-specific *miR-99* overexpression zebrafish. The RAR Related Orphan Receptor A (RORα) in zebrafish is identified as a direct target of *miR-99* in neutrophils. Neutrophil velocity and their response to acute inflammation are dampened upon chemical or genetic inhibition of RORα in zebrafish neutrophils. Chemotaxis of human polymorphonuclear leukocytes (PMN) are also reduced when treated with a RORα specific inhibitor. In conclusion, we identified a role of *miR-99* and its direct target RORα in regulating neutrophil motility and chemotaxis. These data suggest a potential of using *miR-99* and RORα as therapeutic targets in treating infection and autoimmune diseases.

Metabolism

P 139: Feeding juvenile zebrafish with specific fatty acids in order to affect their body fatty acid profile. A tool to investigate the impact of specific fatty acid enrichments on disease development

TOPIC: METABOLISM

Apolline Goudmaeker[1], *Morgane Thibaut*[2], *Elise Dormal*[1], *Hadelin Bodart*[1], *Cathy Debier*[1], *Yvan Larondelle*[1]

[1] *UCLouvain, Institute of Biomolecular Sciences and Technology, Louvain-la-Neuve, Belgium*
[2] *UCLouvain, Louvain Drug Research Institute, Woluwe-St-Lambert, Belgium*

ABSTRACT TEXT

Different types of polyunsaturated fatty acids (PUFA), namely ω-3, ω-6, CLA, CLnA, have shown increasing interest in terms of health impact. We propose an approach allowing the enrichment of growing zebrafish (*Danio rerio*) in specific fatty acids, in order to allow deeper investigations on the mechanisms by which changes in PUFA body composition affect the development of diseases.

Purified diets were formulated to meet the essential nutritional requirements of zebrafish and provide an enrichment in a specific lipid source. On a dry matter (DM) basis, they included 20 % lipids, of which 5.6 % was made of a mix of sunflower oil (SUN) and linseed oil (LIN), to cover the zebrafish essential ω-6 and ω-3 fatty acid requirements. The remaining 14.4 % were made by specific oils or pure triglycerides.

First, we evaluated the impact of trilinolein and trilinolenin on the body lipid profile of juvenile zebrafish. Fish fed for three weeks with trilinolein accumulated linoleic acid (LA) and intermediates of the ω-6 pathway, while the intermediates of the ω-3 pathway showed a net reduction. Fish receiving trilinolenin accumulated α-linolenic acid (ALA) and intermediates of the ω-3 pathway, while the intermediates of the ω-6 pathway remained stable or decreased.

In a second experiment, we investigated the impact of SUN and LIN. After three weeks, the SUN diet showed a specific enrichment in LA while the concentration of ALA slightly exceeded the concentration of LA for the LIN diet.

A last experiment dealt with *Ricinodendron heudelotii* or *Punica granatum* seed oils. These oils respectively contain high levels of α-eleostearic acid and punicic acid, two members of the CLnA family. Zebrafish fed on these oils showed a rise in their body content in CLnA. Interestingly, this rise appeared to stimulate the Δ-6 desaturase activity since C18:4 ω-3 accumulated. In addition, rumenic acid (C18:2c9,t11) accumulated in the fish body, indicating the presence of a Δ-13 reductase activity in zebrafish.

P 140: Analysis of the bone state of the Danio rerio axial skeleton after space flight

TOPIC: METABOLISM

Nadezhda Lukicheva[1], *Galina Vassilieva*[1], *Olga Kabitskaya*[1], *Kirill Gordienko*[1]

[1] *Russian Federation State Research Center Institute of Biomedical Problems RAS IBMP, Research group of bone and metabolic effects of microgravity, Moscow, Russian Federation*

ABSTRACT TEXT

Throughout the history of space flights a large number of species of living organisms have been exposed in space. In particular these are different terrestrial vertebrates with revealed similar changes in the musculoskeletal system: muscle atrophy and loss of bone mineral density.These changes are associated with the absence of gravity in space flights. Scientists are wondering whether microgravity has a similar effect on organisms for which the aquatic environment is the habitat?

Fish more than once became the subjects for studying space flight factors. Experiments on biosatellites, orbital stations, and Shuttles have shown that in the early stages of flight, fish have disorientation in space similar to motion sickness in astronauts.

A series of experiments on *Oryzias latipes* and *Danio rerio* (Roscosmos and JAXA) was conducted on board the ISS in 2012–2016, which showed the change in the expression of genes responsible for the formation of bone and cartilageous tissue.

Histomorphometric analysis was performed using ADF Image Capture software, which allows to get and analyze in real-time the images from a microscope using a digital camera. The non-parametric Mann–Whitney U-test was used to assess the differences between the control and flight groups.

The main purpose of our work was to find those parts of the skeleton which are most informative for assessment of the space factors effects. At the present stage we selected 4 parts in the vertebral column (9 indices for each), and one part in the cranium (37 indices). We conducted the preliminary analysis of the bone and cartilageous tissues of *Danio rerio* on the histological sections in flight (1.5 months) and control groups. Based on the results obtained, we did not receive credible differences between the groups in morphology of skeletal tissues. The work to study fish bone tissue continues. We are looking for most informative (gravity depending) segments of bone and cartilageous tissues.

P 141: Live Tracking of Inter-organ Communication by Endogenous Exosomes In Vivo

TOPIC: METABOLISM

Frederik Verweij[1], *Guillaume Van Niel*[1]

[1] *Institute for Psychiatry and Neurosciences of Paris, U1266 Inserm, Paris, France*

ABSTRACT TEXT

Extracellular vesicles (EVs) are released by most cell types but providing evidence for their physiological relevance remains challenging due to a lack of appropriate model organisms. Here, we developed an in vivo model to study EV function by expressing CD63-pHluorin in zebrafish embryos. A combination of imaging methods and proteomic analysis allowed us to study biogenesis, composition, transfer, uptake, and fate of individual endogenous EVs. We identified a subpopulation of EVs with exosome features, released in a syntenin-dependent manner from the yolk syncytial layer into the blood circulation. These exosomes are captured, endocytosed, and degraded by patrolling macrophages and endothelial cells in the caudal vein plexus (CVP) in a scavenger receptor- and dynamin-dependent manner. Interfer- ence with exosome biogenesis affected CVP growth, suggesting a role in trophic support. Altogether, our work represents a system for studying endogenous EV function in vivo with high spatiotemporal accu- racy, demonstrating functional inter-organ communication by exosomes.

Morphogenesis and Organogenesis

P 142: Regulation of lymphatic development by mafba and mafbb depends on regional differences across lymphatic beds

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Hannah Arnold[1], *Beata Filipek Gorniok*[1], *Renae Skoczylas*[1], *Katarzyna Koltowska*[1]

[1] *Uppsala University, Immunology- Genetics and Pathology, Uppsala, Sweden*

ABSTRACT TEXT

Lymphangiogenesis is the formation of lymphatic vessels from pre-existing veins and is a dynamic process that requires cell migration. Regardless of the location in which lymphangiogenesis occurs, the face or the trunk, lymphatic endothelial cell (LEC) progenitors actively probe their surroundings while migrating to form the lymphatic vascular network. The transcription factor Mafba is expressed in LEC

progenitors and is essential for their migration in the trunk. However, the mechanism by which it orchestrates the migration of LEC progenitors remains elusive. Here, we uncover unexpected regional differences in the requirements for the paralogues of Mafb for lymphatic cell migration.

We have characterised the phenotype of Mafbb mutants alone and in combination with Mafba to dissect the cellular mechanisms of lymphatic development. Our in-depth analysis, using confocal microscopy in combination with lymphatic-specific transgenic lines has revealed differences in the development of two lymphatic beds, the face and the trunk. Surprisingly, LEC migration is dose-dependent on these genes in a tissue-specific manner. Using live-imaging we have further dissected the cellular behaviours of LEC progenitors in these mutants to understand the processes that are regulated downstream of Mafb. Together, this work will contribute to our understanding of how cell migration is controlled discretely in two different environments by the same regulators.

P 143: Role of collagen XV-B in motor neuron axon development

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Sandrine Bretaud[1], *Laurie Nemoz-Billet*[1], *Elisabeth Vaganay*[1], *Florence Ruggiero*[1]

[1] *Institut de Génomique Fonctionnelle de Lyon, ENS de Lyon- CNRS UMR5242, LYON, France*

ABSTRACT TEXT

The extracellular matrix provides local positional information to guide motor neuron axons toward their muscle target. Collagen XV is a basement membrane component mainly expressed in skeletal muscles. We have identified two *zebrafish* collagen XV gene *paralogs, col15a1a* and *col15a1b* that displayed distinct expression patterns. *Col15a1b* was expressed by slow muscle precursors, adaxial cells. Using newly generated antibodies to collagen XV-B (COLXV-B), we showed that the myotomal collagen XV-B (COLXV-B) is deposited in the motor path before the onset of adaxial cell migration. Here, we interrogated the function of *col15a1b* during development. Loss of function of *col15a1b* in two mutant lines provoked truncation of primary motor neuron axons and modified their matrix environment. On the opposite, microinjection of *Shh* mRNA in embryos which resulted in an excess of COLXV-B deposition in the myotome, caused anarchic growth of the primary motor neuron axons. Absence of COLXV-B also provoked defects in 2ndary motor neuron development and neuromuscular junction formation. These results suggest that COLXV-B impacts axon guidance by affecting the matrix protein structural network.

P 144: New type of TRAP-positive cell contributes to actinotrichia degradation during fin fold formation

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Masahiro Chatani[1], *Shunsuke Kobayashi*[1], *Takashi Takaki*[2], *Masamichi Takami*[1]

[1] *Showa University, Pharmacological Research Center, Tokyo, Japan*
[2] *Showa University, Division of Electron Microscopy, Tokyo, Japan*

ABSTRACT TEXT

The zebrafish fin fold consists of unmineralized fibrils called actinotrichia, which keep the fold straight and function as an axial skeleton. Recent discovery of actinodin 1 (Zhang J et al., 2010), a component of actinotrichia, has led to additional research findings revealing dynamics of the actinotrichia bundling structure. Although several important concepts regarding tissue shaping during development have been shown, such as bone modeling that cases degradation of the mineral and organic matrix by osteoclasts in bone, cell death in the space between embryonic hand fingers, and a negative feedback loop by Ihh in endochondral ossification, the fin formation process including actinotrichia remains largely unknown. Here, we present a concept of tissue coordination in the fin fold based on findings of previously unidentified tartrate resistant acid phosphatase TRAP-positive cells, termed "*u-trap*$^{+}$", that participate in degradation of actinotrichia for support of bundling in the fin fold of medaka fish. We observed *u-trap*$^{+}$ cells in locations other than bone (fin ray) in *trap* promoter-EGFP transgenic medaka, which are mainly used for investigation of osteoclasts. Double-immunofluorescent staining with anti-And1 and anti-EGFP antibodies revealed *u-trap*$^{+}$ cells localized in the gap space of actinotrichia bundles. Confocal microscope imaging also showed *u-trap*$^{+}$ cell localization along with actinotrichia fibrils. In *osteoprotegerin* (*opg*)-deficient medaka, which lack the

inhibitor factor for osteoclast differentiation, the numbers of *u-trap*$^{+}$ cells as well as osteoclasts were significantly increased, indicating differentiation of *u-trap*$^{+}$ cells by *opg*. Furthermore, electron microscopy analysis indicated disassembly of actinotrichia fibrils in *opg*-deficient medaka, while *opg/rankl* double-deficient medaka did not show over-induction of *u-trap*$^{+}$ cells. Based on our findings, we propose that *u-trap*$^{+}$ cells provide support for shaping of actinotrichia as the fin fold skeleton.

P 145: A sheath of motile cells supports collective migration of the Zebrafish posterior lateral line primordium under the skin

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Damian Dalle Nogare[1], *Naveen Natesh*[2], *Ajay Chitnis*[3]

[1] *National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda, USA*
[2] *Duke University, Center for Genomics and Computational Biology and Department of Biomedical Engineering, Durham, USA*
[3] *National Institutes of Health, Eunice Kennedy Shriver National Institute of Child Heath and Human Development, Bethesda, USA*

ABSTRACT TEXT

During embryonic development, cells must navigate through diverse three-dimensional environments robustly and reproducibly. The zebrafish posterior lateral line primordium (PLLp) which migrates from the otic vesicle to the tip of the tail is an excellent model to study such collective migration. This system migrates in a channel formed by the underlying horizontal myoseptum and somites, and the overlying skin. Cells in the PLLp progressively reorganize to form epithelial rosettes, called protoneuromasts. These epithelial cells extend basal cryptic lamellipodia in the direction of migration in response to both chemokine and FGF signals. In this study, we show that, in addition to these cryptic lamellipodia, the core epithelial cells are in fact surrounded by a population of motile cells which extend actin-rich migratory processes apposed to the overlying skin. These cells wrap around the protoneuromasts, forming a continuous sheath of cells around the apical and lateral surface of the PLLp. The processes extended by these cells are highly polarized in the direction of migration and this directionality is dependent on FGF signaling. Consistent with these interactions contributing to migration, removal of the skin stalls migration. This is accompanied by a profound change in the morphology of the sheath cells, with directional superficial lamellipodia being replaced with the appearance of undirected blebs. Furthermore, removal of the skin not only affects underlying lamellipodia, it simultaneously alters the morphology and behavior of the deeper basal cryptic lamellipodia, even though these cells do not directly contact the skin. Directional actin-rich protrusions on both the apical and basal surface and migration are completely restored upon regrowth of the skin over the PLLp. We suggest that this system utilizes a circumferential sheath of motile cells to allow the internal epithelial cells to migrate collectively in the confined space of the horizontal myopseptum.

P 146: From fish to mice, the journey of Nrf2 in thyroid functional maturation

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Pierre Gillotay[1], *Mirian Romitti*[1], *Benjamin Dassy*[1], *Achim Trubiroha*[1], *Benoit Haerlingen*[1], *David Gacquer*[1], *Panos Ziros*[2], *Gerasimos Sykiotis*[2], *Sabine Costagliola*[1]

[1] *Université Libre de Bruxelles, Institut de recherche interdisciplinaire en biologie humaine et moléculaire, Brussels, Belgium*
[2] *Lausanne University Hospital, Service of Endocrinology- Diabetology and Metabolism, Lausanne, Switzerland*

ABSTRACT TEXT

Congenital hypothyroidism (CH) represents the most common congenital endocrine disorder in humans, affecting around 1 in every 3000 live births. To date, the cause of most cases of CH remains unexplained due to poor understanding of the molecular regulation of thyroid development. Although recent studies described the mechanisms driving thyroid specification, little is known about the actors driving the functional maturation of the thyroid. From a F0 Crispr-cas9-based screening approach, we recently identified *nrf2a*, a transcription factor implicated in the cell's oxidative stress response, as a potential driver of the thyroid maturation. Analysis of our *nrf2a* stable mutant line revealed defect in thyroid functional maturation. Indeed, quantitative analysis of thyroxine (T4, most abundant thyroid hormone TH) level indicates that homozygous mutants have a complete absence of T4. This absence correlates with the lack

of iodinated thyroglobulin, the precursor of TH. Despite those defects, folliculogenesis occurs normally suggesting a primary defect in the TH production machinery. QPCR analysis of the genes encoding this machinery showed a dysregulation of their expression. To understand the molecular mechanism of this dysregulation and to translate our finding into a mammalian model, we generated, using mouse embryonic stem cells, *in vitro* thyroid follicles lacking functional Nrf2. Analysis of thyroid differentiation of the Nrf2 KO organoids revealed a lack of follicular organization and subsequent functional maturation, marked by a strong downregulation of thyroglobulin expression. Our results show that Nrf2 plays a crucial role in thyroid functional maturation across species and our in vitro data suggest that this Nrf2 control could be thyroid cell autonomous. This suggests that a dysregulation of Nrf2-controlled signalling pathways during embryonic development could lead to congenital hypothyroidism and thus opens new diagnostic and therapeutic perspectives.

P 147: Understanding the mechanism of liver growth and size control during development and regeneration

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Ronja Heyne[1], *Elke Ober*[1]

[1] *Novo Nordisk Foundation Center for Stem Cell Biology DanStem, Faculty of Health and Medical Sciences, Copenhagen, Denmark*

ABSTRACT TEXT

The liver has a unique capacity to regenerate following injury, nonetheless if the injury is too severe the liver will fail to regenerate and organ transplantation is necessary. As donor livers are rare and liver failure fatal, it is of high interest to find new treatments that can aid resolving the issue. It is, therefore, essential to identify the mechanisms that control and trigger organ growth. In the past years, several studies have identified and described the role of different signaling pathways in organ size regulation and regeneration. Still, many questions remain, like how organs sense their size, how growth is triggered and how organs know when they have to stop growing. In this project we investigate these questions using a zebrafish mutant, in which fewer liver progenitors are specified during early development, but then starts to recover and gains a normal liver size later due to compensatory outgrowth. We have determined the critical time points of compensatory growth, marked by a strong peak of proliferation in mutant livers, indicating tight regulation of the start of outgrowth. In addition, recovery to wildtype liver size is observed corroborating the presence of a hepatostat. Interestingly, analysis of the differentiation status of mutant livers revealed, that compensatory growth seems to be driven by differentiated cell types rather than by progenitor-like cells. To identify the molecular regulators controlling compensatory growth, we have performed cell type specific bulk RNA sequencing of mutant and wildtype livers at key time points of the outgrowth response. Top candidates will be investigated and validated *in vivo*. The results of this project will provide important insights into the mechanisms controlling organ growth and possibly new entry points for regenerative therapies.

P 148: Hyperspectral mapping of zebrafish embryos using microscope-based acousto-optical imaging spectrometer

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Demid Khokhlov[1], *Alexander Machikhin*[1], *Alexander Burlakov*[2], *Sergey Shirokov*[1]

[1] *Scientific & Technological Center of Unique Instrumentation- Russian Academy of Sciences, Laboratory of acousto-optic spectroscopy, Moscow, Russian Federation*

[2] *Lomonosov Moscow State University, Department of Ichthyology, Moscow, Russian Federation*

ABSTRACT TEXT

We propose a novel approach to non-invasive morphological study of zebrafish embryo based on in vivo hyperspectral mapping of its structure. We have developed an acousto-optical imaging module for transmission light microscope and conducted multiple experiments on zebrafish embryos. Experiments show that morphological transformations influence the transmission spectrum. Hyperspectral data clustering allows to detect and track the type, dimensions and shape of the main tissues. This approach may provide valuable information during continous studies on organogenesis.

P 149: The role of titin in atrial fibrillation

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Marie Kongsted[1], *Morten Salling Olesen*[2], *Pia Rengtved Lundegaard*[2]

[1] *University of Copenhagen, Department of Biomedical Sciences, Copenhagen, Denmark*
[2] *University of Copenhagen- Rigshospitalet, Department of Biomedical Sciences- Laboratory of Molecular Cardiology, Copenhagen, Denmark*

ABSTRACT TEXT

The purpose of this study is to elucidate the role of titin as well as other structural proteins in relation to atrial fibrillation (AF). AF is the most prevalent cardiac arrhythmia, affecting more than 7 million people in the USA and Europe alone and more than 30 million people world-wide. The disease displays various severe comorbidities such as stroke, heart failure and overall increased mortality. Current treatments are limited and inefficient emphasizing the need for mechanistic understanding of the disease and patient stratification.

Titin truncating variants (TTNtv) occur in ~1 % of the general population and while it is well established that TTNtv is highly associated with dilated cardiomyopathy, our group has demonstrated that TTNtv is a disposing factor for AF. Titin is a sarcomeric protein that functions as a signaling platform and provides passive tension and elasticity expressed in striated muscle cells. The pathophysiological processes of TTNtv will be investigated using zebrafish disease models. Our aims are to: 1) Characterize the electrophysiological conduction in adult *ttn.2* zebrafish genetic mutant hearts, 2) Identify and characterize key cellular pathways involved in the AF pathology of *ttn.2* mutant zebrafish, 3) Investigate the metabolic effects of *ttn.2* mutations in zebrafish – all with the overall aim to identify the mechanism of how TTNtv cause AF. Preliminary data indicates that zebrafish that are heterozygous for TTNtv exhibit higher respiratory rate than their wild-type siblings.

P 150: Vinculin controls endothelial junctional tethers during vascular lumen formation

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Maria Kotini[1], *Miesje M. van der Stoel*[2], *Markus Affolter*[1], *Stephan Huveneers*[2], *Heinz-Georg Belting*[1]

[1] *University of Basel, Biozentrum, Basel, Switzerland*
[2] *Amsterdam UMC- University of Amsterdam, Department of Medical Biochemistry, Amsterdam, Netherlands*

ABSTRACT TEXT

The dynamic properties of endothelial cell-cell junctions are essential for vascular development. The formation and turnover of endothelial junctions is controlled at the level of cell-cell adhesion receptors such as VE-cadherin and PECAM-1. Force-dependent signals are transmitted through the endothelial junctions to coordinate angiogenesis. Here, by using live imaging of angiogenesis in zebrafish, we demonstrate that the onset of lumen opening in developing intersegmental vessels is accompanied by the formation of transient actin-based junctional tethers between endothelial cells. These junctional tethers are prolonged by increased blood flow produced by norepinephrine stimuli, whereas they regress by decreased blood pressure upon treatment with tricaine. Interestingly, the junctional mechanosensor vinculin is localised at the junctional tethers. Furthermore, we show that the genetic depletion of both its functional isoforms in zebrafish, vinculin A and vinculin B, prevents tether formation. Mosaic rescue experiments with endothelial-driven vinculin restored the formation of junctional tethers. These findings reveal the importance of junction-based endothelial mechanosensing during lumen formation of developing blood vessels.

P 151: Mef2c factors are required for early but not late addition of cardiomyocytes to the ventricle

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Duvaraka Kula-Alwar[1], Michael S. Marber[2], Simon M. Hughes[1], Yaniv Hinits[1]

[1] *King's College London, Randall Centre for Cell and Molecular Biophysics- School of Basic and Medical Biosciences and British Heart Foundation Centre of Research Excellence, London, United Kingdom*

[2] *King's College London, Department of Cardiology- School of Cardiovascular Medicine and Sciences- British Heart Foundation Centre for Research Excellence- The Rayne Institute, London, United Kingdom*

ABSTRACT TEXT

During heart formation, the heart grows and undergoes dramatic morphogenesis to achieve efficient embryonic function. Both in fish and amniotes, much of the growth occurring after initial heart tube formation arises from second heart field (SHF)-derived progenitor cell addition to the arterial pole, allowing chamber formation. In zebrafish, this process has been extensively studied during embryonic life, but it is unclear how larval cardiac growth occurs beyond 3 days post-fertilisation (dpf). By quantifying zebrafish myocardial growth using live imaging of GFP-labelled myocardium we show that the heart grows extensively between 3 and 5 dpf. Using cellular development timing assays and Kaede photoconversion, we demonstrate that ventricle growth continues to grow from cardiomyocyte (CM) addition as well as from hypertrophy of existing CMs and from CM proliferation. Mechanistically, we show that reduction in activity of Mef2c (*mef2ca*$^{+/-}$; *mef2cb*$^{-/-}$), downstream of Nkx2.5 and upstream of Ltbp3, prevents some CM addition and differentiation, resulting in a significantly smaller ventricle by 3 dpf. After 3 dpf, however, growth of CM number in *mef2ca*$^{+/-}$; *mef2cb*$^{-/-}$ mutants recovers to a normal pace, and the heart size gap between mutants and their siblings diminishes into adulthood. Thus, as in mice, there is an early time window when SHF contribution to the myocardium is particularly sensitive to loss of Mef2c activity.

P 152: Mechanisms Controlling Primordial Germ Cell Behaviour During Early Gonad Formation

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Zahra Labbaf[1], Michal Reichman-Fried[1], Bernhard Wallmeyer[1], Timo Betz[1], Jan Philipp Junker[2], Erez Raz[1]

[1] *Institute for Cell Biology, University of Muenster, Muenster, Germany*

[2] *Institute for Medical Systems Biology, Max Delbrueck Center for Molecular Medicine, Berlin, Germany*

ABSTRACT TEXT

An essential phase in organ development involves accurate spatial arrangement of the different cell types within it. To define the mechanisms responsible for this step of organogenesis we use the positioning of primordial germ cells (PGCs) within the early developing zebrafish gonad as an *in vivo* model.

Following their specification, PGCs migrate towards the region where the gonad develops in response to directional cues encoded by the chemokine *cxcl12a*. Upon arrival at their target by the end of the first day of embryonic development, the motile PGCs maintain their position, although the chemokine *cxcl12a* ceases to be expressed at this location. Thus, chemokine-independent mechanisms maintain the germ cells at the site of gonad development. To identify tissues and molecules that could contribute to the positioning of germ cells at this stage, we conducted a screen for genes whose mRNA is expressed within this region of the embryo. This screen in which we employed genome-wide RNA tomography (Tomoseq), led us to focus on the pronephros as a tissue that could control PGC positioning. Currently, we are generating genetic tools for eliminating the pronephros to investigate the role that this tissue could play in the positioning of PGCs at the gonad region.

In parallel, we characterised the behaviour of the germ cells during the first and second days of development. This analysis revealed progressive reduction in migration speed until the cells stop migrating. Interestingly, we found that this behaviour is independent of cues provided by somatic gonadal cells, suggesting that PGC-autonomous processes control this behaviour.

P 153: In vivo imaging and morphological study of zebrafish cardiovascular system

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Alexander Machikhin[1], Alexander Burlakov[2], Michail Volkov[3], Demid Khokhlov[1]

[1] *Scientific & Technological Center of Unique Instrumentation- Russian Academy of Sciences, Laboratory of acousto-optical spectroscopy, Moscow, Russian Federation*
[2] *Lomonosov Moscow State University, Department of Ichthyology, Moscow, Russian Federation*
[3] *ITMO University, Department of Applied Optics, Moscow, Russian Federation*

ABSTRACT TEXT

We report on the method for in vivo study of zebrafish's cardiovascular system, i.e. the heart and the structure of vessels that carry blood throughout the body. Proposed approach is based on photoplethysmographic microscopic imaging and enables non-contact two-dimensional mapping of blood volume changes. We demonstrate that the obtained data allows precise measurements of heartbeat, blood flow velocity and other important parameters. This approach provides an objective measure of morphology and dynamics, is free of variability due to contrast agents and may have many practical applications related to developmental biology and study of human cardiovascular disease.

P 154: Cardiac myosin light chain kinase (MYLK3) is a novel effector of the planar cell polarity (PCP) pathway required for cardiac morphogenesis

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Kevin Manuel Méndez Acevedo[1,2], Ismail Özcan[3], Alexander Meyer[1], Daniela Panáková[1,2]

[1] *Max Delbrück Center for Molecular Medicine, Cardiovascular and Metabolic Research, Berlin, Germany*
[2] *German Centre for Cardiovascular Research DZHK- Partner Site Berlin., Cardiovascular Research, Berlin, Germany*
[3] *Max Delbrück Center for Molecular Medicine, Medica Systems Biology, Berlin, Germany*

ABSTRACT TEXT

Planar Cell Polarity (PCP) signaling, a major morphogenetic pathway, drives cardiac chamber formation through regulation of actomyosin tension. The Myosin Regulatory Light Chain (MRLC) activity is regulated through the interplay of Myosin Light Chain Kinase (MLCK), Rho-associated Protein Kinase (ROCK) and Myosin Phosphatase (MYPT). The Myosin Light Chain Kinase 3, encoded by the mylk3 gene, is a cardiac-specific kinase that is highly expressed in the zebrafish heart during the time of cardiac chamber formation. Its role and signaling pathway interaction during heart development remain unclear. Here, using zebrafish as a model organism, we show that the reduced levels of mylk3 cause cardiac abnormalities, including cardiac edema and looping defects resembling PCP-deficient hearts. We found a genetic interaction between Mylk3 and the PCP core components and propose Mylk3 as a novel effector protein of the PCP pathway.

P 155: Understanding the role of CCM3 in endothelial development and disease

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Tvisha Misra[1]

[1] *The Hospital for Sick Children- Sickkids, Department of Developmental and Stem Cell and Biology, Toronto, Canada*

ABSTRACT TEXT

Cerebral cavernous malformations (CCMs) are focal dilations in the cerebral vasculature leading to haemorrhaging, strokes and in extreme cases death. Of the three proteins associated with CCMs, CCM1/2/3, CCM3, a scaffold protein highly conserved through species, is the least understood and proposed to have the most detrimental effects. Though various models have been used to study endpoint vascular

defects, not much is known about the earliest cellular events which eventually lead to CCMs. We use the zebrafish as a vertebrate model to understand the role of Ccm3 in early vascular development and disease progression. With CRISPR/CAS9 we generated a c*cm3a/b* double mutant. *ccm3a/b*$^{(-/-)}$ embryos exhibit cardiac edemas, loss of blood flow, and are lethal. Time lapse imaging was used to characterise defects in endothelial cell migration, lumen formation, blood flow, and membrane dynamics. To explore the mechanism of Ccm3 function, BioID was used to determine the potential interactome of Ccm3. Cellular Ccm3 resides mostly in the striatin interacting phosphatases and kinase (STRIPAK) complex. We generated CRISPR/CAS9 mutants of these components of the STRIPAK complex, consisting of largely unstudied genes, to assess their role in vascular development and their relationship to Ccm3, thus uncovering the novel role of STRIPAK component Mob4 in early vascular development. We also know that CCM disease progression is linked to RhoGTPase activity. We determined Cdc42 is implicated in Ccm3 function: *ccm3a/b* KO embryos show aberrant Cdc42 activity and KO/KD of *cdc42* leads to transient cerebral haemorrhages in embryos. Altogether, we have established a model to study early changes in Ccm3 deficient endothelial cells and probe mechanisms of function of Ccm3 *in vivo*.

P 156: Myosin regulatory light chain genes differentially contribute to development and growth of slow-twitch fibres in zebrafish muscle

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Yosuke Ono[1,2,3], *Steffen Scholpp*[1], *Philip Ingham*[1,2,3]

[1] *University of Exeter, Living Systems Institute, Exeter, United Kingdom*
[2] *Nanyang Technological University, Lee Kong Chian School of Medicine, Singapore, Singapore*
[3] *ASTAR, Institute of Molecular and Cell Biology, Singapore, Singapore*

ABSTRACT TEXT

Vertebrate skeletal muscles consist of morphologically and physiologically distinct types of fibre that are characterised by their expression of different isoforms of the myosin light and heavy chain proteins. We previously showed that the gene encoding myosin regulatory light chain 10, *myl10,* is expressed specifically in primary slow-twitch fibres in the zebrafish embryo. In situ analysis has revealed that expression of this gene is also restricted to the slow-twitch fibres in juvenile and adult fish. Using bioinformatic analysis, we have identified an additional eight genes in the zebrafish genome encoding myosin regulatory light chain proteins. Two of these, *myl2a* and *myl2b*, which are most closely related to *myl10*, are expressed exclusively in small diameter fibres at the interface between the slow-twitch and the intermediate fibres in juvenile fish; these likely correspond to the so-called Red Muscle Rim (RMR) fibres. Mutation of the *myl10* gene disrupts the sarcomeric organisation of slow twitch fibres, reducing locomotor activity of larvae and juveniles. Remarkably, locomotor activity recovers in young mutant adults, a recovery that coincides with the emergence of a new population of slow-twitch fibres at the interface between the intermediate and slow-twitch muscle fibres. Like the presumptive RMR fibres in wild-type animals, these fibres specifically express the *myl2a* and *2b* genes. We are currently investigating the origin of these fibres using lineage tracing and genetic analysis.

P 157: Neuropeptides URP1 and URP2 are required for spine formation

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Guillaume Pézeron[1], *Anne-Laure Gaillard*[1], *Hervé Tostivint*[1]

[1] *Muséum National d'Histoire Naturelle - CNRS, Laboratory of Molecular physiology and adaptation - UMR7221, Paris, France*

ABSTRACT TEXT

We previously identified Urotensin II Related Peptide 1 and 2 (URP1 and URP2) as new member of the Urotensin II (Uts2) neuropeptide family, and shown that these two cyclic neuropeptides are produced in ventral CSF-contacting neurons in the spinal cord[1,2]. More recently these two peptides were suggested to be required to straighten the body axis during embryogenesis in zebrafish[3]. To gain insight into URP1 and URP2 function in zebrafish, we have produced mutant lines for these two genes. Double mutants for these two genes did not show any embryonic phenotype but a progressive body axis deformation, beginning in young larvae (circa 10 dpf), was observed.

In adults, these mutants shown a dramatic spine deformation reminiscent of scoliosis. Interestingly, the loss of function of only one gene has no or little consequence showing redundancy between the two genes. In zebrafish, we previously reported the occurrence of five genes encoding for URP receptors (Uts2R)[4]. We have induced loss of function of these five genes and shown that only one, Uts2R3, seems involved to relay URP1/2 signalling. Uts2R3 is expressed in dorsal muscle progenitor in the embryo and mutant for this gene completely phenocopy URP1/2 double mutant phenotype. We are currently characterizing the phenotype of these mutants to understand what are URP1/2 functions in spine formation and /or maintenance. We are also studying how URP1/2 genes are regulated.

1 Parmentier, C., … and Tostivint, H. (2011). *Endocrinology* 152, 2330-41.
2 Quan, F. B., … and Tostivint, H. (2015). *PLoS One* 10, e0119290.
3 Zhang, X., …, and Zhao, C. (2018). *Nature Genetics* 50, 1666-1673.
4 Tostivint, H., … and Larhammar, D. (2014). *Journal of Molecular Endocrinology*, 52, T61-T86.

Our work is supported by MNHN (ATM blanche) and CNRS

P 158: The Smith-Magenis Syndrome-associated gene LLGL1 is required for early heart morphogenesis and cardiac trabeculation in zebrafish

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Eric Pollitt[1], *Emily Noël*[1]

[1] *University of Sheffield, Biomedical Science, Sheffield, United Kingdom*

ABSTRACT TEXT

The cell polarity protein Lethal(2) giant larvae homolog 1 (Llgl1) regulates formation of apical cell junctions and apicobasal polarity, and is located within the Smith Magenis Syndrome (SMS) microdeletion region in humans. SMS is a developmental disorder which includes intellectual disability, sleep disorders, facial abnormalities and cardiac defects, include Tetralogy of Fallot, a morphological heart disorder which includes septal defects and right ventricular hypertrophy. Although loss of another SMS microdeletion gene *RAI1* is associated with multiple aspects of SMS, it likely does not account for all clinical features including specific heart, eye and hearing defects, suggesting loss of an alternative gene within this region drives these disease phenotypes. While a role for *llgl1* has been identified in eye and brain development, its function in heart development has not been investigated.

We generated an *llgl1* zebrafish mutant using CRISPR-Cas9 and characterised distinct aspects of heart development. We found that *llgl1* mutants exhibit abnormal heart looping morphology at 2 dpf, with relative mispositioning of the chambers. These morphological defects are accompanied by cardiac oedema that resolves by 5 dpf, and *llgl1* mutants are adult viable. Interestingly from 80 hpf *llgl1* mutants show a disorganised ventricular myocardium and abnormal trabeculae, suggesting Llgl1 supports cardiomyocyte cell polarity and/or junction formation during initiation of trabeculation. We are currently investigating further the role of *llgl1* in ventricular wall development, and these analyses may provide mechanistic insights into a link between *Llgl1* deletion and cardiac defects in Smith Magenis Syndrome.

P 159: Resolving mesothelium formation at single cell level and in toto

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Karin D. Prummel[1], Helena Crowell[2], Susan Nieuwenhuize[2], Eline C. Brombacher[2], Stephan Daetwyler[3], Charlotte Soneson[2], Jan Huisken[4], Mark D. Robinson[2], Christian Mosimann[1]

[1] *University of Colorado School of Medicine- Anschutz Medical Campus, Department of Pediatrics- Section of Developmental Biology, Aurora, USA*
[2] *University of Zürich, Institute of Molecular Life Sciences, Zürich, Switzerland*
[3] *MPI-CBG, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany*
[4] *Morgridge Institute for Research, Department of Medical Engineering, Madison, USA*

ABSTRACT TEXT

The mesothelium lines the peritoneal cavities, closes the body wall, and surrounds the intra-abdominal organs. Widely distributed within the body as peritoneal membranes, mesothelium and mesothelial stem cells contribute to organ homeostasis and regeneration, and aberrant regulation of mesothelial cells can result in tumors and congenital herniation syndromes. Nonetheless, the embryonic ontogeny of mesothelia and their developmental regulation remain unresolved. Here, we combine genetic lineage tracing, *in toto* live imaging, and single-cell transcriptomics in zebrafish to track mesothelial progenitor origins from lateral plate mesoderm (LPM). We consistently found LPM-derived genetic lineage labeling in the mesothelial layers covering the body cavities and surrounding the major internal organs. Single-cell transcriptomics revealed a post-gastrulation gene expression signature centered around *hand2* that is distinct from other LPM-derived progenitors. Combining gene expression analysis and live imaging, we charted the origin of mesothelial progenitors to the *hand2*-expressing lateral-most stripe within the emerging LPM, consolidating previous observations in zebrafish, chick, and mice. In lightsheet-based time-lapse imaging, we captured zebrafish mesothelium formation *in toto*, documenting the coordinated cell movements that close the body wall and the pericardium. Functionally, we found that loss of *hand2* in zebrafish causes mesothelial defects characterized by ventral herniation as a result of perturbed migration of mesothelium progenitors. Taken together, our findings chart the development of mesothelial progenitors from uncommitted LPM and indicate that Hand2 function provides critical cellular properties to a highly specialized mesodermal progenitor pool that forms mesothelia.

P 160: Transcription factors Meis1 influence craniofacial development via neural crest in zebrafish

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Viktorie Psutková[1], Ondřej Machoň[1]

[1] *Institute of Experimantal Medicine CAS, Developmental biology, Prague, Czech Republic*

ABSTRACT TEXT

TALE-class homeodomain transcription factors Meis are necessary for expression of *Hox* genes. Previous results have demonstrated that *Hox* genes influence neural crest migration via Meis transcription factors. Neural crest cells migrate along the antero-posterior axis and create a lot of tissues such as craniofacial and hematopoietic tissues. It was shown that *Meis1* genes is also important during development of viscerocranium and differentiation of hematopoietic cells in zebrafish. However, the relationship between genes which are responsible for specification, migration, differentiation of neural crest cells and *Meis* is still unknown. We focus on the role of *Meis1* genes during embryogenesis of zebrafish *(Danio rerio)*. We have generated inactivation mutations in zebrafish *Meis1a* and *Meis1b* genes using CRISPR/Cas9 technique. Despite high sequence similarity between *Meis1a* and *Meis1b* paralogues, only *Meis1b* mutant line is lethal. We describe the phenotype in *Meis1a* mutants with the focus on neural crest pattern.

P 161: Selective requirements for vascular endothelial cells and circulating factors in regulation of retinal differentiation

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Shahar Rotem-Bamberger[1], Mya Beitsch[1], Meyrav Sabban[1], Susov Dhakal[2], Deborah Stenkamp[2], Adi Inbal[1]

[1] *The Hebrew University of Jerusalem, Medical Neurobiology, Jerusalem, Israel*
[2] *University of Idaho, Biological Sciences, Moscow, USA*

ABSTRACT TEXT

Blood vessels are necessary for tissue perfusion, supplying oxygen and transporting metabolites, but vascular endothelial cells also have non-metabolic roles. Very little is known about non-metabolic roles of blood vessels in eye development. We have used the ability of zebrafish embryos to develop without a functional cardiovascular system and without vasculature, to discover how ocular blood vessels influence retinal development.

We compared retinal development between normal embryos and embryos that: 1) lack ocular vessels (*npas4l* mutants and embryos that underwent endothelial-specific ablation); 2) have ocular vessels but lack circulation (*tnnt2a* mutants); 3) have ocular vessels and circulation but lack erythrocytes and hence oxygen-carrying capacity (*gata1* mutants).

We found that absence of ocular vessels causes severe defects in retinal neurogenesis and lamination, whereas embryos with ocular blood vessels but no circulation show much milder abnormalities that include reduced neurogenesis. In sharp contrast, embryos with vasculature and circulation but no erythrocytes show almost normal retinal development, suggesting retinal neurogenesis requires factors both from endothelial cells and circulating factors. Cell cycle analyses suggest that the reduced neurogenesis seen in *npas4l* mutants is due to failure of retinal progenitors to exit the cell cycle.

To identify molecular mechanisms underlying the abnormal development of *npas4l* and *tnnt2a* mutant retinas, we compared transcriptomes of retinas of mutant and normal embryos. We find that *npas4l* mutant eyes have many more alterations in gene expression levels compared to *sih* mutants, consistent with the retinal phenotype. We further identified increase in Notch and Wnt signaling, pathways that promote a progenitor fate over retinal neurogenesis, providing a likely mechanism for the disruption of retinal neurogenesis. Together, our data uncovers new roles for the vasculature in regulation of retinal differentiation.

P 162: 3D reconstructions of the cardiac jelly, a tool to unravel the role of ECM asymmetry in the developing zebrafish heart

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Juliana Sanchez-Posada[1], Emily S. Noël[1]

[1] *The University of Sheffield, Department of Biomedical Science, Sheffield, United Kingdom*

ABSTRACT TEXT

During embryonic development, the zebrafish heart tube is formed by an outer layer of myocardial cells and an inner layer of specialised endothelial cells, between which lies a layer of extracellular matrix (ECM) known as the cardiac jelly. Classic studies have demonstrated that specific ECM components have distinct roles in organ morphogenesis, while recent studies have shown how regionalised processes such as ECM remodelling and degradation help to spatially fine-tune organ morphology, particularly of tubular and asymmetric organs like the heart.

Using live *in vivo* light-sheet imaging of zebrafish embryos, we observe an asymmetric expansion of the cardiac jelly on the left side of the heart tube at the onset of cardiac looping, suggesting that early regional differences in the ECM regulate cardiac morphogenesis. To understand the spatiotemporal dynamics of the ECM during heart development and its role in cardiac morphogenesis, an image analysis pipeline using high-resolution live images with state-of-the-art fluorescence microscopy was developed. This pipeline allows not only the visualisation and morphological study of 3D reconstructions of the myocardium and endocardium but also the extraction of a 3D model of the cardiac jelly, facilitating the analysis and quantification of the ECM throughout development. 3D renders of the ECM at different developmental stages confirm the cardiac jelly is dynamic, highly regionalised and precisely deposited and degraded during morphogenesis.

This represents the first temporal 3D morphological characterisation of all three layers of the developing heart: the myocardium, endocardium and ECM. Moreover, this detailed 3D characterisation of cardiac jelly distribution and regionalisation through time, combined with functional analysis of its various components, will provide invaluable insights into ECM dynamics in heart development, and deepen our understanding of its role in the context of cardiac morphology in the embryonic heart.

P 163: Drivers of thyroid cell specification and fate mapping of thyroid progenitors

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Meghna Shankar[1], *Benoit Haerlingen*[1], *Pierre Gillotay*[1], *Sumeet Pal Singh*[1], *Sabine Costagliola*[1]

[1] *Universite libre de Bruxelles, Institut de Recherche Interdisciplinaire en Biologie Humaine et Moleculaire IRIBHM, Brussels, Belgium*

ABSTRACT TEXT

The thyroid is an endocrine gland derived from the foregut endoderm. It is responsible for production of hormones which control growth and metabolism of the organism. Errors in thyroid development cause thyroid dysfunction. The transcription factors, *pax2a, hhex, nkx2.4b* and *foxe1,* form a self-regulatory system, enabling specification, proliferation and maintenance during thyroid formation. We are focusing on the early stages of thyroid development, i.e thyroid progenitor cells. *Pax2a*, one of the first transcription factors to get expressed, marks the thyroid progenitor cells at 18 hours post fertilization(hpf) in zebrafish. 50 % of these pax2a-positive cells, express *nkx2.4b* at 22 hpf. This subset, of double positive cells, has thyroid fate and lies close to the cardiac mesoderm. Could the cardiac mesoderm be signalling thyrocyte specification?

Firstly, to understand the fate of these pax2a positive nkx2.4b negative cells, we are performing multicolour lineage tracing using transgenic lines Tg(tg: brainbow) and Tg(pax2a:CreER). We use the Cre-lox system for unique labelling of these cells to understand their contribution and fate. Do they change fate or undergo apoptosis? We have set up pilot experiments using the transgenic lines and generated multicoloured thyroid follicles.

Secondly, due to the proximity of these double positive cells, to the cardiac mesoderm we hypothesize, that the cardiac mesoderm may be driving thyroid cell specification. To validate this, we use the transgenic line Tg(CM:Gal;UAS:Casp8), an inducible system for caspase mediated apoptosis in the cardiac cells. Targeted ablation should then bring about defective thyroid development. Our experimental setup induces cardiac cell death by tamoxifen induction in zebrafish embryos with loss of cardiac cells and severe pericardial edema. Identifying what drives the thyrocyte specification will allow us to understand the molecular mechanism/signalling involved in early stages of thyroid development.

P 164: Origins of primitive zebrafish erythropoiesis

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Ondřej Svoboda[1], *Olga Machonova*[1], *David Traver*[2], *Petr Bartunek*[1]

[1] *Institute of Molecular Genetics of the Czech Academy of Sciences, Laboratory of Cell Differentiation, Prague, Czech Republic*

[2] *University of California- San Diego, Department of Cellular and Molecular Medicine, San Diego, USA*

ABSTRACT TEXT

Primitive erythrocytes are the first blood cells generated during embryogenesis. They are thought to arise from lateral plate mesoderm, however tracking their emergence has remained challenging. Origin of primitive erythrocytes has been related to the specification of endothelial cells, but the exact relationship between these cell types is still unknown. According to the most prevalent hypotheses, both cell types are thought to be specified either directly from multipotent mesoderm during gastrulation, or later during development from bi-potent endothelial and blood progenitors, called hemangioblast. According to the third hypothesis, the primitive erythrocytes are transdifferentiated from a subset of already committed and differentiated endothelial cells, termed hemogenic endothelium. Although all three hypotheses have been already postulated, the exact relationship between primitive erythrocytes and endothelial cells is still unknown.

Here, we utilized zebrafish as a model organism to study the primitive erythropoiesis. We coupled in vivo fatemapping techniques along with single cell RNA sequencing experiments of early mesodermal population and we were able to track the earliest erythroid fingerprints

at the beginning of gastrulation (between 50 % epiboly and the shield stage that corresponds to mouse E6.5). Importantly, we show that population of these cells express markers of intermediate mesoderm. Although, the pool of these progenitors seem to be already specified at this early stage and even though these cells express endothelial markers, they seem to be relatively distinct from endothelial progenitors.

In summary, on the onset of gastrulation, we identified population of cells that gives rise to precursors of primitive erythrocytes and that does not seem to contribute to general endothelium. Thus, our results provide experimental support for erythroid specification from multipotent intermediate mesoderm during zebrafish gastrulation period.

P 165: Fgfrl1b suppresses FGF signaling to ensure equator-specific onset of lens fiber differentiation in zebrafish

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Yuki Takeuchi[1], *Ichiro Masai*[1]

[1] *Okinawa Institute of Science and Technology Graduate University, Developmental Neurobiology Unit, Okinawa, Japan*

ABSTRACT TEXT

In vertebrate lens, lens epithelium covers the anterior half of lens fiber core. During lens development, lens epithelial cells proliferate, migrate toward the lens equator, and start to differentiate into lens fiber cells after passing through the lens equator. FGF regulates multiple steps of lens fiber differentiation in a dose-dependent manner. However, mechanism underlying the equator specific onset of lens fiber differentiation is not fully understood. FGF receptor-like 1 (Fgfrl1) has three extracellular immunoglobulin domains, which bind to FGF ligands, but lacks an intracellular kinase domain. In mammals, proteolytic cleavage of Fgfrl1 releases its ectodomain, which subsequently binds FGF ligands. Here we focus on zebrafish Fgfrl1b, which is exclusively expressed in lens epithelium, and elucidate its role in lens fiber differentiation. To inhibit the function of Fgfrl1b, we injected its morpholino antisense into wild-type embryos. In Fgfrl1b morphant, expression of an FGF target, pea3, was elevated, suggesting that Fgfrl1b suppresses FGF signaling. Consistently, the expression of a lens fiber differentiation marker, Prox1, was also enhanced and ectopic Prox1 expression occurred in lens epithelium of Fgfrl1b morphant. These data suggest that FGF-dependent lens fiber differentiation is enhanced in the absence of Fgfrl1. Next, to elucidate the mode of action of Fgfrl1b, we overexpressed Fgfrl1b tagged with GFP and mCherry at N- and C-terminus, respectively. GFP expression was observed in plasma membrane or extracellular space, whereas mCherry expression was detected as intracellular foci. This result suggests that Fgfrl1b ectodomain is cleaved and released into extracellular space, resulting in inhibition of FGF signaling. Thus, Fgfrl1b is essential for equator-specific onset of lens fiber differentiation by tuning FGF signaling.

P 166: Mapping tissue properties in developing embryos employing single migrating cells as bioprobes

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Lukasz Truszkowski[1,2], *Sargon Gross-Thebing*[1,2], *Daniel Tenbrinck*[3,4], *Héctor Sánchez-Iranzo*[5], *Carolina Camelo*[2,6], *Kim Westerich*[1,2], *Amrita Singh*[1,2], *Paul Maier*[1,2], *Pia Lange*[3], *Jan Hüvel*[3], *Fjedor Gaede*[3], *Ramona Sasse*[3,7], *Bart Vos*[2,8], *Timo Betz*[2,8], *Maja Matis*[1,2], *Robert Prevedel*[5], *Stefan Luschnig*[2,6], *Alba Diz-Muñoz*[5], *Martin Burger*[3,4], *Erez Raz*[1]

[1] *Institute for Cell Biology, University of Münster, Münster, Germany*
[2] *Cells in Motion, Interfaculty Center, Münster, Germany*
[3] *Applied Mathematics, University of Münster, Münster, Germany*
[4] *current address - Department of Mathematics, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany*
[5] *Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany*
[6] *Institute of Animal Physiology, University of Münster, Münster, Germany*
[7] *Institute for Computational and Applied Mathematics, University of Münster, Münster, Germany*
[8] *Mechanics of Cellular Systems Group- Institute of Cell Biology, University of Münster, Münster, Germany*

ABSTRACT TEXT

Interactions among cells and tissues determine the distribution and shape of structures within the developing embryo. In general, such pattering events depend on biochemical signaling cascades and on biophysical properties of tissues. Here we report on a novel

methodology for identifying tissue properties relevant for morphogenesis, employing Primordial Germ Cells (PGCs) as "bioprobes" for tissue features. Using a software platform we developed, we generated maps of cell distribution derived from a large number of embryos within which PGCs lacking the guidance receptor Cxcr4b migrate non-directionally.

This analysis pipeline allowed us to identify differences among domains within the embryo that are relevant for single-cell migration. Specifically, we identified medial tissue structures that exclude the single migrating cells. We attributed this distribution to deflection of the cells from tissue borders and analyzed the border between the notochord and somitic mesoderm in more detail. We find that this border is characterized by the presence of structured ECM and differences in the tissues' viscoelastic properties.

Furthermore, we show that this methodology is also useful for analyzing the effect of mutations on tissue patterning events and can be used in embryos other than those of zebrafish.

P 167: Studying meningeal development and function using the zebrafish

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Marina Venero Galanternik[1], Ryan D. Gober[1], Andrew E. Davis[1], Daniel Castranova[1], Steven L. Coon[2], Ryan Dale[3], Sydney Hertafeld[3], James Iben[2], Joe Zoeller[2], Louis E. Dye[4], Brant Weinstein[1]

[1] *NICHD, Division of Developmental Biology, Bethesda, USA*
[2] *NICHD, Molecular Genomics Core, Bethesda, USA*
[3] *NICHD, Bioinformatics and Scientific Programming Core, Bethesda, USA*
[4] *NICHD, Microscopy & Imaging Core, Bethesda, USA*

ABSTRACT TEXT

The meninges are a complex vascularized connective tissue that surrounds the Central Nervous System, protecting it from mechanical shock and infection, supporting brain buoyancy, and maintaining brain homeostasis. Despite the critical role of this tissue, the molecular identity, developmental origins, and functional properties of different meningeal cell types remain poorly characterized. We are using the zebrafish to carry out a comprehensive anatomical, molecular, and genetic characterization of the meninges, their cellular constituents, and their roles in brain homeostasis. To examine the anatomical structure of the meninges and the morphology of its resident cell types, we are using histology, electron microscopy, and super-resolution confocal imaging. Our studies have shown that the adult zebrafish meninges are a complex highly vascularized multi-layered tissue containing several unusual cell types. Using single-cell RNA-seq from dissected meninges, we are profiling these cells and correlating expression and anatomical data to define the morphological and molecular identities and interrelationships between the different meningeal cell populations. We have identified "Fluorescent Granular Perithelial" cells (FGPs), a novel scavenger perivascular cell population residing within the inner meningeal layer and clears waste from the cerebrospinal fluid. We have also discovered an inner meningeal cell population expressing high levels of *ependymin (epd)*, a meningeal cerebrospinal fluid glycoprotein with a poorly understood function. Ependymin-expressing cells (EPDs) are large flat cells that ensheath meningeal blood vessels and FGPs. We have found additional novel cell types and structures in the outer meningeal layers. Together, our ongoing studies using the powerful tools and methods available in the fish are facilitating comprehensive understanding of meningeal development and function.

P 168: On the trail of zebrafish pacemaker cells

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Cecilia Winata[1,2], Karim Abu Nahia[1], Tomasz Obrebski[1], Rashid Minhas[1], Katarzyna Piwocka[3], Vladimir Korzh[4]

[1] *International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Zebrafish Developmental Genomics, Warsaw, Poland*
[2] *Max Planck Institute for Heart and Lung Research, Laboratory of Zebrafish Developmental Genomics, Bad Nauheim, Germany*
[3] *Nencki Institute of Experimental Biology- Polish Academy of Sciences- Warsaw- Poland, Department of Biochemistry, Warsaw, Poland*
[4] *International Institute of Molecular and Cell Biology in Warsaw, Laboratory of Neurodegeneration, Warsaw, Poland*

ABSTRACT TEXT

Cardiac pacemaker cells are responsible for initiating and coordinating the electrical signals that cause rhythmic and synchronized contractions of the heart. They are set apart from cardiomyocytes early in the course of heart development through the initiation of distinct

molecular programs. To better understand the molecular mechanism underlying pacemaker development and function, we profiled the transcriptome of the sinoatrial ring and atrioventricular canal - regions that are known to possess pacemaker activity in the zebrafish heart. These were FACS-isolated from zebrafish transgenic lines sqet33mi59BEt and sqet31Et expressing EGFP in respective cell populations. Besides genes known as hallmarks of the pacemaker cells such as *hcn4*, *shox2*, and *tbx18*, the analyses also revealed new candidate genes and signaling pathways underlying pacemaker development and function. Comparative analyses between the transcriptomes of cardiomyocytes and the two pacemaker sites revealed distinct molecular profiles, which provide insights into the mechanism of their diversification and suggests the heterogeneity of cell types composing the pacemaker regions. I will present the latest results from our ongoing work on this topic.

The project First TEAM/2016 -1/8 is carried out within the First Team program of the Foundation for Polish Science co-financed by the European Union under the European Regional Development Fund.

P 169: Mechanisms and patterns of activation of Matrix Metalloproteinase 2 during embryonic development

TOPIC: MORPHOGENESIS AND ORGANOGENESIS

Rachael Wyatt[1], *Matthew Mabey*[1], *Emma Jeffrey*[2], *Bryan Crawford*[1]

[1] *University of New Brunswick, Biology, Fredericton, Canada*
[2] *Dalhousie University, Medicine, Saint John, Canada*

ABSTRACT TEXT

In the context of vertebrate embryonic development, the emergence of tissue architecture and morphogenesis are a function of cellular proliferation, changes in cell shape adhesion and cytoskeletal architecture, cellular motility, and remodelling of the extracellular matrix (ECM). The matrix metalloproteinases (MMPs) are the primary effectors of ECM remodelling, and the regulation of their activities underlies embryonic morphogenesis, physiological tissue homeostasis and wound healing, and their mis-regulation underlies a plethora of pathologies ranging from arthritis to tumour metastasis. Matrix metalloproteinase 2 (Mmp2) is expressed ubiquitously in vertebrates from early somitogenesis, but as an inactive proMMP (zymogen) whose biologically relevant activity is regulated post-translationally by the proteolytic removal of an auto-inhibitory pro-peptide. We have developed a technique (the 'epitope mediated MMP activation' (EMMA) assay) and transgenic zebrafish expressing doubly epitope tagged catalytically inactive 'EMMA-Mmp2' under the regulation of the heat shock promoter such that we can visualize and quantitatively characterize the proteolytic activation of Mmp2 during embryonic development in zebrafish. We observe developmentally dynamic patterns of Mmp2 activation that correlate with patterns of collagen degradation in vivo, particularly in somite boundaries and associated with notochord straightening and elongation. Mmp2 is thought to be activated primarily by other MMPs, particularly Mmp14 (MT1-MMP), but surprisingly, inhibitors of metalloproteinases have no significant effects on EMMA-Mmp2 processing. In contrast, AEBSF (a serine proteinase inhibitor) significantly inhibits EMMA-Mmp2 processing in the notochord, and also causes severe notochord kinking and disruption of tail uncurling in zebrafish embryos, suggesting that serine proteinases may be more important in Mmp2 activation during early vertebrate development than previously appreciated.

Neurobiology

P 170: Inhibition of lymphocyte-specific protein tyrosine kinase affects microglia function

TOPIC: NEUROBIOLOGY

Aitana Almodóvar Payá[1], *Petronella Kettunen*[1]

[1] *University of Gothenburg Sahlgrenska Academy, Neuroscience and Physiology, Gothenburg, Sweden*

ABSTRACT TEXT

Alzheimer's disease (AD) is the most common cause of dementia and is generally thought to be caused by the extracellular accumulation of amyloid-beta (Aβ) in the brain, resulting in neurodegeneration and memory loss at its later stages. It has been shown that microglia are involved in the pathogenesis of AD as they are part of clearing and degrading Aβ from the brain. However, accumulation of Aβ peptide is also thought to cause microglia activation leading to chronic inflammation and the consequent neuronal loss, but the exact mechanisms are not yet known.

Lymphocyte-specific protein tyrosine kinase (*LCK*) is a risk gene for AD and is thought to regulate inflammatory pathways that could contribute to the disease. This study aimed at understanding how Lck is affecting neuronal survival as well as microglia function *in vivo*. To this end, we used a transgenic zebrafish marker line for microglia, Tg(*ApoE:GFP*) in combination with brain injections of labeled Aβ. In this preparation, we investigated effects of the inhibition of Lck on microglia function. In parallel, experiments were done in a wild-type line for cell death analysis. Confocal imaging was used to evaluate neuronal cell death, microglia number, morphology and phagocytosis, as well as properties of microglia lysosomes.

Our results indicate that blocking of Lck in the zebrafish leads to increased cell death, morphological and functional changes in microglial cells and possibly malfunctioning microglia lysosomes. We conclude that the effects seen from blocking Lck could be related to the disease processes leading to AD in humans. Our data invite further investigation on possible AD treatments focusing on the Lck pathway.

P 171: Defective excitatory/inhibitory synaptic balance and increased neuron apoptosis in a zebrafish model of Dravet Syndrome

TOPIC: NEUROBIOLOGY

Alexandre Brenet[1], *Rahma Hassan-Abdi*[1], *Julie Somkhit*[1], *Constantin Yanicostas*[1], *Nadia Soussi-Yanicostas*[1]

[1] *Inserm U1141, NeuroDiderot, Paris, France*

ABSTRACT TEXT

Epilepsy is one of the most common neurological disorder that affect around 1 % of the world's population. This disease is characterized by recurrent and spontaneous electrical discharge of a group of neurons in the brain. Current anti-epileptics therapeutic approaches, which focus only neurons, are fully inefficient in one-third of the patients, highlighting the need to find new approaches. In recent years, zebrafish disease models with Scn1Lab sodium channel deficiency that mimic Dravet Syndrome have been generated to seek novel anti-epileptic drug candidates, some of which are currently undergoing clinical trials. However, the spectrum of neuronal deficits observed in scn1Lab$^{-/-}$ larvae is poorly know. To fill this gap and gain a better understanding of the mechanisms underlying neuron hyperexcitation in Scn1Lab-depleted larvae, we analyzed the neuronal activity *in vivo* of these larvae by combining local field potential recording with transient calcium uptake imaging, and show for the first time in a genetic model of epilepsy the correlation between calcium imaging and local field potential, opening a new non-invasive way to study spatial and temporal spread of epileptic seizures. We also looked at the genesis of the disease by studying the distribution of excitatory and inhibitory neurons during the development, which showed a specific loss of inhibitory neurons, appearing just before the beginning of the seizure. This neuronal imbalance lead to a decreased density of inhibitory post-synaptic markers and an increase density of excitatory post-synaptic markers. Finally we demonstrate that this

neuronal hyperactivity causes a marked increase of neuronal death. Our results thus provide *in vivo* evidences suggesting that Scn1Lab loss of function causes a specific inhibitory neuronal loss, a synaptic imbalance toward excitation, probably leading to the apparition of neuronal hyperexcitability and neuron damage observed in Dravet syndrome.

P 172: Wif1 mediated Wnt signaling feedback control in habenular neuron formation and axonal targeting

TOPIC: NEUROBIOLOGY

Anja Bühler[1], Luca Guglielmi[2], Enrico Moro[3], Francesco Argenton[3], Lucia Poggi[1], Matthias Carl[1]

[1] *University of Trento, Department of Cellular- Computational and Integrative Biology- CIBIO, Trento, Italy*
[2] *The Francis Crick Institute, Developmental Signalling Laboratory, London, United Kingdom*
[3] *University of Padova, Department of Biomedical Sciences, Padova, Italy*

ABSTRACT TEXT

Bilateral clusters of habenular neurons in the forebrain of vertebrates relay cognitive information into the interpeduncular nucleus and the median raphe in the ventral mid- and hindbrain, respectively. This neurotransmitter system has been implicated in behaviours from fear and social behaviour to reward responses and addiction. It is also linked to pathophysiological syndromes such as depression, autism and schizophrenia. Indeed, our studies in zebrafish have revealed that the Wnt/beta-catenin signalling pathway gene Tcf7l2, which was formerly identified as a risk factor for schizophrenia, is pivotal for the establishment of habenular neuron diversity. In this process, Tcf7l2 mediated Wnt signalling functions only in post-mitotic habenular neurons, although many Wnt pathway genes including tcf7l2 mRNA are expressed far earlier. We now find that premature activation of Wnt signalling delays habenular neuron differentiation, severely perturbs correct habenular neuron identities and abrogates the laterotopic segregation of habenular efferent axons in the IPN target. Our gene expression and functional analysis provide strong evidence that the secreted tumour suppressor Wnt inhibitory factor 1 (Wif1) is mediating the temporal control of Wnt signalling. Intriguingly, once initiated, *Wif1* expression in turn depends on Wnt signalling itself similar to findings in cancer cells in vitro suggesting a conserved mechanism underlying different processes. We propose that a Wif1 mediated regulatory feedback loop dynamically buffers Wnt signalling within nascent habenular progenitors before they develop into neurons. Thus, both early inhibition and subsequent activation of Wnt/beta-catenin signalling are equally important for correct generation of habenular neurons. Intriguingly, like habenular function Wif1 has been linked to autism, paving the path for further exploring the link between molecule, neural circuit and pathophysiological syndrome.

P 173: The role of quaking b in neuronal development and behavior

TOPIC: NEUROBIOLOGY

Adrian Ekehorn Gimdal[1], Petronella Kettunen[1], Lina Emilsson[2]

[1] *University of Gothenburg, Neuroscience and Physiology, Gothenburg, Sweden*
[2] *Uppsala University, Faculty of Science and Technology, Uppsala, Sweden*

ABSTRACT TEXT

Schizophrenia is a multifactorial mental disorder with a normal onset in early adulthood. Defective development of the central nervous system has been suggested as a cause and downregulation of the gene quaking (*QKI*) has been observed in the brains of patients with schizophrenia. QKI is a RNA-binding protein that is involved in regulating the translation of several oligodendrocyte genes. The main role of oligodendrocytes is to form myelin sheaths around axons in the CNS and it is therefore possible that a non-functioning *QKI* gene could lead to impaired myelination which in turn could cause some of the symptoms associated with schizophrenia. In this study, we used a newly developed mutant *qkib* zebrafish line to investigate the role of *qkib* in CNS development and behavior. We used both *in vivo* confocal imaging of the brain and spinal cord as well as behavioral tests to assess effects on e.g. locomotion, drug-induced seizures and learning. Preliminary data indicate that *qkib* is involved in the development of the CNS and introduction of mutations in *qkib* affects specific behaviors in the zebrafish. Further research is required to fully understand how *qkib* regulates neuronal structure and function in the healthy and diseased brain.

P 174: Neuropilin1a is required for proper axonal targeting in the zebrafish Optic Tectum

TOPIC: NEUROBIOLOGY

Beatriz Furones Cuadrado[1], Helene Juul Belling[1], Alison Barker[2], Bettina Kirchmaier[1], Amparo Acker-Palmer[1]

[1] *Institute of Cell Biology and Neuroscience, Neuroscience and Buchmann Institute for Molecular Life Sciences BMLS, Frankfurt am Main, Germany*

[2] *Max Delbrück Center for Molecular Medicine, Molecular Physiology of Somatic Sensation, Berlin, Germany*

ABSTRACT TEXT

Neuropilin1 (nrp1), which is duplicated in zebrafish (*nrp1a* and *nrp1b*), is a neurovascular molecule that acts as co-receptor for VEGFR2 and plexins by binding VEGFs or class-3 semaphorins. Here, we investigated the role of *nrp1a* in the formation of the retinotectal map by analyzing axonal navigation of the retinal ganglion cells (RGCs) from the retina to the optic tectum (OT) in the *nrp1a*^{hu10012} zebrafish mutant. Silencing *nrp1a* with morpholinos has previously been shown to result in defective midline axonal crossings, but lipophilic eye injections in 5 dpf of our *nrp1a* mutant did not reproduce any aberrant ipsilateral projections. Interestingly, *nrp1a* mutants crossed with the reporter line Tg(-2.7shh:GFP)^{t10} for visualizing RGCs, exposed a robust phenotype in the sublaminar layering of the OT, as increased axonal invasion could be observed aberrantly positioned between the SO and the SFGS layers. Using brainbow labeling we could show that *nrp1a* mutant axons entered the SFGS layer and later arborized into both the SFGS and SO. We next performed a visual behavior assay as a functional readout of the aberrant retinotopic layering. Importantly, a size discrimination assay demonstrated that 5 dpf *nrp1a* mutant larva respond abnormally to small dots, suggesting that the mislayering of the axons also give rise to a defect in visual function. To delineate the molecular mechanism regulating the RGC pathfinding in the OT mediated by *nrp1a* signaling, we performed rescue experiments with different Nrp1 signaling mutants. We injected RNA corresponding to Nrp1Δa1+7 (Sema3- signaling deficient *Nrp1*) and Nrp1Δb2 (VEGF-binding deficient *Nrp1*). Only Nrp1Δb2 was able to rescue the mislayering phenotype of the *nrp1a* mutants, suggesting that binding to class-3 semaphorins is required for the function of *nrp1a* during OT axonal targeting. In summary, our results unveiled that *nrp1a* plays a novel role in OT layering and visual function mediated by the interaction with class-3 semaphorins.

P 175: Zebrafish knockout of the autism risk gene Setd5 leads to neurotransmission-associated gene alterations and social impairments

TOPIC: NEUROBIOLOGY

Chiara Gabellini[1], Cecilia Pucci[1], Davide Martini[1], Caterina Di Lauro[1], Matteo Digregorio[1], Diletta Sidoti[1], Chiara De Cesari[1], Silvia Marini[1], William Norton[2], Alessio Zippo[3], Vania Broccoli[4], Alessandro Sessa[5], Massimiliano Andreazzoli[1]

[1] *University of Pisa, Department of Biology, Pisa, Italy*

[2] *University of Leicester, Department of Neuroscience- Psychology and Behaviour-, Leicester, United Kingdom*

[3] *University of Trento, Center for Integrative Biology, Trento, Italy*

[4] *CNR, Institute of Neuroscience, Milan, Italy*

[5] *San Raffaele Scientific Institute, Stem Cell and Neurogenesis Unit- Division of Neuroscience, Milan, Italy*

ABSTRACT TEXT

SETD5 loss-of-function (LoF) mutations in humans have been recently associated to intellectual disability (ID) and autistic spectrum disorders (ASD). *SETD5* gene encodes for a putative histone H3 methyltransferase highly expressed in the brain and it falls within the critical interval deleted in the "3p25.3 microdeletion syndrome", characterized by ID, microcephaly and congenital heart defects. The aim of this study is to characterize *setd5* LoF zebrafish models generated by morpholino injection and CRISPR-Cas9 gene editing technique. *setd5* expression is localized in the developing central nervous system of larvae and in specific brain areas of adults. *setd5* morphants show reduced methylation of Lysine 36 of Histone 3 (H3K36), indicating a catalytic activity of *setd5* as H3K36 methyltransferase. Moreover, *setd5* LoF zebrafish display reduced expression of mRNAs coding for synaptic proteins and enzymes of neurotransmitter metabolism, associated to microcephaly, a significant reduction of body length and locomotor activity in both larvae and adults. In addition, *setd5* LoF adults are characterized by a reduced social interaction which is ameliorated by risperidone, an antipsychotic drug commonly used to treat behavioral traits in ASD patients.

The validation of the zebrafish *setd5* LoF mutants as reliable models for ASD/ID might have an important therapeutic impact. Indeed, the characterization of the molecular pathways altered by setd5 LoF may support the screening for targeted compounds able to rescue the developmental and behavioral defects observed in these zebrafish mutants. The future perspective is to identify promising therapeutics to ameliorate the behavioral alterations in individuals affected by ASD/ID due to *SETD5* haploinsufficiency.

P 176: Understanding the role of chromatin remodeler chd7 in GABAergic network development using a zebrafish loss-of function model

TOPIC: NEUROBIOLOGY

Priyanka Jamadagni[1], *Maximilian Breuer*[2], *Eric Samarut*[3], *Kessen Patten*[1]

[1] *INRS - Center Armand-Frappier Santé Biotechnologie, Biology, Laval, Canada*
[2] *INRS- Center Armand-Frappier Santé Biotechnologie, Biology, laval, Canada*
[3] *Centre de recherche du centre hospitalier de l'Université de Montréal CRCHUM- Montréal- Canada, Biology, Montreal, Canada*

ABSTRACT TEXT

Mutations in the ATP-dependent chromatin remodeller chromodomain, helicase, DNA binding (CHD) 7 are the primary cause of CHARGE syndrome and have been associated with autism spectrum disorder (ASD) . However, the mechanisms by which mutations in CHD7 affect brain development and function are poorly understood. We have developed a zebrafish chd7 CRISPR/Cas9 knockout model, employing the suitability of this vertebrate model for the study of early neurodevelopment. chd7 knockout zebrafish larvae exhibit a small head phenotype, defects in craniofacial cartilage development, heart defects and had no swim bladder. We also found that chd7-/- fish display aberrant axonal network development. Interestingly, the mutant fish displayed hyperactivity particularly during the dark phase of the light-dark cycle. Reports suggest, an aberrant inhibitory signaling in the brain could be a mechanism underlying this phenotype in a zebrafish model for ASD. Interestingly, treatment with the GABA-A receptor antagonist PTZ showed that chd7 mutants exhibit an increased sensitivity to PTZ-induced seizures. Further, we observed a significant decrease in GABAergic neurons in chd7 mutants, that in certain regions of the brain is due to a failure in the migration of these cells. Using an unbiased whole transcriptomic approach, genes involved in cell proliferation, migration and cell adhesion that are dysregulated in the chd7 mutants were identified. Our findings indicate loss of chd7 results in a deficit of inhibitory neurons, suggesting an essential role of chd7 in the brain neuronal network development.

P 177: Are the fish inner ear endorgans differently affected by noise exposure?

TOPIC: NEUROBIOLOGY

Ieng Hou Lau[1], *Lukas Breitzler*[2], *Paulo J. Fonseca*[3], *Raquel O. Vasconcelos*[2]

[1] *Rua de Londres 106- Macau- China, ISE, Macau, Macao*
[2] *University of Saint Joseph, ise, Macau, Macao*
[3] *Universidade de Lisboa, ce3c, Lisboa, Portugal*

ABSTRACT TEXT

Teleost fishes rely on their auditory systems to interpret crucial information from the acoustic environment. However, the contribution of each inner ear otolithic endorgans (saccule, utricle and lagena) for hearing and their sensitivity to noise exposure is still not clear. While the saccule has often been assumed as the main auditory endorgan in teleosts, some authors suggest that all three may serve mixed auditory-vestibular functions, and hence they might all be affected by acoustic trauma.

This study aims to evaluate the sensitivity of the three otolithic endorgans based on the impact of noise on the hair cells and their synaptic activity in the zebrafish Danio rerio, a widely used model organism in hearing research.

Adult zebrafish were subject to either 24 hours of white noise (168 ± 4 dB re re 1 µPa) or quiet conditions (c. 105 dB). Some of the noise-treated individuals were allowed to recover for additional 24 hours in silent conditions. The sensory epithelia morphological damage, hair cell number, and the presynaptic function (based on Ribeye b) were quantified.

The saccule was the only endorgan exhibiting obvious cellular damage and loss of receptors, which confirms its main auditory role in the zebrafish. Preliminary data also indicated a potential decrease in presynaptic function of saccular hair cells. Moreover, results pointed to a more pronounced loss of both hair cell receptors and synaptic function in the specimens that were analyzed 24 hours post treatment, probably due to subsequent oxidative stress, inflammatory processes, and activation of cellular death pathways.

P 178: The specification of quiescent neural stem cells in the vertebrate zebrafish brain

TOPIC: NEUROBIOLOGY

Yuanyuan Liu[1], *Nynke Oosterhof*[1], *Judith Paridaen*[2]

[1] *European Research Institute for the Biology of Ageing ERIBA/UMCG University of Groningen, Postdoc, Groningen, Netherlands*

[2] *European Research Institute for the Biology of Ageing ERIBA/UMCG University of Groningen, Junior PI/Assistant professor, Groningen, Netherlands*

ABSTRACT TEXT

In the adult vertebrate brain, neural stem cells (NSCs) constitute astroglial cells that generate a limited number of neuronal and glial cell types. They are crucial components of brain homeostasis and repair. Essential parameters of NSC maintenance include stemness (capacity to self-renew) and quiescence (undergo cell division rarely). There are two main study result of adult NSCs, the first is the adult NSCs emerge during embryonic neurogenesis, it was identified a slowly dividing subpopulation of embryonic NPCs that later give rise most young adult NSCs; the second is adult NSCs are randomly selected from the entire population of NPCs after embryogenesis, the cells that later become adult NSCs slow down the cell cycle and are set aside as a reserve pool early in brain development, when other NPCs are dividing rapidly to build the brain within a limited time period. However, we currently want to understand how and why this subset of cells is selected as adult NSCs.

In order to solve this problem, zebrafish will be used as model organism. Importantly, zebrafish are a great model organism for live imaging and genetic manipulations. We propose to study how embryonic neural stem cells in the developing zebrafish telencephalon are selected to enter quiescence and remain as the adult NSCs. In the adult zebrafish pallium, RGCs are located along the pallial ventricle. RG cell bodies organize into a monolayer in contact with the CSF, while their long processes extend into the pallial parenchyma to reach the pallial surface of the brain in contact with large blood vessels.

In addition, the experiment also would like to investigate whether qNSCs arise in specific spots in the developing telencephalon; how the number of selected qNSC is determined, and there are specific cell biological properties that are important for their maintenance into adulthood.

P 179: Synapsin III knockdown affects neuron development in Zebrafish

TOPIC: NEUROBIOLOGY

Alessia Muscò[1], *Francesca Longhena*[1], *Gaia Faustini*[1], *Maurizio Memo*[1], *Arianna Bellucci*[1], *Daniela Zizioli*[1]

[1] *Università degli Studi di Brescia, Dipartimento di Medicina Molecolare e Traslazionale, Brescia, Italy*

ABSTRACT TEXT

Synapsins are a family of neuron-specific phosphoproteins highly conserved in vertebrate and invertebrate species. Although they were first described and characterized as synaptic vesicles (SV)associated proteins, reversibly tethering SVs to presynaptic's actin cytoskeleton thus modulating neurotransmitter release, many data suggest their involvement in synaptic plasticity processes and synapse formation. In mammals, Synapsin III (SynIII) temporal expression pattern, with an onset during the early stages of nervous system development, is emerging as an important factor in the regulation of neurogenesis. Moreover, recent data have focused on SynIII because of its presence in Lewy bodies, the main histological hallmark of Parkinson's disease (PD), where it plays a crucial role for ∝-synuclein pathological deposition. To further understand the role of this protein during neurogenesis in a vertebrate model, we used a specific morpholino (MO) to knock down *synIII* in zebrafish embryos. The *synIII* morpholino was injected at one-cell stage in a fluorescent transgenic line for neurons, Tg(*neurod1*/EGFP). Our work shows that the lack of *synIII* induces a clear phenotype with perturbed brain morphology. WISH analysis, using specific neural markers such as *isl1* and *ngn1*, of *synIII* morphants reveals a clear diffuse signal reduction in telencephalic

and diencephalic cell populations. Moreover, the organization of axons originated from *neurod1*+ spinal cord neurons within the somites, appears to be disrupted and axon branches display a significant reduction in length. *SynIII* mRNA from Rattus Norvegicus was co-injected with *synIII*-MO at one-cell stage, the mRNA was able to restore the phenotype of morphants, demonstrating both the specificity of our morpholino and the conserved function of *synIII* among different species. Our findings highlight the role and the importance of zebrafish *synapsin III* as a key factor in neuron development and axon branching.

P 180: Potential neuronal biomarkers of pain in zebrafish larvae

TOPIC: NEUROBIOLOGY

Nils Ohnesorge[1]

[1] *German Federal Institute for Risk Assessment, Experimental Toxicology and ZEBET, Berlin, Germany*

ABSTRACT TEXT

For fish as for all vertebrates it is required by European law to reduce pain, suffering and distress to the unavoidable minimum in husbandry and experiments. It has been well established that fish have excellent capabilities of nociception to perceive potentially harmful conditions but the discussion whether they are capable to experience negative emotions to fulfill the definition of pain is not yet settled. One problem is that in contrast to mammals the brain regions for pain perception and processing have not been identified, yet.

In the project presented here, a model of the panneuronal line Tg(elavl3:H2B-GCaMP6s) was established to screen for neuronal biomarkers of pain and nociception in zebrafish larvae. Different nociceptive and potentially painful stimuli were applied to unanaesthetized 4 dpf larvae and brain activity was recorded based on calcium imaging in the scale of seconds in timelapse imaging for 30 min. Both whole brain scans and single slice scans for higher resolution in time have been performed. The search for neuronal biomarkers of pain was narrowed down to a few brain regions comprising in total around 1000 active cells, which is small enough that analysis of single cell activity patterns becomes possible.

On these selected active cells, tools of bioinformatic analysis will be applied to identify patterns of neuronal activity specific for pain perception and processing.

P 181: A new candidate gene involved in nervous system myelination: functional in-vivo validation using a zebrafish model system

TOPIC: NEUROBIOLOGY

Alex Pezzotta[1], *Stefania Magri*[2], *Francesca Balistreri*[2], *Daniela Di Bella*[2], *Cinzia Gellera*[2], *Ettore Salsano*[3], *Anna Pistocchi*[1], *Franco Taroni*[2]

[1] *University of Milan, Medical Biotechnology and Translational Medicine, Milan, Italy*
[2] *IRCCS Foundation Neurological Institute Carlo Besta, Unit of Medical Genetics and Neurogenetics, Milan, Italy*
[3] *IRCCS Foundation Neurological Institute Carlo Besta, Department of Clinical Neurosciences, Milan, Italy*

ABSTRACT TEXT

Leukodystrophies (LDs) represent a heterogeneous group of inherited neurodegenerative disorders characterized by demyelinated or hypomyelinated forms of myelin in the central nervous system (CNS). The aetiology of these diseases is often unknown and advances in the field of diagnostic research are an emerging topic for the searching of new candidate genes. In line with this, our preliminary data obtained from NGS approach identify one gene (named *GENEA*) as a possible candidate gene for LDs insurgence. Due to the conservation of the signaling pathway governing central nervous system development between vertebrates, we decided to use zebrafish (*Danio rerio*) as an in vivo model to investigate the role of the identified gene in the formation of myelin. In a *geneA* knockdown zebrafish model generated with the injection of an antisense oligo called morpholino (*geneA*-MO), we found a defective response in a touch-response motility assay in 2.5 and 4 days post fertilization (dpf) embryos. In addition, at the molecular level (RT-qPCR and in situ hybridization) we observed that *geneA* knock-down reduced the expression of the *oligodendrocyte transcription factor 2* (*olig2*) and *myelin basic promoter* (*mbp*), the markers commonly used as a read-out for the screening of defects in myelin formation. Moreover, we found that the injection of the zebrafish and human mRNA of *GENEA* into *geneA*-MO embryos was able to rescue both the motor defects and to recover the *olig2* and *mbp* expressions, confirming that the observed phenotype was specifically due to *geneA* downregulation. On the contrary, the injection

of the human mRNA of *GENEA* carrying the mutation identified in the LD patient did not rescue the phenotype of *geneA*-MO injected embryos, confirming the pathogenicity of the mutation. In conclusion, our data point to the role of geneA in myelinating processes confirming its role, when mutated, in LDs insurgence.

P 182: Neural auditory encoding, auditory sensitivity and tonotopy in larval zebrafish

TOPIC: NEUROBIOLOGY

Rebecca Poulsen[1], *Lena Constantin*[1], *Leandro Scholz*[1], *Itia Favre-Bulle*[2], *Gilles Vanwalleghem*[1], *Ethan Scott*[1]

[1] *University of Queensland, Queensland Brain Institute, St Lucia, Australia*
[2] *University of Queensland, Queensland Brain Institute & School of Mathematics and Physics, St Lucia, Australia*

ABSTRACT TEXT

To date, we have a partial and inconsistent knowledge base regarding the hearing abilities of larval zebrafish. The question of how the brain responds to the various properties of sound and the heterogeneity of frequency organization remains unanswered. Here, we present an experimental setup using speakers coupled directly to a water-filled imaging chamber, permitting precise ensonification to 6dpf larval zebrafish. We use this with GCaMP6f and selective plane illumination microscopy (SPIM) to extract neuronal population profiles of responses to the different properties of sound. We found auditory responses to frequencies reaching 2.5kHz brain-wide. Using K-means clustering, we found profiles of neurons that respond selectively to "simple" pure tones, "complex" white noise, short onset versus long onset sounds, and varying combinations of these. Our supervised analysis showed frequency-selective neuronal populations, with the majority residing in the mid- and hindbrain. We found spatial heterogeneity of frequency-selective neurons in several brain regions namely the telencephalon, tectum, torus semicircularis, cerebellum, octavolateral nuclei and remaining hindbrain, although this has not been arranged into coherent patterns of tonotopy. We then tested auditory sensitivity on the Fragile X *fmr1* zebrafish model. Fragile X is the most common monogenetic form of autism, with humans showing hypo- and hyper-responsiveness to sensory stimuli. We showed auditory hypersensitivity in the *fmr1* fish in the ON (homologous to the cochlea nuclei), combined with reduced filtering in the ascending auditory pathway, particularly the thalamus and the tegmentum. We constructed a model of brain-wide auditory networks that revealed that the network is engaging at lower volumes in the *fmr1* animals, providing possible network-level mechanisms by which sensory processes is altered subcortically.

P 183: Chronic hyperammonemia causes hypoglutamatergic and hyperGABAergic neurotransmission associated with neurobehavioral abnormalities in zebrafish larvae

TOPIC: NEUROBIOLOGY

Joris Probst[1], *Stefan Kölker*[1], *Jürgen Okun*[1], *Amrish Rajendra Kumar*[2], *Eduard Gursky*[2], *Roland Posset*[1], *Georg Hoffmann*[1], *Ravindra Peravali*[2], *Matthias Zielonka*[1]

[1] *University Hospital Heidelberg, Center for Pediatric and Adolescent Medicine- Division of Pediatric Neurology and Metabolic Medicine, Heidelberg, Germany*
[2] *Karlsruhe Institute of Technology, Institute for Biological and Chemical Systems-Biological Information Processing, Eggenstein-Leopoldshafen, Germany*

ABSTRACT TEXT

Chronic hyperammonemia is a common condition affecting individuals with inherited urea cycle disorders, which is associated with progressive cognitive impairment and behavioral abnormalities. Altered neurotransmission has been proposed as a source of neuronal dysfunction, but the molecular pathomechanisms induced by chronically elevated ammonium (NH_4^+) concentrations remain only incompletely understood.We established a zebrafish model of chronic hyperammonemia by exposing zebrafish larvae to 0.5 mM ammonium acetate starting at developmental age of 2 days post fertilization (dpf) until 10 dpf and investigated the impact of elevated NH_4^+ concentrations on genetic, biochemical, morphological and behavioral outcome parameters. Chronic hyperammonemia caused locomotor dysfunction and abnormal feeding behavior, indicative for an impairment of higher brain functions. Elevated NH_4^+ concentrations were associated with increased formation of GABA with concomitant depletion of glutamate by enhanced activity of glutamate decarboxylases, ultimately leading to dysfunctional hypoglutamatergic and hyperGABAergic neurotransmission. Elevated GABA concentrations were

accompanied by increased expression of $GABA_A$ receptor subunits alpha-1, gamma-2 and delta, supporting the notion of an increased "GABA tone" in chronic hyperammonemia. Altered glutamatergic and GABAergic neurotransmission is an important pathophysiological factor causing neurocognitive impairment in chronic hyperammonemia, which might have direct implications for future treatment approaches.

P 184: Role of microglia on seizure activity after organophosphate exposure

TOPIC: NEUROBIOLOGY

Julie Somkhit[1], *Alexandre Brenet*[1], *Nadia Soussi-Yanicostas*[1]

[1] *Inserm U1141, NeuroDiderot, Paris, France*

ABSTRACT TEXT

Organophosphorus (OP) compounds are widely used as pesticides and chemical warfare agents. They represent a real threat in the event of terrorist attacks or in situations of armed conflict and this threat remains relevant. OPs are irreversible cholinesterase inhibitors that cause a cholinergic syndrome associating peripheral manifestations and epileptic seizures that worsen in status epilepticus if they are not rapidly treated. As the cholinergic status epilepticus becomes drug-resistant in less than 30 minutes, the search for new innovative treatments is a priority. In our team, we use a zebrafish model to study the role of an organophosphorus compound, diisopropylfluorophosphate (DFP), in the induction of epileptic seizures. In particular, we are interested in the role of microglial neuroinflammation in the status epilepticus induced by DFP. Thus, we developed a zebrafish model of DFP poisoning. This model reproduces characteristics of epileptic seizures observed in existing murine models such as generalized seizures and induction of neuronal death. We then looked at the behavior of microglia following DFP intoxication. Microglial cells are resident macrophages of the vertebrate brain that maintain its homeostasis and protect neurons against injuries and pathogens. They engulf or phagocytize debris and dying cells and also contribute to neuroinflammation by releasing cytokines and neurotoxic proteins. Using live imaging of the zebrafish model of DFP poisoning that we developed, we observed drastic changes of the phenotype and the dynamics of microglial cells. This change correlates with an extreme increase in pro-inflammatory cytokine expression. Our results suggest that modulating microglial activation and neuro-inflammation may be considered as a potential treatment approach to acute organophosphorus poisoning.

P 185: An innovative inducible gene silencing technology to study schizophrenia risk genes in zebrafish

TOPIC: NEUROBIOLOGY

Alisha Tromp[1], *Kate Robinson*[1], *Haitao Wang*[1], *Sathish Periyasamy*[1,2], *Bryan Mowry*[1,2], *Jean Giacomotto*[1,2]

[1] *University of Queensland, Queensland Brain Institute, Brisbane, Australia*

[2] *University of Queensland, Queensland Centre for Mental Health research, Brisbane, Australia*

ABSTRACT TEXT

Schizophrenia (SCZ) is a highly heritable and complex mental health disorder currently affecting 1 % of the population worldwide with an estimated cost of more than $5 billion AUD annually. The present working hypothesis is that SCZ is a neurodevelopmental disorder with a multifactorial origin and highly polygenic genetic predisposition.

Investigating each gene is a major global challenge given the large number of identified SCZ risk genes predicted to act through a partial loss of function (pLOF) rather than a total loss of function (tLOF). To respond to this challenge, we have developed and optimized a novel innovative genetic approach to create single and multigenic pLOF zebrafish transgenic animals. It is envisaged that this material will be helpful to the scientific community to investigate the pathological role of SCZ-associated genes compared to its wild-type counterpart. Here, I will demonstrate the use of these genetic tools (in synergy to a traditional approach such as CRISPR-Cas9) to decipher the functional role of one of the most recurrently identified SCZ risk gene, neurexin1 *(NRXN1)*.

P 186: Gdnf affects ventral diencephalic dopaminergic neuron development in zebrafish

TOPIC: NEUROBIOLOGY

Chee Ern David Wong[1,2], *Khang Hua*[2], *Vishal Saxena*[2], *Anwar Norazit*[1], *Suzita Mohd Noor*[1], *Marc Ekker*[2]

[1] *University of Malaya, Department of Biomedical Science- Faculty of Medicine, Kuala Lumpur, Malaysia*
[2] *University of Ottawa, Department of Biology- Faculty of Science, Ottawa, Canada*

ABSTRACT TEXT

Glial cell line-derived neurotrophic factor (GDNF) is reported to be protective towards dopaminergic neurons and a potential therapeutic agent for Parkinson's Disease. However, the physiological role of endogenous GDNF is still poorly understood. GDNF is highly conserved in zebrafish and has been shown to play a role in the enteric nervous system function. Little is known about *gdnf* function in the zebrafish brain, particularly its role in the dopaminergic system. Here, we sought to decipher *gdnf* function during dopaminergic neuron development in zebrafish. CRISPR/Cas9 system with multiple gRNAs targeting targeting the *gdnf* coding region was then used to impede *gdnf* function in the zebrafish dopaminergic neuron reporter line, Tg(*dat*:EGFP), producing G0 crispants. Live imaging revealed a ~20 % reduction in ventral diencephalic dopaminergic neuron numbers in *gdnf* crispant larvae. Whole-mount double immunolabeling with tyrosine hydroxylase and EGPF corroborated these results. These deleterious effects could be partly attributed to deregulation of dopaminergic neuron fate specification-related transcription factors (*otp*, *lmx1b*, *shha*, *and ngn1*) in both crispants and established homozygous mutants with whole mount in-situ hybridization (WISH) on *gdnf* mutants showing reduced *otpb* and *lmx1b.1* expression in the ventral diencephalon. As expected, in *gdnf* crispants, dopaminergic neurons were shown to be about 17 % more susceptible to neurotoxic damage. In conclusion, these findings demonstrate that *gdnf* plays a role in dopaminergic neurogenesis, possibly through regulation of differentiation-associated transcription factors in zebrafish.

P 187: Long-term effects of Habenula injury

TOPIC: NEUROBIOLOGY

Chun-Shiu Wu[1,2], *Shih-Hsian Wen*[1,2], *Chun-Long Mui*[1], *Yung-Shu Kuan*[1,2]

[1] *College of Life science- National Taiwan University- Taipei- Taiwan R. O. C., Institute of Biochemical Sciences, Taipei, Taiwan*
[2] *Academia Sinica- Taipei- Taiwan R. O. C., Institute of Biochemical Sciences, Taipei, Taiwan*

ABSTRACT TEXT

The habenula is an evolutionary conserved brain structure that is present in all vertebrates. In response to environmental stimulations, the habenula merges information received from the limbic, sensory, and basal ganglia, then modulates the level of dopamine, serotonin, norepinephrine, and acetylcholine in the brain, and eventually contributing to pain, stress, anxiety, sleep, and/or reward behaviors. Studies in animal models and human mental diseases have indicated that a hyperactive habenula is associated with depressive phenotypes and hypoactive habenula is associated with the negative and cognitive symptoms of schizophrenia. However, there is a lack of comprehensive studies on how vertebrate brain responds to a dysfunctional habenula long after habenular was damaged. To address this, zebrafish habenula were damaged at larval stages (8 or 10 dpf by laser ablation or electroablation) and raised to juvenile (6-week-old) or adult (12-week-old) stages. After examining novel tank behavior or sleep behavior, the zebrafish brains were excised out for subsequent transcriptomic or metabolomic analyses. Increased anxiety behavior in a novel environment and sleep disorder were observed in the habenula-ablated groups. Furthermore, a comparison of microarray data between the ablated and normal brains identified substantial differences in gene expression involved in redox equilibrium, monoaminergic activity and circadian rhythms. LC-MS results also confirmed that levels of downstream products of some of these genes were markedly altered. It demonstrates that the damage during the larval stage can persist to the juvenile and adult stage and continuously affect the gene expression and metabolism of the brain, and this can be an ideal model for long-term brain trauma studying.

P 188: Automatic high-throughput screening of the zebrafish brain using VAST

TOPIC: NEUROBIOLOGY

Hanqing Zhang[1], Laura Waldmann[2], Tiffany Klingström[3], Joao Campos Costa[4], Beata Filipek-Gorniok[3], Johan Ledin[3], Åsa Mackenzie[2], Tatjana Haitina[2], Amin Allalou[1]

[1] *Uppsala University, Division of Visual information and interaction- Department of Information Technology- BioImage Informatics Facility at SciLifeLab, Uppsala, Sweden*
[2] *Uppsala University, Department of Organismal Biology, Uppsala, Sweden*
[3] *Uppsala University, Department of Organismal Biology- Genome Engineering Zebrafish- SciLifeLab, Uppsala, Sweden*
[4] *Uppsala University, Department of Immunology- Genetics and Pathology, Uppsala, Sweden*

ABSTRACT TEXT

The Zebrafish Image Informatics (Zii), a joint collaboration between the Genome Engineering Zebrafish (GEZ) and BioImage Informatics (BIIF) facilities at SciLifeLab Uppsala, Sweden, has establish an automatic high-throughput phenotypic screening pipeline using the VAST system. The system can automatically load fish and acquire images in both bright-field and fluorescence. Using a convolutional neural network we segment the bright-field images to get an estimate of length and shape. For the fluorescence data we rotate the sample and acquire high-resolution images from different angles. For each view, images are aligned using an intensity-based registration algorithm and the results can be used to compare average patterns and get regions of statistically significant differences. In addition, utilizing multiple views for each fish enables us to identify the 3D position of the detected phenotypes based on the optical projection tomography technique.

We have evaluated our pipeline using a CRISPR/Cas9 *dbx1a;dbx1b* double mutant line in the transgenic *Tg(dat:EGFP)* background and detected multiple statistically significant phenotypes in the zebrafish brain including pretectum and ventral diencephalon.

Patterning

P 189: Notochordal signals establish phylogenetic identity of the teleost spine

TOPIC: PATTERNING

Brianna Peskin[1], Katrin Henke[2], Stephen Treaster[2], Nicolás Cumplido[3], Gloria Arratia[4], Matthew Harris[2], Michel Bagnat[1]

[1] *Duke University, Cell Biology, Durham, USA*
[2] *Harvard Medical School- Boston Children's Hospital, Department of Genetics, Boston, USA*
[3] *University of Chile, FONDAP Center of Genoma Regulation- Faculty of Sciences, Santiago, Chile*
[4] *University of Kansas, Department of Ecology and Evolutionary Biology and Biodiversity Institute, Lawrence, USA*

ABSTRACT TEXT

The spine is a defining feature of the vertebrate body plan. However, broad differences in vertebral structures and developmental strategies occur across vertebrate groups, clouding homology. Analysis of a zebrafish mutant, *spondo,* whose spine is dysmorphic, promoted us to reconstruct paleontological evidence, highlighting specific transitions during teleost evolution. Interestingly, the *spondo* mutant recapitulates characteristics present in basal fishes, not found in extant teleosts. The alteration of a notochordal protein, *Calymmin* (*cmn),* in the mutant results in defective notochordal patterning, altering osteoblast migration to developing vertebrae, and increasing sensitivity to pathways associated with scoliosis. Our data demonstrate that signals from the notochord define the evolutionary identity of the teleost spine and demonstrate how simple shifts in development can unmask ancestral traits hidden for at least 250 million years.

Regeneration

P 190: Optic nerve regeneration in the fast-aging killifish is functionally impaired by aging

TOPIC: REGENERATION

Steven Bergmans[1], *Sophie Vanhunsel*[1], *Lieve Moons*[1]

[1] *KU Leuven, Neural Circuit Development and Regeneration Research Group- Department of Biology, Leuven, Belgium*

ABSTRACT TEXT

Today, a growing number of elderly is suffering from age-associated neuropathies. Research efforts therefore focus on stimulating repair in the degenerating central nervous system (CNS). Inducing neuroregeneration, a capacity that is very limited in adult mammals, remains challenging, especially in an aging environment. The fast-aging African turquoise killifish is very well-suited to investigate the underlying mechanisms that support successful restoration in an aging context, as it has a remarkable regenerative potential in its adult CNS and displays aging features similar to humans.

A detailed study of the aging hallmarks in the killifish visual system, our preferred model to study regeneration, has not yet been performed. Therefore, we evaluated age-related changes in the visual system of young, middle-aged, old and very old killifish, and revealed several senescence-associated manifestations in the old fish, such as decreased visual acuity, retinal atrophy, increased β-galactosidase expression, and declined neurogenesis. Additionally, we are investigating other age-associated changes, including oxidative stress, inflammaging and neurodegeneration. To unravel the effect of these aging processes on regenerative ability, we compared optic nerve regeneration in killifish of various ages subjected to optic nerve crush (ONC). Strikingly, both the number of regenerating retinal ganglion cells and the level of tectal reinnervation are reduced in old fish, which results in old fish not recovering their sight. To study why there is no functional recovery and where in the regenerative process old fish are failing, we are currently performing experiments in which neurodegeneration, synaptic repair and neuronal activity upon ONC are being examined.

In summary, our results urge further investigations into the underlying aging processes affecting the regenerative potential, thereby contributing to the search for effective neuroregenerative therapies in the aged mammalian CNS

P 191: ankrd1a in zebrafish development and heart regeneration

TOPIC: REGENERATION

Srdjan Boskovic[1], *Rubén Marín-Juez*[2], *Nemanja Stamenkovic*[1], *Jovana Jasnic*[1], *Emilija Milosevic*[1], *Mirjana Novkovic*[1], *Dragica Radojkovic*[1], *Didier YR Stainier*[2], *Snezana Kojic*[1]

[1] *Institute for Molecular Genetics and Genetic Engineering, Laboratory for Molecular Biology, Belgrade, Serbia*

[2] *Max Planck Institute for Heart and Lung Research, Department of Developmental Genetics, Bad Nauheim, Germany*

ABSTRACT TEXT

ANKRD1 encodes a muscle stress-responsive transcriptional cofactor, member of the muscle ankyrin repeat proteins family (MARP). This protein is mainly expressed in cardiac muscle where it participates in transcriptional regulation, sarcomere assembly and mechanosensing in the heart. *ANKRD1* expression is induced in various cardiomyopathies and heart failure, while mutations in this gene are associated with cardiac developmental defects and cardiomyopathy occurrence in adults. To gain deeper insights into the function of this clinically relevant gene we employed the zebrafish model. Previously we described the developmental expression patterns of the zebrafish orthologue, *ankrd1a*, and showed that, upon increased physical activity, *ankrd1a* is upregulated in the adult zebrafish. To study its expression with greater detail, we have now generated a *TgBAC(ankrd1a:EGFP)* line. Here we show the peculiar *TgBAC(ankrd1a:EGFP)* expression pattern during development, in cranial and trunk muscle fibers which were immunostained with MF20, but not with F310, suggesting that *ankrd1a* expression is restricted to a subpopulation of slow muscle fibers. Following ventricular cryoinjury in adults, increased levels of *ankrd1a* mRNA were detected in the injury border zone at 24 hours after injury, and remained elevated at 7 days post injury. Our results

suggest the involvement of *ankrd1a* during myocardial regeneration and provide a novel tool to further study its function as an early response gene in stress conditions and tissue remodeling.

P 192: Autophagy Activation in Zebrafish Heart Regeneration

TOPIC: REGENERATION

Myra Chavez[1]

[1] *Institute of Anatomy- University of Bern, Institute of Anatomy, Bern, Switzerland*

ABSTRACT TEXT

Autophagy is an evolutionarily conserved process that plays a key role in the maintenance of overall cellular health. While it has been suggested that autophagy may elicit cardio-protective and pro-survival modulating functions, excessive activation of autophagy can also be detrimental. In this regard, the zebrafish is considered a hallmark model for vertebrate regeneration, since contrary to adult mammals, it is able to faithfully regenerate cardiac tissue. Interestingly, the role that autophagy may play in zebrafish heart regeneration has not been studied yet. In the present work we hypothesize that, in the context of a well-established injury model of ventricular apex resection, autophagy plays a critical role during cardiac regeneration and its regulation can directly affect the zebrafish regenerative potential. We studied the autophagy events occurring upon injury using electron microscopy, *in vivo* tracking of autophagy markers, and protein analysis. Additionally, using pharmacological tools, we investigated how rapamycin, an inducer of autophagy, affects regeneration relevant processes. Our results show that a tightly regulated autophagic response is triggered upon injury and during the early stages of the regeneration process. Furthermore, treatment with rapamycin caused an impairment in the cardiac regeneration outcome. These findings are reminiscent of the pathophysiological description of an injured human heart, and hence put forward the zebrafish as a model to study the poorly understood double-sword effect that autophagy has in cardiac homeostasis.

P 193: Transcriptomic profiling of proliferating cardiomyocytes during heart regeneration

TOPIC: REGENERATION

Hessel Honkoop[1], *Phong Nguyen*[1], *Jeroen Bakkers*[1,2]

[1] *Hubrecht Institute, Cardiac Development and Genetics, Utrecht, Netherlands*
[2] *University Medical Center Utrecht, Pediatric Cardiology, Utrecht, Netherlands*

ABSTRACT TEXT

Zebrafish have the remarkable capacity to regenerate the heart. This is achieved by cell cycle re-entry of existing cardiomyocytes in a zone close to the injury site which we call the border zone. The cardiomyocyte intrinsic mechanisms that drive this cell cycle re-entry remain largely unknown. We recently showed that these proliferating cardiomyocytes undergo metabolic reprogramming. Whereas adult cardiomyocytes depend on fatty acid oxidation as a primary energy source, proliferating cardiomyocytes switch their primary energy source to glycolysis. Not only were glycolytic genes upregulated, also glucose uptake was increased in the border zone. Most importantly, blocking glycolysis impairs cardiomyocyte proliferation in the regenerating heart. Altogether, these findings highlight the importance of a metabolic switch in cardiomyocytes during heart regeneration. While metabolic reprogramming is also important for cancer and stem cells, very little is known about how it is regulated and what the consequences are. Here, we have employed more detailed transcriptomic profiling of these proliferating cardiomyocytes at key timepoints to gain new insights on these outstanding questions. These results will be presented here.

P 194: Expression of heparan sulfate proteoglycans (HSPGs) in regenerating zebrafish fins

TOPIC: REGENERATION

Sebastian Keil[1], *Mansi Gupta*[2], *Michael Brand*[1], *Franziska Knopf*[1]

[1] *CRTD - Center for Regenerative Therapies, TU Dresden, Dresden, Germany*
[2] *Merus N.V., Merus N.V., Utrecht, Netherlands*

ABSTRACT TEXT

Zebrafish have the capacity to completely restore fins after amputation. To successfully regenerate fins FGF signaling is crucial. Here, we have examined the expression of HSPGs, known co-factors of FGF signaling facilitating receptor-ligand binding, during the process of fin regeneration.

To do so, we have performed RT-PCR and RNA in situ hybridization (ISH) of selected HSPGs, providing a detailed overview about potential co-localizations with FGF signaling molecules. Whole mount ISH was performed on regenerates at different regenerative stages, and expression has been assessed in known domains of the regenerate after cryosectioning. At 3 days post amputation, *glypican 1a* (*gpc1a*) is specifically expressed in the lateral domain of the basal layer of the wound epidermis (BLWE), overlying areas of new bone formation. *gpc1b* and *gpc6b* are expressed in the distal blastema, which is thought to function as an organizing center for signaling in the regenerate. In contrast, *gpc5c* and *gpc6a* are expressed in the more proliferative proximal blastema. *gpc5a* is expressed in the distal blastema, as well as distal and lateral parts of the BLWE. Furthermore, we find expression of *syndecan 2* (*sdc2*) in the regenerate. To conclude, several HSPGs are expressed during fin regeneration, which may be necessary for FGF signaling to drive successful fin regeneration.

P 195: Communication between microglia and adult neural stem cells for neural tissue regeneration in zebrafish model of traumatic brain injury Manana Kutsia, Ichiro Masai

TOPIC: REGENERATION

Manana Kutsia[1]

[1] *Okinawa Institute Of Science and Technology Graduate University, Developmental Neurobiology, Tancha- Onna Son Kunigami District, Japan*

ABSTRACT TEXT

Neural regeneration in response to brain damage is an important topic in the field of medical science. In general, humans have low regenerative capability. On the other hand, zebrafish show remarkable ability to regenerate neural tissue in response to various types of brain injury. One of important factors that inhibit neural regeneration in human injured brain is immune response. In human brain injury, microglia keep an activated state for a prolonged period, which leads to secondary degeneration. On the other hand, Michael Brand group reported that acute inflammation causes reactive proliferation of neural stem cells and production of new born neurons in zebrafish. These data raise an important question on whether microglia/macrophage activity can solely promote neural regeneration in zebrafish.

To address this question, we examined behavior of microglia in traumatic brain injury. Applying vertical needle insertion, we introduced traumatic injury into telencephalon of 3 months old zebrafish. This method resembles the traumatic brain injury in human patients. Morphological features of microglia/macrophages are once activated at 1 dpi; however, they returned to control conditions by 3dpi, suggesting the possibility that short activation of microglia facilitates neural regeneration in zebrafish. Next, we used 3–6 month old zebrafish transgenic line Tg[mpeg1:Nitroreductase (NTR)] combined with metronidazole (MTZ) treatment to eliminate microglia/macrophage population. The number of proliferating adult neural stem cells significantly increased in wild-type damage-induced hemisphere at 3 dpi, compared with undamaged control hemisphere, whereas their number in NTR/Mtz-treated damaged hemisphere is not significantly different from that of undamaged control hemisphere. Thus, communication between microglia/macrophages and neural stem cells is important to initiate proliferation of neural stem cell response and neural neuronal regeneration program.

P 196: The role of SPARC in the regenerating zebrafish spinal cord

TOPIC: REGENERATION

Elizabeth Lake[1], *Marcus Keatinge*[1], *Themistoklis Tsarouchas*[2], *Thomas Becker*[1], *Catherina Becker*[1]

[1] *University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, United Kingdom*
[2] *University of Edinburgh, Scottish Centre for Regenerative Medicine, Edinburgh, United Kingdom*

ABSTRACT TEXT

Regeneration of the spinal cord following injury requires the formation of an "axonal bridge" to re-establish neuronal continuity and function. We have demonstrated the pivotal role of the collagenous extracellular matrix (ECM) in the axonal bridging that enables the remarkable regenerative capacity of the zebrafish spinal cord (Wehner, 2017). Here, we aim to analyse the function of Secreted Protein, Acidic, and Rich in Cysteine (SPARC) for spinal cord regeneration. Sparc is a known protein ECM organiser and signal modulator. Our unpublished single-cell RNA sequencing of macrophages recruited to spinal lesion sites in the larval zebrafish model has indicated an upregulation in *sparc* mRNA expression by these cells. We have generated a *sparc* mutant zebrafish which is adult-viable and shows a reduced capacity for spinal regeneration after lesion, indicating pivotal functions of Sparc for successful spinal cord regeneration. We will now analyse the immune response in *sparc* mutants and changes to the configuration of the lesion site ECM. This study will expand our knowledge of the pro-regenerative ECM environment that allows functional recovery after spinal injury in zebrafish.

Wehner, D., Tsarouchas, T., Michael, A., Haase, C., Weidinger, G., Reimer, M., Becker, T.., Becker, C. (2017). Wnt signalling controls pro-regenerative Collagen XII in functional spinal cord regeneration, Nature Communications, 8, article 126.

P 197: Cellular Drivers of Heart Repair

TOPIC: REGENERATION

Sara Lelek[1], *Bo Hu*[2], *Jan Philipp Junker*[2], *Daniela Panakova*[1]

[1] *Max Delbrück Center for Molecular Medicine, Cardiovascular & Metabolic Diseases, Berlin, Germany*
[2] *Max Delbrück Center for Molecular Medicine, Medical Systems Biology, Berlin, Germany*

ABSTRACT TEXT

Heart failure is one of the most common causes of death worldwide. While humans are not able to regenerate their heart after myocardial infarction, zebrafish heart can fully recover after an injury, making it an excellent model organism to study how to overcome limited regenerative response in humans.

Using single-cell RNA sequencing we investigated the single-cell responses in gene expression of adult zebrafish heart after cryoinjury at the crucial steps of regeneration

(3-, 7-, 15- and 30- days post-injury). We identified specific responses and dynamics in the various cell types comprising adult heart including fibroblasts, macrophages, and cardiomyocytes.

We were also able to observe the distinct spatial distribution of activated fibroblasts and macrophages using RNAscope assay at different stages post-injury. We propose these activated fibroblasts and macrophages acquire specific functions during heart regeneration and contribute to heart repair.

Our strategies will allow us in the future to identify new targets to limit adverse cardiac remodeling after myocardial infarction.

P 198: An intraneuronal energy restriction in neurite regeneration: which way to grow?

TOPIC: REGENERATION

Lieve Moons[1], *An Beckers*[1], *Annelies Van Dyck*[1], *Luca Masin*[1], *Lien Andries*[1], *Lies De Groef*[1]

[1] *KU Leuven, Biology Department, Leuven, Belgium*

ABSTRACT TEXT

Up to now neuroregenerative research mainly focused on improving axonal regrowth, leaving the dendrites, which form an essential component of the neuronal circuit, largely unstudied. Nevertheless, both axonal as well as dendritic regeneration are needed to re-establish a functional neuronal circuit. Using an optic nerve injury model, we demonstrated, in the spontaneously regenerating retina of adult zebrafish, an antagonistic axon-dendrite interplay, wherein an early dendritic deterioration phase boosts axonal regrowth and retinal ganglion cell (RGC) dendrites only regenerate after proper axonal repair and innervation of the target cells in the brain. This orderly sequence of neurite outgrowth is a recapitulation of development, where axogenesis also precedes dendritogenesis.

One of the mechanisms underlying this segregation of dendritic and axonal growth during regeneration might be a neuronal energy trade-off that prevents simultaneous (re)growth of axons and maintenance/regrowth of dendrites. To test this hypothesis, a combination of state-of-the-art in vitro and in vivo research approaches are being pursued to investigate RGC mitochondrial dynamics upon axonal injury. Our data reveal a timed intraneuronal dendrite-axon-dendrite mitochondrial translocation after injury. Moreover, we found increased fission in the dendrites and soma after injury and a biphasic upregulation of mitochondrial biogenesis, respectively matching the start of axon and dendrite regrowth.

We are now further characterizing, in the axonal *versus* dendritic cell compartment, the subcellular and molecular players modulating energy production and enabling spontaneous regeneration and functional recovery in adult zebrafish neurons. Overall, our findings could generate pivotal insights into how re-directing intraneuronal energy channelling may promote neuronal repair in the mammalian CNS.

P 199: Protein networks of heart regeneration

TOPIC: REGENERATION

Mira Pronobis[1], *Susan Zheng*[1], *Sumeet Pal Singh*[2], *Ken Poss*[1]

[1] *Duke University, Cell Biology, Durham, USA*
[2] *IRIBHM, Developmental Biology, Brussels, Belgium*

ABSTRACT TEXT

Heart disease continues to be the leading cause of death worldwide. Rather than producing new functional muscle tissue, the injured human heart forms scar tissue which often leads to heart arrhythmias and heart failures. Zebrafish, on the other hand, can regenerate the heart with minimal scarring.

Transcriptional profiling in adult zebrafish revealed profound changes in gene expression during heart regeneration. How these changes impact protein networks in cardiomyocytes (CMs) is unknown. Monitoring the proteome of CMs with standard methods is challenging due to an abundance of structural proteins. Here we used BioID2 to capture cell type-specific protein networks during heart regeneration. This recently established technique probes interacting networks by using a promiscuous Biotin ligase, BirA2 which covalently attaches biotin to adjacent proteins. This allows capture of transient protein interactions, while at the same time avoiding isolation of structural proteins from CMs.

In adult zebrafish, CM proliferation is activated by injury or by experimentally increased presence of cardiac mitogens like Nrg1, Vegfaa, or Vitamin D. Proteome comparisons of CMs after injury or overexpression of any of the 3 cardiac mitogens identified elevated levels of several proteins that are likely to represent proteome networks of heart regeneration.

Nrg1 signaling is sufficient to induce growth of heart muscle, and inhibition of its coreceptor ErbB2 reduces CM division. While the signaling network of ErbB2 has therapeutic potential, its downstream network has not been defined. We used BioID2 targeting of ErbB2 to identify Rho A as a direct downstream target of Nrg1-activated Erbb2 signaling in CMs. Rho A association with Erbb2 is increased during heart regeneration, and Rho A inhibition impairs proliferation of Nrg1-overexpressing CMs and blocks heart regeneration after injury. Our findings implicate an ErbB2-Rho A axis in CMs during innate heart regeneration.

P 200: The ageing zebrafish heart

TOPIC: REGENERATION

Hanna Reuter[1], *Christoph Englert*[1]

[1] *Leibniz Institute on Aging – Fritz Lipmann Institute FLI, Molecular Genetics, Jena, Germany*

ABSTRACT TEXT

Ageing is accompanied by organ failure and degenerative diseases. It is therefore important to understand molecular mechanisms underlying the functional decline of organs such as the heart. The aim of this project is to address whether the highly regenerative zebrafish heart is a good model to study organ homeostasis in the elderly.

We performed RNA-Sequencing of ventricles of young (7 months) and old (4 years) zebrafish and identified 1233 genes as differentially regulated in old vs. young. Actin-based processes were enriched among terms associated to down-regulated genes and terms linked to the immune system where enriched among up-regulated genes. Comparing markers for different immune cell lineages, many marker genes were associated with the myeloid lineage. Immunofluorescence analysis confirmed the increase of immune cells in old hearts. These data suggest the presence of 'inflammaging' in the zebrafish heart.

Since macrophages are known to be important for regenerative processes and are linked to the cardiac scaring process, it is of interest to understand to which extent the increased presence of immune cells might modulate regenerative responses to cardiac injury in old fish.

P 201: Vascular repair after spinal cord injury in zebrafish

TOPIC: REGENERATION

Ana Ribeiro[1], *Carmen de Sena*[1], *Mariana Costa*[1], *Leonor Saúde*[1]

[1] *Instituto de Medicina Molecular- Faculdade de Medicina da Universidade de Lisboa, Leonor Saúde Lab, Lisboa, Portugal*

ABSTRACT TEXT

Spinal cord injuries have dramatic and irreversible effects on motor and sensory functions in mammals. By contrast, zebrafish are able to repair the spinal cord and restore motility and are increasingly used to study successful strategies of regeneration. In this study we investigate if the vascular system is reestablished after spinal cord injury in zebrafish and whether the vasculature is important for the efficient recovery of spinal cord function.

We show that the zebrafish spinal cord has a similar organization and specialised blood-spinal cord barrier modifications as observed in mammals. We followed the vascular response over the course of spinal cord regeneration and confirmed that zebrafish, unlike mammals, are able to restore the vascular network. The repair of the damaged blood vessels occurs through the activation of angiogenesis, in response to the expression of pro-angiogenic factors detected in the injury site. The new blood vessels are also able to rapidly recruit pericytes, thus contributing to the reestablishment of the blood-spinal cord barrier.

To address the role of the vasculature during spinal cord regeneration we are inhibiting the formation of new blood vessels. Our preliminary results show that interfering with the spinal cord re-vascularisation results in impaired functional recovery.

This work reveals the enhanced capacity of zebrafish to repair the spinal cord vasculature when compared to mammals and highlights the importance of tissue re-vascularisation during regeneration.

P 202: Role of menaquinone producing probiotic bacteria on the development and regeneration of zebrafish bone

TOPIC: REGENERATION

Jerry Maria Sojan[1], *Francesca Maradonna*[1], *Giorgia Gioacchini*[1], *Giorgini Elisabetta*[1], *Oliana Carnevali*[1]

[1] *Università Politecnica delle Marche, Department of Life and Environmental Sciences, Ancona, Italy*

ABSTRACT TEXT

Probiotics are microorganisms (usually live) known to exert many beneficial effects on the host when administered in required quantities. There are many probiotic bacterial species like *Bacillus subtilis* and *Lactococcus lactis* which are known producers of many bio-active forms of menaquinone (vitamin K2), a fat-soluble vitamin considered to have a role in bone health. As vitamin K is the enzyme co-factor for catalysing the carboxylation of glutamate (Gla) residues in many Gla proteins, our hypothesis is that the menaquinone form of vitamin K produced by the probiotic bacteria could also aid in the maturation of Gla proteins like bone Gla protein (BGP), matrix Gla protein (MGP) etc, which are the main proteins involved in skeletal development. Zebrafish is an ideal model for these studies due to its fast development and short fin regeneration period.

In the current study, the extent of skeletal development and fin regeneration were analysed in probiotics treated fishes using double staining, Fourier-transform infrared spectroscopy (FTIR) and gene expression studies. Double staining was done for the visualisation of calcification while FTIR provided the quantification of phosphate, which represents the major component of the bone. Key marker genes involved in the skeletal development pathways such as *runx2b, sp7*, *col10a1a, spp1*, *bglap* and several other genes were analysed for their expression difference among control and probiotics groups.

The multi-disciplinary approach used in the present study to test the efficiency of specific probiotics on early stage skeletal development in larvae and regeneration of caudal fin in juvenile zebrafish, provided clear evidences of the positive role of menaquinone producing bacteria in the ossification process. Zebrafish is considered an excellent model to translate the current findings into industrial and scientific significance both in aquaculture and bio-medicinal perspectives.

P 203: A switch in myoseptal and perivascular cell-derived extracellular matrix composition is required for axon regeneration in the zebrafish spinal cord

TOPIC: REGENERATION

Vasiliki Tsata[1], *Stephanie Möllmert*[2], *Christine Schweitzer*[2], *Benjamin Böhm*[2], *Gonzalo Rosso*[2], *Julia Kolb*[2], *Christian Lange*[3], *Matthias Lesche*[4], *Juliane Hammer*[3], *Gokul Kesavan*[3], *Dimitris Beis*[1], *Jochen Guck*[2], *Michael Brand*[3], *Daniel Wehner*[2]

[1] *Biomedical Research Foundation of the Academy of Athens, Experimental Surgery- Clinical and Translational Research Center, Athens, Greece*

[2] *Max Planck Institute for the Science of Light, Division Biological Optomechanics, Erlangen, Germany*

[3] *Technische Universität Dresden, Center for Regenerative Therapies TU Dresden, Dresden, Germany*

[4] *Technische Universität Dresden, DRESDEN-concept Genome Center c/o Center for Molecular and Cellular Bioengineering, Dresden, Germany*

ABSTRACT TEXT

In mammals, perivascular cell-derived scarring after spinal cord injury impedes axonal regrowth. In contrast, the extracellular matrix (ECM) in the spinal lesion site of zebrafish is permissive and required for axon regeneration. However, to date, the underlying cellular mechanisms of this interspecies difference have not been investigated. Here, we show that an injury to the zebrafish spinal cord triggers recruitment of *pdgfrb*$^{+}$ myoseptal and perivascular cells in a PDGFR signaling-dependent manner. Interference with *pdgfrb*$^{+}$ cell recruitment or depletion of *pdgfrb*$^{+}$ cells inhibits axonal regrowth and recovery of swimming function. Gene expression profiling and functional experiments reveal that *pdgfrb*+ cells upregulate expression of axon growth-promoting ECM genes and concomitantly reduce synthesis of matrix molecules that are detrimental to regeneration. Our data demonstrates that a switch in ECM composition is critical for axonal regeneration after spinal cord injury in zebrafish and identifies the cellular source and components of the growth-promoting lesion ECM.

P 204: PI3Kγ/Akt signal at wounded site supports the survival of regenerative cells by recruiting the macrophage during zebrafish fin fold regeneration

TOPIC: REGENERATION

Siyu Zhou[1], *Atsushi Kawakami*[1]

[1] *Tokyo Institute of Technology, Life Science and Technology, Yokohama, Japan*

ABSTRACT TEXT

Some vertebrate species retain a high regeneration potential that remold amputated fins or limbs. By using the zebrafish fin fold regeneration as a model, we have previously revealed that the interaction between immune cells and the regenerative cells is crucial for regulating regeneration. We have demonstrated that macrophage recruitment and quick attenuation of inflammation is necessary for the survival of regenerative cells. However, the underlying mechanisms remained unknown.

To obtain the clues, we performed a screening for chemicals that induce apoptosis and abnormal regenerative response during fin fold regeneration. We identified a PI3Kγ inhibitor, AS605240, as one of compounds that induces aberrant apoptosis in wounded site. The AS induced apoptosis mainly in mesenchymal cells and resulted in regeneration defects. The MO-mediated gene knockdowns showed comparative TUNEL phenotypes, confirming that PI3Kγ is responsible for the survival of regenerative cell. Furthermore, analysis of regeneration-response genes and cell proliferation suggested that PI3Kγ is also necessary for a proper initiation of regenerative response.

To analyse the target process of the AS, we next examined the macrophage behavior. Intriguingly, live-imaging of macrophage indicated that their migration to wounded site was impaired by the AS, suggesting that PI3Kγ is required for macrophage recruitment. More significantly, phosphorylation of Akt, a possible downstream effector, was detected in the wound epidermis with a peak at 6 hpa, and downregulated by the AS. The PI3Kγ/Akt signaling in the wound epidermis, but not in the macrophage, is likely to play a critical role for facilitating macrophage recruitment.

Thus, our study revealed the role of PI3Kγ/Akt signal during regeneration. The signal at the injury site is not only necessary for regulating the proper regenerative response, but also crucial for recruiting the macrophage to support the survival of regenerative cells.

Stem Cells

P 205: The cooperation between myeloid and vascular cells favors HSC expansion in the embryonic hematopoietic niche

TOPIC: STEM CELLS

Pietro Cacialli[1], *Julien Y. Bertrand*[1]

[1] *University of Geneva, Department of Pathology and Immunology, Geneva, Switzerland*

ABSTRACT TEXT

During embryonic development, very few hematopoietic stem cells (HSCs) are produced from the hemogenic endothelium, and therefore are expanded in a very specific niche. This fetal HSC niche comprises a complex and dynamic molecular network of interactions across multiple cell types, including endothelial cells and mesenchymal stromal cells. It is known that functional changes in the hematopoietic niche, such as aging, vascular cell remodelling, inflammation can directly affect the fate of HSCs. Among all these inflammatory regulators, the eicosanoid prostaglandin E (PGE2) has been shown to be very important during embryonic life. Prostaglandins are synthetized from arachidonic acid, released from the plasma membrane by phospholipases (PLAs) and metabolized by the sequential actions of cyclooxygenase (COX) and prostaglandin synthases (PTGS). However, the precise source of PGE_2 in the embryo is still elusive. Here we show

that all the genes involved in PGE_2 synthesis are expressed by cells of the caudal hematopoietic tissue (CHT) in the embryonic zebrafish. While neutrophils express high levels of PLAs, macrophages express more COX enzymes and endothelial cells, more PTGS enzymes. This suggests that each cell type is sequentially necessary in PGE_2 synthesis. Indeed, ablation of myeloid cells at the CHT stage induced a loss of HSCs. Moreover, in endothelial cells, we identified the role of an important transporter, *slco2b1*, that mediates the transport of prostaglandins across the cell membrane. We found a defect of HSC proliferation in the CHT of *slco2b1*-deficient embryos, which could be rescued by prostaglandin E_2 (PGE_2) treatment. Taken altogether, our data show that the myeloid cells and the vascular niche cooperate to enhance HSC expansion in the CHT.

P 206: Zebrafish Kit ligands cooperate with erythropoietin to promote erythroid cell expansion

TOPIC: STEM CELLS

Jana Oltová[1], Ondrej Svoboda[1], Olga Machonova[1], Petra Svatonova[1], David Traver[2], Michal Kolar[1], Petr Bartunek[1]

[1] *Institute of Molecular Genetics, Laboratory of Cell Differentiation, Prague, Czech Republic*

[2] *University of California at San Diego, Department of Cellular and Molecular Medicine, San Diego, USA*

ABSTRACT TEXT

Kit ligand (Kitlg) is pleiotropic cytokine with a prominent role in vertebrate erythropoiesis. Although the role of Kitlg in this process has not yet been reported in *Danio rerio* (zebrafish), in this study, we show that its function is evolutionary conserved. Zebrafish possess two copies of Kitlg genes (Kitlga and Kitlgb) due to whole genome duplication. To determine the role of each ligand in zebrafish, we performed a series of *ex vivo* and *in vivo* gain- and loss-of-function experiments. First, we tested the biological activity of recombinant Kitlg proteins in suspension culture from zebrafish whole kidney marrow and we demonstrate that Kitlga is necessary for expansion of erythroid progenitors *ex vivo*. To further address the role of *kitlga* and *kitlgb* in hematopoietic development *in vivo*, we performed gain-of-function experiments in zebrafish embryos, showing that both ligands cooperate with erythropoietin (Epo) to promote erythroid cell expansion. Finally, using the *kita* mutant (*kita*$^{b5/b5}$ or *sparse*), we show that Kita receptor is crucial for Kitlga/b cooperation with Epo in erythroid cells. In summary, using optimized suspension culture conditions with recombinant cytokines (Epo, Kitlga), we are reporting for the first time *ex vivo* suspension cultures of zebrafish hematopoietic progenitor cells, which can serve as an indispensable tool to study normal and aberrant hematopoiesis in zebrafish. Furthermore, we conclude that although partial functional diversification of Kit ligands has been described in other processes, in erythroid development, both paralogs play a similar role and their function is evolutionary conserved.

P 207: Dynamic properties of noise and her6 levels are optimized by miR-9, allowing the decoding of the her6 oscillator

TOPIC: STEM CELLS

Ximena Soto[1], Veronica Biga[1], Nancy Papalopulu[1], Robert Lea[1]

[1] *University of Manchester, Faculty of Biology Medicine and Health, Manchester, United Kingdom*

ABSTRACT TEXT

Noise is prevalent in biology and has been widely quantified using snapshot measurements. This static view obscures our understanding of dynamic noise properties and how these affect gene expression and cell state transitions. Using a CRISPR/Cas9 Zebrafish Her6::Venus reporter combined with mathematical and *in vivo* experimentation, we explore how noise affects the protein dynamic of Her6, a basic helix-loop-helix transcriptional repressor. During neurogenesis, Her6 expression transitions from fluctuating to oscillatory at single cell level. We identify that absence of miR-9 input generates high frequency noise in Her6 traces, inhibits the transition to oscillatory protein expression and prevents the downregulation of Her6. Together, these impair the upregulation of downstream targets and lock cells into a normally transitory state where progenitor and early differentiation markers are co-expressed. Computational modelling and double smFISH of *her6* and its downstream target, *elavl3*, suggest that the change in Her6 dynamics precedes the downregulation in Her6 levels. This sheds light onto the order of events at the moment of cell state transition and how this is influenced by the dynamic properties

of noise. Our results suggest that Her/Hes oscillations, facilitated by dynamic noise optimization by miR-9, endow progenitor cells with the ability to make a cell state transition.

P 208: As far as the eye can't see: Stem cells in the non-visual retina are continuous with the ciliary marginal zone

TOPIC: STEM CELLS

Erika Tsingos[1], *Mai Thu Nguyen*[1], *Joergen Benjaminsen*[1], *Joachim Wittbrodt*[1]

[1] *Ruprecht-Karls-Universität Heidelberg, Centre for Organismal Studies, Heidelberg, Germany*

ABSTRACT TEXT

The mammalian ciliary and iris epithelia contain adult retinal stem cells, and are considered homologous to the ciliary marginal zone (CMZ), the retinal stem cell niche active throughout life in amphibians and fish. However, homology of these structures has not been established. We show that the non-visual retina (NVR) of the model fish medaka (*Oryzias latipes*) grows peripherally to the CMZ during post-embryonic development and is structurally homologous to the mammalian ciliary and iris epithelia, while the CMZ is homologous to the *ora serrata*. The fish NVR maintains proliferative potential throughout the animal's life and molecular and clonal continuity with the CMZ stem cells that contribute to the neural retina and the retinal pigmented epithelium. The molecular and clonal continuum of CMZ and NVR suggests that the peripheral retina in fish comprises a singular niche whose evolutionary relict is seen in adult retinal stem cells in the peripheral retinal epithelia of mammals.

P 209: Stem cell quiescence allows rapid adaptation to growth demands in the retinal pigmented epithelium

TOPIC: STEM CELLS

Erika Tsingos[1], *Mai Thu Nguyen*[1], *Joachim Wittbrodt*[1]

[1] *Ruprecht-Karls-Universität Heidelberg, Centre for Organismal Studies, Heidelberg, Germany*

ABSTRACT TEXT

Quiescence is believed to maintain a reserve population of adult stem cells in a non-cycling state that can be rapidly reactivated in response to injury, and has also been implicated in the long-term survival of therapy-resistant cancer cells that go on to generate metastases. In this work, we uncover dynamic entry and exit into quiescence during homeostasis of the stem cell niche of the retinal pigment epithelium (RPE) of the teleost fish medaka (*Oryzias latipes*).

To meet the optical requirements for vision during post-embryonic growth, the RPE must grow proportionally to all other tissues in the eye. Previous computational work predicted that growth cues from the surroundings instructed RPE stem cells to modulate their cell division frequency. We hypothesized that this modulation occurred at the level of individual stem cells temporarily leaving the cell cycle via quiescence. In this work, we combined simulations with experimental DNA label retention to test the impact of quiescence and intrinsic population variability on the proliferation dynamics of RPE stem cells.

Surpassing our expectations, we identified frequent label retention indicating that a large proportion of RPE stem cells was quiescent for up to several weeks. At the same time, another fraction of RPE stem cells maintained the actively cycling state. These two populations coexisted in adjacent territories and dynamically switched roles throughout the life of the organism. Surprisingly, sister cells displayed similar cell cycle dynamics, suggesting low intrisic variability in the population's cell cycle timing. Thus, quiescence played the major role in modulating the proliferative output of the tissue. The dynamic state of the RPE niche allows the tissue to rapidly modulate its growth rate in response to fluctuating environmental demands.

Toxicology

P 210: The role of propylparaben in zebrafish brain development

TOPIC: TOXICOLOGY

Giulia Caioni[1], Carmine Merola[2], Michele Amorena[2], Gloria Panella[1], Annamaria Cimini[1,3], Elisabetta Benedetti[1], Monia Perugini[2]

[1] *University of L'Aquila, Department of Life- Health and Environmental Sciences, L'Aquila, Italy*
[2] *University of Teramo, Faculty of Bioscience and Agro-Food and Environmental Technology, Teramo, Italy*
[3] *Temple University, Sbarro Institute for Cancer Research and Molecular Medicine and Center for Biotechnology, Philadelphia, USA*

ABSTRACT TEXT

Humans are exposed to increasing amount of chemicals, and the concern about these substances is related to their unknown effects. In particular, recent studies focused on the controversial role of parabens, used as preservatives in food and cosmetics. They have estrogenic properties [1], which could influence the brain development in Vertebrates. In this work we examine the effect of (PrP) on brain development during early-life stages of zebrafish. PrP treatment induces an alteration in brain morphology evaluated by NeuN staining; particularly we detect an increase in nuclei positive for NeuN in PrP treated embryo respect to control. This morphological anomaly may be related to the altered lipid metabolism and development of head cartilage observed by us in embryos exposed to PrP [2]. In the present study we also investigate the expression in genes involved in neurodevelopment and synapse formation, such as Shank3a, Nrxn1 and Ngln3. Our preliminary data open to further studies to explore the toxicity associated with parabens. Since PrP acts as agonist for peroxisome proliferator–activated receptor g (PPARg) [3] that it is strongly involved in neurogenesis we hypothesize its connection in PrP action.

1 Darbre P.D., & Harvey P.W. (2008). Paraben esters: review of recent studies of endocrine toxicity, absorption, esterase and human exposure, and discussion of potential human health risks. J Appl Toxicol, *28*(5), 561–578.
2 Perugini M,, Merola C., Amorena M., D'Angelo M., Cimini A., Benedetti E. (2019). Sublethal exposure to propylparaben leads to lipid metabolism impairment in zebrafish early-life stages. J Appl Toxicol, 1–11. https://doi.org/10.1002/jat.3921.
3 Hu P., Chen X., Whitener R.J., Boder E.T., Jones J.O., Porollo A., Chen J., Zhao L. (2013). Effects of parabens on adipocyte differentiation. Toxicol Sci, 131(1):56-70. doi:10.1093/toxsci/kfs262.

P 211: Usefulness of the transgenic cyp19a1b-GFP zebrafish model to refine the zebrafish embryo acute toxicity test (ZFET)

TOPIC: TOXICOLOGY

Armelle Christophe[1], Marc Léonard[2], Pascal Pandard[1], Noémie De Corzé[2], Francois Brion[1]

[1] *INERIS, Direction of Chronic Risk Assessment, Verneuil en Halatte, France*
[2] *L'Oréal Research & Innovation, Environmental Research Department, Aulnay-sous-Bois, France*

ABSTRACT TEXT

During the past few years, specific zebrafish embryo-based assays were validated at the OECD level for assessing the hazard of test chemicals. Among them, the zebrafish embryo acute toxicity test (ZFET) is used for aquatic toxicity testing and the EASZY assay allows to quantify the estrogenic activity of chemicals by measuring the induction of the GFP driven by the ER-regulated zebrafish brain aromatase *cyp19a1b* promoter. Herein, our objective was to evaluate the usefulness of using cyp19a1b-GFP model for combining the assessment of toxicity and endocrine potency of test chemicals in the FET (OCDE TG236). We first evaluated the acute and developmental toxicity of eleven chemicals on wildtype (AB strain) and cyp19a1b-GFP embryos. The selected chemicals were chosen among those used for the OECD validation of the FET test. Comparison of the acute and developmental toxicity data revealed that both wild type and transgenic embryos had similar sensitivity to chemicals and showed similar lethal and developmental effects. The usefulness of the cyp19a1b-GFP model was further evaluated by performing *in vivo* 2D fluorescence imaging at the end of the FET at nontoxic concentrations of tested chemicals. For most of them, no GFP induction was measured which agrees with their known lack of estrogenic activity. In contrast, strong up-regulations of *cyp19a1b* expression were measured for 4 tert-octylphenol, indicating an estrogenic activity which was further quantify using

EASZY. Altogether, our data indicates that the cyp19a1b-GFP zebrafish embryo model can be used in the FET to assess the toxicity of test chemicals and provide preliminary information on their endocrine potency. In that respect, our data support the use of the cyp19a1b-GFP model to develop an efficient testing strategy combining the FET and the EASZY assays in a hazard assessment perspective.

Acknowledgements: funded by L'Oréal (C060509_A110135), the ANR FEATS (CES34-19 to FB) and the DRC-58 (to PP, FB).

P 212: Investigation of hormone-disrupting substances in slurry-based irrigation waters: the combined application of yeast and fish test

TOPIC: TOXICOLOGY

Zsolt Csenki[1], *Edina Garai*[1], *Eduárd Gubó*[2], *Katalin Bakos*[1], *Erna Vásárhelyi*[1], *Dóra Kánainé S.*[1], *Béla Urbányi*[1], *Judit Plutzer*[3]

[1] *Szent István University, Department of Aquaculture, Gödöllő, Hungary*
[2] *Széchenyi István University, Faculty of Agricultural and Food Sciences, Mosonmagyaróvár, Hungary*
[3] *National Public Health Institute, Vízhigiénés Osztály, Budapest, Hungary*

ABSTRACT TEXT

Among the compounds released into our environment, there are several EDC chemicals primarily estrogenic substances. The chemical diversity of the substances makes it difficult to test as they require different analytical methods to detect them. This problem is solved by the use of effect directing methods, such as biomonitor/bioindicator organisms.

In our work, we analyzed 30 slurry-based irrigation water using two models suitable for estrogen detection. Our aim was to test the potential of combining estrogen detection methods based on yeast (YES-Yeast Estrogen Screen) and zebrafish model (Tg (vtg1: mCherry)).

The liquid fraction of slurry-based irrigation water samples was first passed through filtration columns (SPE), and the hormone-active substances were extracted from the slurry with methanol in an ultrasonic bath. For yeast tests, genetically modified *Saccharomyces cerevisiae* cells were used. Fish tests were performed on transgenic zebrafish embryos, which were microinjected. 96 hours after the treatment, the fluorescence signal in the livers of juveniles was examined under a fluorescence microscope.

During the yeast test, samples were tested in 7 different dilutions. By increasing dilutions, a stronger estrogen effect was measured. In the cases of transgenic fish line tests, similarly to yeast, estrogen activity was demonstrated in a significant proportion of the samples, which was manifested in the induction of the fluorescent signal.

Based on our results, it can be concluded that slurry-based irrigation water samples may contain significant amounts of estrogenic compounds. Both yeast and fish tests have been proved to be suitable for the detection of these compounds, the simultaneous use of the two models complemented each other well. It can also be concluded that the application of slurry to soil, may entail serious risks due to its possible EDC content.

The work was supported by: EFOP 3.6.3.-VEKOP 16.-2017-00008, NVKP_16-1-2016-0003, NKFIH-831-10/2019.

P 213: Zebrafish as a Model for Chemical Induced Adipogenesis and Related Metabolic Diseases

TOPIC: TOXICOLOGY

Marjo Den Broeder[1], *Nikki Habraeken*[1], *Leonie Kamminga*[2], *Jorke Kamstra*[1], *Juliette Legler*[1]

[1] *Institute for Risk Assessment Sciences- Department of Population Health Sciences, Utrecht University, Utrecht, Netherlands*
[2] *Wageningen University, Research Library, Wageningen, Netherlands*

ABSTRACT TEXT

Humans are widely exposed to endocrine disrupting chemicals (EDCs) which are defined as any an exogenous chemical, or mixture of chemicals, that can interfere with any aspect of hormone action. Increasing research in recent years has indicated that developmental exposure to specific EDCs, the so-called metabolism disrupting chemicals (MDCs), may play a role in the latent onset of obesity and other metabolic disorders like type 2 diabetes mellitus (T2DM) and non-alcoholic fatty liver disease (NAFLD). Within the European H2020 project

GOLIATH (Generation Of NoveL, Integrated and Internationally Harmonised Approaches for Testing Metabolism Disrupting Compounds) we aim to get a better understanding of molecular mechanisms of MDCs, and work towards integrated testing and assessment of this class of chemicals.

Zebrafish is emerging as a valuable model organism to study human metabolic diseases. The goal of this study is to analyze the effects of early developmental exposure to chemicals on adipogenesis, and pancreas and liver development in the zebrafish (*Danio rerio*). We have developed a zebrafish model of adipogenesis and obesity, and use advanced imaging technology and fluorescent lipid stains to visualize the development of adipocytes in living larvae at 15 dpf. With this approach we have shown that developmental exposure to the pesticide tributyltin stimulates adipogenesis, similar to previous reports in rodent models. Here, we will take this approach further and use transgenic fish lines to examine effects of MDCs on the endocrine and exocrine pancreas, as well as lipid metabolism in the liver,in combination with a lipid staining to assess alterations in adipogenesis. In addition, we will analyze molecular mechanisms by which MDCs can disrupt metabolism using a multi-omics approach.

This project (GOLIATH) has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 825489.

P 214: Zebrafish (Danio rerio) Oat1 and Oat3 transporters and their interaction with endo- and xenobiotics

TOPIC: TOXICOLOGY

Jelena Dragojević[1], *Ivan Mihaljević*[1], *Petra Marić*[1], *Jovica Lončar*[1], *Marta Popović*[1], *Tvrtko Smital*[1]

[1] *Ruđer Bošković Institute, Division for Marine and Environmental Research, Zagreb, Croatia*

ABSTRACT TEXT

Organic anion transporters (OATs) are membrane proteins within the Solute carrier family 22 (SLC22). They play important roles in cellular uptake of various organic compounds, and due to their expression in barrier tissues of major excretory and non-excretory organs are considered as crucial elements in absorption and distribution of a wide range of endobiotic and xenobiotic compounds. Based on our previous work and initial insights on SLC22 members in zebrafish (*Danio rerio*), in this study we aimed at *in vitro* characterization of Oat1 and Oat3 transporters and understanding of their interaction with potential physiological and xenobiotic substrates. We first performed phylogenetic and synteny analysis to reveal the orthological relationship of zebrafish *oat1* and *oat3* genes. We then developed stable cell lines overexpressing Oat1 and Oat3, and identified Lucifer yellow as Oat1 model fluorescent substrate and 6-carboxyfluorescein as Oat3 model substrate. Initial interaction screening performed using the developed assays revealed interaction with various endo- and xenobiotics, including Kreb's cycle intermediates, bilirubin, bile salts, steroid hormones, NSAIDs, chemoterapeutics, pesticides and industrial chemicals. After the IC50 values for the most potent interactors were determined, the type of interaction with zebrafish Oat1 and Oat3 was revealed by observing the change in Michaelis-Menten kinetics parameters of model substrates uptake in the presence and absence of a tested interactor. Finally, to further confirm the type of interaction and initially evaluate toxic potential of the most potent interactors, the cytotoxicity assays were performed. In conclusion, using the approach described structural and functional similarities of both transporters to human and mammalian orthologs are revealed, their broad ligand selectivity confirmed and potent interactors among endo- and xenobiotic compounds identified.

P 215: New testing and screening in vivo zebrafish based methods to identify additional key events and potential treatments for organophosphorus poisoning

TOPIC: TOXICOLOGY

Leslie E. Dubrana[1], *Anja Knoll-Gellida*[1], *Laure M. Bourcier*[1], *Patrick J. Babin*[1]

[1] *University of Bordeaux - INSERM U1211, Rare Diseases : Genetic and Metabolism MRGM, Bordeaux, France*

ABSTRACT TEXT

Organophosphorus (OPs) are organic compounds widely used, for example, as pesticides, plasticizers or nerve agents, which poses a serious public health problem because of their lethality or their induced neurological alterations. The acute toxicity of OPs results from the irreversible inhibition of acetylcholinesterase, whose inactivation leads to a major cholinergic syndrome associating peripheral manifestations and epileptic seizures. Currently, the antidote cocktail (2-PAM/atropine) is not sufficiently efficient to counteract the OP toxicity; that is why it is necessary to discover new medical countermeasures for human protection. In addition, exposure to neuropathic OPs is well-known to lead to OP-induced delayed neuropathy, a neurodegenerative disorder characterized by a delayed onset of prolonged ataxia and upper motor neuron spasticity. Neuropathic OPs may target the neuropathic target esterase (NTE/PNPLA6) but their mechanism of action is not entirely resolved and still controversial. Selected OPs were used for discovering additional key events involved in the neurotoxicity of these molecules and new antidote treatments. Visual motor response and an electric field pulse motor response tests enabled to discriminate between central and peripheral nervous system functions. Complementary biochemical assays allowed us to evaluate the inhibition of target esterases and to compare *in vivo* antidote effectiveness. In conclusion, zebrafish larvae are valuable tools for identifying new drugs for multifunctional drug therapy against OP poisoning.

P 216: Investigation of the biodegradation efficiency of T-2 mycotoxin-degrading bacteria by microinjection on zebrafish (Danio rerio) embryos

TOPIC: TOXICOLOGY

Garai Edina[1], *Flora Kerekes*[1], *Anita Kozár-Risa*[2], *Mátyás Cserháti*[2], *Emese Varga*[3], *Erna Vásárhelyi*[1], *Dóra Kánainé S.*[1], *Illés Bock*[1], *Balázs Kriszt*[2], *Béla Urbányi*[2], *Zsolt Csenki*[1]

[1] *Szent István University, Department of Aquaculture, Gödöllő, Hungary*

[2] *Szent István University, Department of Environmental Safety and Ecotoxicology, Gödöllő, Hungary*

[3] *Szent István University, Department of Applied Chemistry, Budapest, Hungary*

ABSTRACT TEXT

As a result of climate change, extreme weather conditions greatly affect the presence of microscopic molds and the amount of mycotoxins produced. Mycotoxins are not only responsible for economic losses but also a source of health threats. Degradation and detoxification by microorganisms or by their enzymes is one of the most promising methods of mycotoxin decontamination. According to EFSA's recommendation, the metabolites from the parent compound should be studied by ecotoxicological tests too. Not all toxins and microbes have a fast, reliable and cost-effective bioassay, this is also relevant for the T-2 toxin we tested, which is one of the most important mycotoxins from an agricultural point of view. Due to its immunological, dermato- and neurotoxic effects, it means a potential hazard to human and animal health.

Our study covers if the seven selected T-2 degrading microbial strains have biodetoxification potential. The aim of our experiments was the bacterial degradation and biodetoxification of T-2. The residual biological activity of the degradation samples was tested on zebrafish embryos by microinjection (120 hour test). Based on the results of our experiments, it can be stated that only the normal metabolite and the degradation by-product of *R. erythropolis* NI1 were not toxic to the treated fish. The normal metabolite of *R. rhodochrous* NI2 was found to be harmful, whereas the degradation by-product was not. As a conclusion, metabolic products produced by degrading bacteria under

normal living conditions are also important to study, and considering the EFSA recommendation it is recommended in all similar cases. In addition, the strain NI1 may be used in the future to decontaminate contaminated feed indoors.

Our work was supported by EFOP-3.6.3-VEKOP-16-2017-00008, NVKP_16-1-2016-0035, NKFIH-831-10/2019.

P 217: Possible mechanism of PHMG-P's harmful effects was assessed with zebrafish embryo/larvae model

TOPIC: TOXICOLOGY

Kojo Eghan[1], Jeong-ah Song[2], Sangwoo Lee[1], Seokjoo Yoon[1], Woo-Keun Kim[1]

[1] *Korea Institute of Toxicology, Department of Predictive Toxicology, Daejeon, Korea Republic of*
[2] *Korea Institute of Toxicology, Department of Inhalation Research, Jeong-up, Korea Republic of*

ABSTRACT TEXT

Polyhexamethylene guanidine phosphate (PHMG-P) has a strong penetration in our households, particularly due to its resilient bactericidal properties. This guanidine-based cationic antimicrobial polymer, is a potent antimicrobial biocide, even at low concentrations. It was safely used extensively in homes until its use in humidifiers led to a catastrophic event in South Korea. Epidemiological studies have linked the use of PHMG-P as a humidifier disinfectant to pulmonary fibrosis. However, because little is known about the mechanisms of its harmful impacts, we applied a zebrafish embryo/larva model to help bridge this research gap. Zebrafish embryos were exposed to 0.1, 0.2, 0.3, 0.4, 0.5, 1, and 2 mg/L concentrations of PHMG-P for 96 h post fertilization. A concentration of 2 mg/L resulted in total mortality and an LC_{50} value of 1.18 mg/L at 96 hours. The heart rate of zebrafish larvae was significantly altered. In transcriptome analysis, immune and inflammatory responses were significantly affected similarly to those in epidemiological studies. Our qPCR analysis (*Itgb1b*, *TNC*, *Arg1*, *Arg2*, *IL-1β*, *Serpine-1*, and *Ptgs2b*) also confirmed this following a 96-hour exposure to 0.4 mg/L of PHMG-P. These discoveries on adverse effects of PHMG-P and its putative mechanism should help in mitigating adverse effects.

This study was supported by the Ministry of Environment (2018002490004).

P 218: Protective effect of beta-cyclodextrin on zearalenon-induced toxicity in Tg(vtg1:mcherry) zebrafish embryos

TOPIC: TOXICOLOGY

Edina Garai[1], Zelma Faisal[2], Katalin Bakos[1], Lajos Szente[3], Béla Urbányi[1], Miklós Poór[2], Zsolt Csenki[1]

[1] *Szent István Egyetem, Department of Aquaculture, Gödöllő, Hungary*
[2] *University of Pécs, Department of Pharmacology, Pécs, Hungary*
[3] *Cyclolab, Cyclodextrin Research & Development Laboratory, Budapest, Hungary*

ABSTRACT TEXT

Solving the problem of production and quality degradation caused by mycotoxins is increasingly urgent. Several physical, chemical and biological methods for their removal have been described, of which toxin fixation is a practical option.

Cyclodextrins are ring-shaped molecules made of glucose units that are capable of forming so-called "host-guest" inclusion complexes with various apolar compounds. The formation of highly stable cyclodextrin-ligand complexes may limit the cellular uptake of the guest molecule and its biological effects. Thus, cyclodextrin technology may be able to reduce the toxicity of xenobiotics, even after exposure.

In our study, the toxicitiy of zearalenone was studied in the presence of native and chemically modified (sulfobutyl-, methyl- and succinyl-methyl-substituted) beta-cyclodextrins on transgenic bioindicator zebrafish embryos (Tg (vtg1: mCherry)) using various experimental settings.

The formation of stable zearalenone-cyclodextrin complexes reduces or even eliminates the significant morphological changes induced by zearalenone. In addition, the presence of cyclodextrins caused the increase of estrogen production in the case of treated individuals at the same concentration of zearalenone as compared to the mycotoxin treatment without contaning cyclodextrin. This latter observation is probably due to the reduced hepatotoxicity of zearalenone, since zearalenone-induced vitellogenin production is partially inhibited by the hepatotoxicity of mycotoxin caused in zebrafish.

Overall, zearalenone binds to some cyclodextrins with high affinity, thereby cyclodextrins significantly reduce the toxic effects of mycotoxins. However, further studies are needed considering the potential *in vivo*use of cyclodextrins as a mycotoxin binding agent.

The research was supported by the following projects: FK125166, NKFIH-831-10/2019, NKFIA, NVKP_16-1-2016-0003, EFOP-3.6.3-VEKOP-16-2017-00008, KA-2018-17.

P 219: Biological evaluation of microbial toxin degradation by microinjected zebrafish (Danio rerio) embryos

TOPIC: TOXICOLOGY

Edina Garai[1], Anita Kozár-Risa[2], Mátyás Cserháti[2], Katalin Bakos[1], Erna Vásárhelyi[1], Dóra Kánainé S.[1], Bokor Zoltán[1], Flora Kerekes[1], Illés Bock[1], Balázs Kriszt[2], Béla Urbányi[1], Zsolt Csenki[1]

[1] *Szent István University, Department of Aquaculture, Gödöllő, Hungary*
[2] *Szent István University, Department of Environmental Safety and Ecotoxicology, Gödöllő, Hungary*

ABSTRACT TEXT

The aim of our study was to investigate the use of microinjection of newly fertilized zebrafish eggs as a suitable tool for qualifying the biodetoxification properties of toxin-degrading microbes.

Ochratoxin A (OTA), bacterial degradation products of OTA and bacterial metabolites of the *Cupriavidus basilensis* ŐR16 strain were microinjected. Results demonstrated that variations in the injected droplet size, and thus treatment concentrations, remained within ±20 %. Furthermore, embryo mortality did not exceed 10 % in controls either, which is in accordance with the recommendations of the OECD 236 guideline. The highest lethality was documented in the case of OTA, with a significantly higher toxicity than that of bacterial metabolites or OTA degradation products. However, toxicity of the latter two did not show any statistical differences. It means that the observed mortality was caused by the intrinsic toxicity of bacterial metabolites (and not OTA degradation products), thus, the strain is effectively able to degrade OTA to nontoxic products. Sublethal symptoms also affirmed this conclusion. Results confirmed that microinjection of zebrafish embryos could be a proper tool for testing the toxin-degrading properties of microbes. By the help of this method comparisons among microbial strains able to degrade the same toxin, helping the selection of effective and environmentally safe microbial strains for the biodetoxification of mycotoxins in large scale. Our work was supported by EFOP-3.6.3-VEKOP-16-2017-00008, NVKP_16-1-2016-0009, projects, NKFIH-831-10/2019.

P 220: Safety assessment of compounds after in vitro metabolic conversion using Zebrafish eleuthero embryos

TOPIC: TOXICOLOGY

Arianna Giusti[1], Xuan-Bac Nguyen[1], Stanislav Kislyuk[2], Mélanie Mignot[2], Cecilia Ranieri[1], Johan Nicolai[3], Marlies Oorts[3], Wu Xiao[1], Pieter Annaert[3], Noémie De Croze[4], Marc Léonard[4], Annelii Ny[1], Deirdre Cabooter[2], Peter de Witte[1]

[1] *University of Leuven, Laboratory for Molecular Biodiscovery- Department of Pharmaceutical and Pharmacological Sciences, Leuven, Belgium*
[2] *University of Leuven, Pharmaceutical Analysis- Department of Pharmaceutical and Pharmacological Sciences, Leuven, Belgium*
[3] *University of Leuven, Drug Delivery and Disposition- Department of Pharmaceutical and Pharmacological Sciences, Leuven, Belgium*
[4] *L'Oréal, Research & Innovation, Aulnay-sous-Bois, France*

ABSTRACT TEXT

Zebrafish-based platforms have recently emerged as a useful tool for toxicity testing as they combine the advantages of in vitro and in vivo methodologies. Nevertheless, the capacity to metabolically convert xenobiotics by zebrafish eleuthero embryos is supposedly low. To circumvent this concern, a comprehensive methodology was developed wherein test compounds (i.e., parathion, malathion and chloramphenicol) were first exposed in vitro to rat liver microsomes (RLM) for 1 h at 37 °C. After adding methanol, the mixture was ultrasonicated, placed for 2 h at -20 °C, centrifuged and the supernatant evaporated. The pellet was resuspended in water for the quantification of the metabolic conversion and the detection of the presence of metabolites using ultra high performance liquid chromatography-Ultraviolet-Mass (UHPLC-UV-MS). Next, three days post fertilization (dpf) zebrafish eleuthero embryos were exposed to the metabolic mix diluted in Danieau's medium for 48 h at 28 °C, followed by a stereomicroscopic examination of the adverse effects induced,

if any. The novelty of our method relies in the possibility to quantify the rate of the in vitro metabolism of the parent compound and to co-incubate three dpf larvae and the diluted metabolic mix for 48 h without inducing major toxic effects. The results for parathion show an improved predictivity of the toxic potential of the compound.

P 221: Effects of embryonic exposure to non-lethal concentrations of aflatoxin B1 on zebrafish immune system

TOPIC: TOXICOLOGY

Bence Ivánovics[1], *Gyöngyi Gazsi*[1], *Zsolt Csenki*[1], *Izabella Berta*[1], *Márta Reining*[1], *András Ács*[1], *Béla Urbányi*[1], *Zsolt Czimmerer*[2]

[1] *Szent István University, Department of Aquaculture, Gödöllő, Hungary*
[2] *University of Debrecen, Department of Biochemistry and Molecular Biology, Debrecen, Hungary*

ABSTRACT TEXT

Aflatoxin B1 (AFB1) is a highly carcinogenic mycotoxin produced by *Aspergillus spp.* that commonly contaminates foods and feeds. Exposure to AFB1 during pregnancy pose a significant threat to the developing embryo, because the toxin can cross the placental barrier.

The aim of this study was to investigate the harmful effects of non-lethal AFB1 exposure in zebrafish embryos applying combined molecular, toxicological and immunological methods.

We performed Fish Embryo Acute Toxicity (FET) test to determine the sub-lethal ($<LC_{10}$) concentrations. Next, we investigated morphological malformations, total body length and swimbladder area following 120 hours toxin exposition at four different sub-lethal AFB1 concentrations. Drastic malformations have not been observed at the selected concentrations, but both reduced total body length and decreased swimbladder area were detected. Next, we performed global transcriptome analysis in whole larvae to identify AFB1 exposition-regulated molecular pathways. Our *in silico* gene ontology (GO) enrichment analysis showed that the inflammation-linked pathways were significantly enriched among AFB1-induced genes. Therefore, we characterized the AFB1 exposure-associated immunomodulatory effects using neutrophil granulocyte-specific zebrafish line and fluorescence-based measurement of nitric-oxide production. Sub-lethal AFB1 exposition induced an altered, diffuse distribution of neutrophil granulocytes in the whole larvae and significantly increased the nitric-oxid production. Finally, we examined AFB1 exposure-modulated neutrophil granulocyte migration in tail fin transection model of local inflammation. Interestingly, decreased neutrophil granulocyte number was observed at the site of injury in AFB1-treated zebrafish larvae.

Our findings suggest that sub-lethal AFB1 exposure has a significant impact on immune system through induction of systemic inflammation and modulation of neutrophil response to a local injury in zebrafish embryos.

P 222: Mixture toxicity analysis in zebrafish embryo – a time and concentration resolved study on mixture effect predictivity

TOPIC: TOXICOLOGY

Gianina Jakobs[1], *Andreas Schüttler*[2], *Rolf Altenburger*[1], *Wibke Busch*[1]

[1] *Helmholtz Centre for Environmental Research GmbH - UFZ, Bioanalytical Ecotoxicology, Leipzig, Germany*
[2] *Helmholtz Centre for Environmental Research GmbH - UFZ, Young Investigators Group Transcriptomics and Bioinformatics, Leipzig, Germany*

ABSTRACT TEXT

Humans and wildlife are continuously exposed to chemical mixtures. These mixtures vary in composition but typically contain hundreds of lowly abundant micropollutants. However, current regulation is based on individual chemical toxicity. As it is not feasible to measure the toxicity of all possibly occurring mixtures, there is a critical need to develop approaches to reliably predict mixture toxicity. Yet, two models, the concept of Concentration Addition (CA) and Independent Action (IA), are discussed to serve as promising tools to predict mixture toxicity. Here, we evaluate the predictability of mixture toxicity in zebrafish. Therefore, we predicted the toxicity of nine mixtures and compared predicted to actually measured toxicity. We further assessed the influence of phenotype (i.e. lethality or malformations), potency (i.e. $EC_{10,50,90}$), or exposure period on model accuracy. In total, 177 measured toxicity values were compared to their predicted counterparts by calculating the prediction deviation ratio. Our results showed that mixture toxicity was correctly estimated by the *prediction window*, hence the concentration-effect space that spanned both prediction models, in 86 % of cases, or was even more potent

as predicted. Importantly, 90 % of mixtures induced a significant toxic effect although mixture constituents were applied in concentrations that failed to evoke individual toxicity. Comparing both models, CA was more sensitive and the prediction deviation ratio did not exceed a factor of 2.5. The CA model showed highest predictability for long-term exposure with highly potent mixtures, whereas IA was more accurate for short-term exposure with lowly potent mixtures. Overall, these data support the *prediction window* to predict mixture toxicity in zebrafish. More broadly, this study demonstrates the importance of mixture toxicity predictions to capture the combined toxicity of diverse chemicals and ultimately improve water quality for humans and wildlife.

P 223: BPAP exposure caused negligible or weak thyroid hormone disrupting effects in zebrafish embryo/larvae model

TOPIC: TOXICOLOGY

Sangwoo Lee[1], *Kojo Eghan*[1], *Seokjoo Yoon*[1], *Woo-Keun Kim*[1]

[1] *Korea Institute of Toxicology, Department of Predictive Toxicology, Daejeon, Republic of Korea*

ABSTRACT TEXT

Bisphenol AP (BPAP) has been used as a bisphenol A (BPA) alternative. BPAP was detected in various environmental samples and also found in food and personal care products. Although thyroid disrupting effect of other BPA analogues, such as bisphenol F and bisphenol S, was recently reported, that of BPAP has not yet been evaluated.The thyroid hormone (TH) is essential for development and growth. Hence, this study was conducted to examine the effects of BPAP on the TH system, development and behavior in zebrafish embryo/larvae model. In this present study, zebrafish embryos were exposed to BPAP (0, 0.04, 0.12, 0.37 and 1.0 mg/L) until 120 hpf. Following the exposure, TH levels (T3, T4 and TSH) and transcription of 16 TH related genes were analyzed. Additionally, enriched gene ontology was identified based on RNA-sequencing. Changes in development (survival, hatching, and morphology) and behavior (moved distance and duration) were also evaluated. In our study, BPAP caused reduction of T4 level at the highest non-lethal concentration but significant changes were not found in T3 and TSH. BPAP did not cause significant changes in transcription of 16 TH related genes although only the transcription of tg showed significantly increasing trend. Gene ontology enrichment related to TH system was not significantly different from control group. In addition, developmental and behavioral changes were not observed up to non-lethal concentration (0.37 mg/L). Despite the T4 level reduction, other markers for TH regulation and zebrafish development and behavior were not affected. Thus, the results in the present study might indicate that BPAP may have negligible or weak potency to disrupt TH system. Thyroid hormone disrupting potency of BPAP might be relatively lower than that of other BPA analogues, though quantitative comparisons among BPA analogues deserve further investigations.

This study was supported by the Ministry of Environment (2018002490004).

P 224: The effect of β-adrenergic receptors ligands on the heart function in zebrafish model

TOPIC: TOXICOLOGY

Monika Maciąg[1,2], *Kinga Bednarz*[1], *Jakub Rusinek*[1], *Artur Wnorowski*[1], *Maciej Maj*[1], *Anita Płazińska*[1]

[1] *Medical University of Lublin, Department of Biopharmacy, Lublin, Poland*
[2] *Medical University of Lublin, Laboratory of Behavioural Studies, Lublin, Poland*

ABSTRACT TEXT

The β-adrenergic receptors (βAR) play an essential role in diverse cellular and biological processes. Both β_1AR and β_2AR are important for the regulation of cardiovascular functions, as well as they are involved in the pathogenesis of heart diseases. Nevertheless, increasing body of experimental evidence gathered over the past two decades indicates that βAR may play opposing roles in the regulation of cardiac responses.

Single β_1AR and two distinct variants of β_2AR have been identified in the zebrafish; however their pharmacology has not been studied extensively. Therefore, we screened a panel of subtype-selective βAR ligands to investigate the effects of β_1AR and β_2AR activation in zebrafish model.

Toxicity profile of selected βAR agonists was assessed in zebrafish larvae for 96 hours post fertilization. Comparison of these compounds in respect to the elicited mortality, changes in heart rate and morphologic alterations indicates compound-specific effects. Isoprenaline, a non-selective βAR agonist, which is a classic inducer of heart failure, triggered both bradycardia and severe cardiac malformation. On the contrary, selective β_2AR agonists have not demonstrated cardiotoxic properties.

Different pharmacological profiles of various βAR ligands are reflected in differences in cardiac responses in zebrafish. Hence, these findings demonstrate that zebrafish constitute good cardiotoxicity model reflecting plethora of pathophysiological effects and offering cost effective screening of the cardiological effects during early drug development.

The authors acknowledge financial support from the National Science Centre, Poland (Grant 2017/25/B/NZ7/02654).

P 225: Spatial and temporal distribution of compounds in zebrafish after different administration routes

TOPIC: TOXICOLOGY

Annelii Ny[1], *Marlly Guarin*[1], *Arianna Giusti*[1], *Noémie De Croze*[2], *Marc Léonard*[2], *Peter de Witte*[1]

[1] *University of Leuven, Laboratory for Molecular Biodiscovery- Department of Pharmaceutical and Pharmacological Sciences, Leuven, Belgium*
[2] *L'Oréal, Research & Innovation, Aulnay-sous-Bois, France*

ABSTRACT TEXT

The zebrafish larvae model is a proven efficient screening tool for safety assessments of pharmaceuticals, cosmetics and environmental samples. Immersion is the most common route of compound delivery, however, compounds with low solubility in aqueous solution or compounds that are poorly up-taken by the exposed larvae, could be at risk of being classified as a false-negative using this approach. To circumvent this, compounds can also be injected. However, how compounds distribute spatially and temporally after the different distribution routes is not well known. Therefore, we performed a study where fluorescent compounds with different physicochemical properties were delivered to 3 dpf zebrafish larvae. Four different exposure routes, i.e. immersion, injections into the pericardial area, the yolk or intraperitoneal, were examined and at several time-points after exposure, the larvae were photographed and the intra-fish compound distribution was quantified in arbitrary units as RFU using the MetaMorph®.

Results show that only highly lipophilic compounds (but not hydrophilic compounds) were well taken up and distributed after immersion and that injections in the yolk resulted in poor distribution in the larvae. These two distribution routes can hence result in false-negative results. Injections into the pericardial and the peritoneal cavities, however, showed well distribution of compounds in a spatial and temporal way irrespectively of the physicochemical properties of the compounds. Therefore, these delivery routes could provide a good alternative to immersion for hydrophilic compounds and compounds with poor solubility.

P 226: Developmental and neurotoxicity of acrylamide to zebrafish

TOPIC: TOXICOLOGY

Jong-Su Park[1], *Palas Samanta*[2], *Sangwoo Lee*[1], *Jae-Woo Cho*[3], *Hang-Suk Chun*[1], *Seokjoo Yoon*[4], *Woo-Keun Kim*[1]

[1] *Korea Institute of Toxicology, Biosystem Research Group, Daejeon, Korea Republic of*
[2] *University of North Bengal, Department of Environmental Science, West Bengal, India*
[3] *Korea Institute of Toxicology, Toxic Pathology Research Group, Daejeon, Korea Republic of*
[4] *Korea Institute of Toxicology, Molecular Toxicity Research Group, Daejeon, Korea Republic of*

ABSTRACT TEXT

Acrylamide is one of the commonly used chemical in various industries such as caulking, food packaging and some adhesives. However, acrylamide was also already well known neurotoxic chemical. To investigating neurotoxicity of acrylamide, most previous study were used traditional rodent animal models, which have some limitations including cost and time. To overcome these limitations we used alternative animal model zebrafish AB and *tg*(*elavl3:eGFP*) line to detect their developmental toxicity, neurotoxicity, and behavioral toxicity within 5 days post acrylamide treatment. At 6 hours post fertilization zebrafish embryos were exposed to four concentrations of acrylamide (10, 30, 100, 300 mg/L) for up to 24 to 120 hours post fertilization. Our results showed that acrylamide can lead developmental toxicity

features such as yolk retention, scoliosis, swim bladder deficiency, heart edema, and curved body shape. Behavioral toxicity showed that acrylamide treated group has significantly impaired locomotor activity including decreased swimming speed and irregular movement. And we also found that acrylamide treated zebrafish larvae brain and spinal cord width has significantly impaired at 100 mg/L. We also confirmed that these results were similar with acrylamide treated rat brain histology data which also shows cerebellum and sciatic nerve neuronal degeneration. In summary, we found that acrylamide can induced developmental toxicity and neurotoxicity in zebrafish larvae and confirmed that these results were similar with traditional animal model rat data. So we can suggests that these zebrafish using methods were one of good way to check acrylamide induced neurotoxicity.

This study was supported by the Ministry of Environment (2019002490006).

P 227: A multi-parametric embryo-larval assay for toxicological analysis in zebrafish

TOPIC: TOXICOLOGY

Chiara Reina[1], *Vincenzo Cavalieri*[2,3]

[1] *University of Palermo, Health Promotion- Mother and Child Care- Internal Medicine and Medical Specialties PROMISE, Palermo, Italy*
[2] *University of Palermo, Department of Biological- Chemical and Pharmaceutical Sciences and Technologies STEBICEF, Palermo, Italy*
[3] *University of Palermo, Advanced Technologies Network ATeN Center, Palermo, Italy*

ABSTRACT TEXT

Due to their small size, high fecundity, rapid development and similarity to humans in genetics and anatomy, zebrafish (*Danio rerio*) embryos and larvae provide a useful platform for *in vivo* pharmacological assessments to identify potential safety liabilities in drug discovery. Embryos are permeable to small molecules, allowing easy administration of drugs directly in their culture medium, and maintain optical transparency into the free-swimming larval stage, facilitating detailed examination of internal organs.

On this basis, we set an experimental workflow combining survival rate, developmental aberrations and behavioural profiling to evaluate toxicity of new drug candidates. Dechorionated zebrafish embryos at 6 hours-post-fertilization (hpf) are placed in a 96-well plate (1 embryo/well), which is then loaded with the compounds of interest, dissolved either in water or organic solvents, at different concentrations. Development progression and larval growth of statistically relevant groups of treated individuals are compared with those of unperturbed controls until 5 days post-fertilization (dpf), and a range of features are examined, including developmental delay, morphological abnormalities (such as edema, skeleton defects, cardiac and vascular dysfunctions), and survival. In parallel, an apoptotic assay is performed by staining control and treated embryos at 48 hpf with the vital fluorescent dye acridine orange. Moreover, locomotor variation induced by drug exposures is identified by measuring differences in distance travelled and average speed between 5 dpf control and treated larvae, using an automated video-tracking system. Optionally, the effects of selected drugs are examined delivering them by microinjection directly into target tissues of the embryo/larvae.

The robustness of this assay has been verified by testing potential pharmaceuticals for several diseases, including complex mixtures such as conditioned media harvested from cultured stem cells.

P 228: Evaluation of Domoic acid toxicity in zebrafish embryo

TOPIC: TOXICOLOGY

Gabriel Mbuta Tchivelekete[1], *Xinhua Shu*[1], *Patricia Martin*[1]

[1] *Glasgow Caledonian University, Biological and Biomedical Sciences, Glasgow, United Kingdom*

ABSTRACT TEXT

Domoic acid (DA), a cyclic amino acid, is a neurotoxin produced by algae. Humans can be exposed to DA through the consumption of contaminated shellfish that have accumulated this toxin during algal blooms. *In vitro* and *in vivo* experiments demonstrate that DA exposure causes neurotoxic symptoms such as seizures and memory loss. It is not clear whether DA exposure affects the development of the retina, a part of the central nervous system. Here we used the zebrafish model to assess the retinal toxicity of DA. Zebrafish embryos at 24 hours post-fertilization (hpf) were exposed to DA at concentrations of 0.1, 0.25, 0.5, 1.0, 2.5, 5.0 and 10.0 µM till 120 hpf. Endpoint measurements included mortality, malformations (curved body axis and oedema), delayed hatching, altered heartbeat or reduced movement. Embryos

exposed to 10 μM DA were dead at 96 hpf. DA treatment did not have a significant effect on hatching but resulted in a decrease in heart rate at higher concentration. Embryos exposed to 5 μM were subjected to histological analysis, measurement of reactive oxygen species (ROS), and gene expression by quantitative real-time polymerase chain reaction (qRT-PCR). Our data showed that DA at high concentration caused increased mortality, decreased hatching and heart rate and abnormal morphology. DA-exposed embryos demonstrated lower antioxidant capacity with increased production of reactive oxygen species (ROS), decreased expression of antioxidant genes and increased expression of inflammatory genes. Zebrafish retinal development was also disrupted by DA treatment at high concentrations.

Keywords: Domoic acid, Zebrafish embryos, oxidative stress, inflammation, retina.

List of Presenters

Albadri, Shahad
Aliti, Geoffrey
Allanki, Srinivas
Bajoghli, Baubak
Baraban, Scott
Bartunek, Petr
Bessa, Jose
Bomont, Pascale
Breuer, Maximilian
Burgess, Shawn
Cardeira-da-Silva, João
Carletti, Alessio
Cherng, Bor-Wei
Crim, Marcus
Demirler, Mehmet Can
Diogo, Patricia
Dona, Margo
Economou, Andrew
Elkouby, Yaniv
Eufrásio, Ana
Finger-Baier, Karin
Gillotay, Pierre
Giuliano, Ferrero
Gore, Aniket
Goudmaeker, Apolline
Guglielmi, Luca
Hans, Stefan
Harish, Rohit Krishnan
Hasan, Kazi Md Mahmudul
Hatta, Kohei
Hoeksma, Jelmer
Hu, Bo
Kamel, Sarah
Kaslin, Jan
Kelsh, Robert
Kim, Hyejeong Rosemary
Kizil, Caghan
Koltowska, Kaska
Kroll, Jens
Lacadie, Scott
Lam, Pui Ying
Lefkopoulos, Stylianos
López-Cuevas, Paco
Lou, Bowen
Malkinson, Guy
Marlow, Florence
Merks, Anne M.
Merola, Carmine
Moravec, Cara
Morizet, David
Münch, Daniela
Nguyen, Phong
Nikolaou, Nikolas
Noël, Emily
Ober, Elke
Ono, Fumihito
Otten, Cecile
Panáková, Daniela
Pantazis, Periklis
Piotrowski, Tatjana
Powell, Gareth
Qin, Wei
Rajewsky, Nikolaus
Ramakrishnan, Lalita
Resseguier, Julien
Scholz, Stefan
Schulte-Merker, Stefan
Sehring, Ivonne
Siauciunaite, Rima
Small, Christopher
Solnica-Krezel, Lilianna
Soussi-Yanicostas, Nadia
Spreafico, Marco
Sugden, Wade
Tarasco, Marco
Tessadori, Federico
Thiessen, Kevin
Torigoe, Makio
Vanwalleghem, Gilles
Vasconcelos, Raquel
Wang, Han
Weber, Michael
Whitlock, Kathleen
Wilhelm, Anke
Wilson, Carole
Wittamer, Valerie
Xu, Bing
Zenagui, Sarra

List of Oral Presentation Co-Authors

Aghaallaei, Narges
Akalin, Altuna
Albadri, Shahad
Alcalay, Myriam
Alexandrov, Yuriv
Aliti, Geoffrey
Alkiraz, Aysu Şevval
Allanki, Srinivas
Alunni, Alessandro
Ambrosio, Elizabeth
Angelozzi, Giovanni
Anticevic, Ivan
Anvar, Mana
Aoki, Tazu
Arnold, Hannah
Arribat, Yoan
Bailey, Charlotte
Baillie, Gregory
Bajoghli, Baubak
Bakken, Todd
Bakkers, Jeroen
Baldera, Davide
Balderas, Enrique
Bali, Burak
Bally-Cuif, Laure
Baraban, Scott
Barnes, Keith
Barooji, Younes
Barreira, Luísa
Barrington, Christopher
Bartolini, Laura
Battaglia, Cristina
Bayramli, Xalid
Beaurepaire, Emmanuel
Bebianno, Maria J.
Benfey, Tillmann
Bennewitz, Katrin
Bensimon-Brito, Anabela
Bergo, Veronica
Bertolucci, Cristiano
Besch-Williford, Cynthia
Bessa, Jose
Bhattarai, Prabesh
Bilić, Ilija
Bjørgen, Håvard
Bläsche, Juliane
Boezio, Giulia
Boizot, Alexia
Bomont, Pascale
Bonnet, Susan
Bordeira-Carriço, Renata
Boudinot, Pierre
Bowman, Teresa V.
Brand, Michael
Brandt, Kerstin
Brenet, Alexandre
Breuer, Maximilian
Bryant, Helen
Burgess, Shawn
Cabrita, Elsa
Cancela, M. Leonor
Candia, Danissa
Cardeira-da-Silva, João
Carletti, Alessio
Carneiro, Fatima
Carpenter, Colleen
Carpinello, Olivia
Castranova, Daniel
Cauchy, Pierre
Celikkaya, Hilal
Clapes, Thomas
Collet, Bertrand
Cordelières, Fabrice P.
Cosacak, Mehmet Ilyas
Costagliola, Sabine
Cox, Andrew
Crawford, Bryan
Crim, Marcus
Cristofoli, Nathana L.
Daley, George
Davis, Andrew
de Boer, Teun
Deflorian, Gianluca
Deis, Rachael
Del Bene, Filippo
del Pino, Victória
Demirler, Mehmet Can
den Hertog, Jeroen
Derrick, Christopher
Detours, Vincent
Di Ruggiero, Elodie
Dick, Advaita
Dinday, Matthew
Diogo, Patricia
Dominguez-Acemel, Rafael
Dona, Margo
Draper, Bruce
Dreher, Marie
Dreier, Jes
Du Pasquier, Louis
Duque, Marta
East, Philip
Economou, Andrew
Edwards, Ameila
Eggeler, Fanny
Eisenhofer, Graeme
Eitan, Hagai
Ekehorn Gimdal, Adrian
El-Khamisy, Sherif
Elkouby, Yaniv
Ergönen, Arda
Eski, Elif
Eski, Sema Elif
Eufrasio, Ana
Faro, Ana
Ferreira, Fabio
Ferrero, Giuliano
Figueroa, Francisco
Finger-Baier, Karin
Firbas, Panos
Fleming, Angeleen
Fleming, Thomas
Foulkes, Nicholas Simon
Freitas, Telmo
French, Paul
Freudenberg, Uwe
Fuchter, Matthew
Fukai, Tomoki
Fung, Chi Chung
Fuss, Stefan H.
Gacoin, Thierry
Galhardo, Mafalda
Gangadhar, Katkam N.
Garfield, David
Gavaia, Paulo J.
Geffarth, Michaela
Gillotay, Pierre
Goessling, Wolfram

Goméz-Skarmeta, Jose Luis
Gordon, Patricia
Gore, Aniket
Gori, Ilaria
Goureau, Olivier
Greenald, David
Griffin, Aliesha
Griffiths, Gareth Wyn
Grone, Brian
Gruszka, Alicja Maria
Guenther, Stefan
Guglielmi, Luca
Hamid, Fursham
Hamling, Kyla
Hammes, Hans-Peter
Han, Areum
Hans, Stefan
Harish, Rohit Krishnan
Hart, Marcia
Hasan, Kazi Md Mahmudul
Hasel, Eva
Hassan-Abdi, Rahma
Hassenklöver, Thomas
Hatta, Kohei
Heather, Riley
Heliot, Claire
Hering, Steffen
Hill, Caroline
Hirsekorn, Antje
Hoeksma, Jelmer
Hogan, Ben
Houart, Corinne
Hu, Bo
Huisken, Jan
Hussein, Farah
Chaudan, Elodie
Chaudhuri, Dipayan
Chen, Cheng
Chen, Jun
Chen, Xiangke
Cherng, Bor-Wei
Chocron, Sonja
Chou, Stephanie
Christoffels, Vincent
Ingber, Donald
Islam, Tanvir
Isomura, Takuya
J. Ferreira, Fábio
Jenuwein, Thomas
Jerez-Longres, Carolina
Jimenez, Erin
Jimpo, Ayumi
Jones, E. Yvonne
Junker, Jan Philipp
Junker, Philipp
JunkerPhilipp, Jan
Kakinuma, Hisaya
Kamel, Sarah
Kaslin, Jan
Kawakami, Koichi
Kelsh, Robert
Kempfer, Rieke
Kesavan, Gokul
Kettunen, Petronella
Khan, Shanawaz
Kießling, Tobias
Kim, Hyejeong Rosemary
Kizil, Caghan
Klaeylé, Lhéanna
Kocagöz, Yiğit
Koltowska, Kaska
Koopman, Charlotte
Kopp, Wolgang
Koppang, Erling Olaf
Kränkel, Annekathrin
Kroll, Jens
Kruse, Fabian
Kucenas, Sarah
Kumar, Sunil
Kumar, Vineet
Lacadie, Scott
Lai, Chih
Laizé, Vincent
Lam, Pui Ying
Lange, Christian
Lefkopoulos, Stylianos
Lekk, Ingrid
Lelek, Sara
Lescouzères, Léa
Leshchiner, Ignat
Levraud, Jean-Pierre
Levy, Karine
Li, Joe
Lin, Shuo
Liu, Feng
Liu, Jing
Liu, Yulong
Livingston, Robert
López-Cuevas, Paco
Lou, Bowen
Lu, Mengjia
Lucon-Xiccato, Tyrone
Lundin, Vanessa
Ma, Alvin Chun Hang
MacCarthy-Morrogh, Lucy
Mahou, Pierre
Machate, Anja
Malkinson, Guy
Mann, Stephen
Manzini, Ivan
Markham, Eleanor
Marks, Alora
Marlow, Florence
Marozzi, Anna
Marques, Joana
Martagon-Calderón, Natalia-Adriana
Martin, Paul
Martins, Gil
Marvel, Miranda
Mattonet, Kenny
Mazzola, Mara
McClain, Melainia
McGarvey, Alison
McGinty, James
Meneses-Giles, Paloma
Merks, Anne
Merks, Anne M.
Merola, Carmine
Metzger, Lena
Meyer, Alexander M.
Milicevic, Diana
Mione, Marina
Miserocchi, Magali
Mizumaki, Yumiko
Moghtadaei, Motahareh
Moog, Maia
Moravec, Cara
Morizet, David

Münch, Daniela
Mysiak, Karolina
Mytlis, Avishag
Nather, Farouq
Navalho, João
Nawroth, Peter Paul
Nguyen, Phong
N'Guyen-Chi, Mai-Eva
Nikaido, Masataka
Nikolaou, Nikolas
Noël, Emily
North, Trista
Ober, Elke
Oesterheld, Willy
Ohler, Uwe
Okamoto, Hitoshi
Okamoto, Hotoshi
Okamoto, Shin-ichi
Okuda, Kazuhide
Onderwater, Yeszamin
Onishi-Seebacher, Megumi
Ono, Fumihito
Ononuju, Chinwendu
Otten, Cecile
Otterlee, Jarred
Ovcharenko, Ivan
Owens, Jennifer
Pal Singh, Sumeet
Palominos, Maria Fernanda
Pan, Y. Albert
Panakova, Daniela
Pantazis, Periklis
Paterno, Rosalia
PattenShunmoogum, Kessen
Pelegri, Francisco
Peloggia, Julia
Pereira, Hugo
Perrod, Chiara
Perugini, Monia
Peterson, Randall
Petronella, Kettunen
Petzold, Andreas
Piotrowski, Tatjana
Pistocchi, Anna
Polidoro Pontalti, Clarissa
Pollitt, Eric
Polyzou, Aikaterini
Pombo, Ana
Popova, Stanislava
Popovic, Marta
Potts, Kathryn S.
Powell, Gareth
Preussner, Jens
Putti, Elena
Qi, Haozhe
Qin, Wei
Quintè, Arianna
Reichman, Sacha
Reinhardt, Susanne
Reischauer, Sven
Resseguier, Julien
Ribeiro, Diogo
Richter, Susan
Rodenburg, Richard
Romano, Shannon
Romero-Carvajal, Andrés
Rondon Galeano, Maria
Roque, Ana
Rossel, Mireille
Ruiz, Maxime
Rummler, Maximilian
Sakızlı, Uğurcan
Salinas, Irene
Santhakumar, Kiran
Santos, Tamára
Santos, Tamára F.
Satoh, Shiori
Sawale, Rajlakshmi
Scott, Ethan
Sehring, Ivonne
Severn, Charlotte
Shankar, Meghna
Shawahny, Amal
Shimazaki, Hideaki
Schier, Alexander F
Scholz, Stefan
Schroeder, Paul
Schulte-Merker, Stefan
Siauciunaite, Rima
Siddiqui, Tohid
Simons, Cas
Small, Christopher
Smith, Kelly
Sneddon, Lynne
Somkhit, Julie
Sonay, Ali
Song, Wei
Sousa, Patricia
Soussi-Yanicostas, Nadia
Spanjaard, Bastiaan
Spreafico, Marco
Stainier, Didier
Stickney, Heather
Strilic, Boris
Sugden, Wade
Supatto, Willy
Supina, Christine
Swearer, Avery
Taimatsu, Kiyohito
Takamido, Daiji
Tarasco, Marco
Taylor, Richard
Teixeira, Joana
Teixido, Elisabet
Tena, Juan
Tessadori, Federico
Testa, Noe
Thauvin, Marion
Thawani, Aditya
Theodore, Lindsay
Thumberger, Thomas
Timmers, Henri
Tinman, Simon
Torigoe, Makio
Tory, Williams
Totaro, Maria Grazia
Toye, Ashley
Trinks, Alexandra
Trompouki, Eirini
Unterweger, Iris
Valli, Debora
van den Boogaard, Malou
van den Brink, Susanne
van Eeden, Freek
van Herwaarden, Antonius
van Opbergen, Chantal
van Veen, Toon
Vanwalleghem, Gilles

Varela, João
Vasconcelos, Raquel
Verkerk, Arie
Voit, Gabriella
Vos, Marc
Vougny, Juliette
Vriz, Sophie
Vučićević, Dubravka
Waaijers, Selma
Wada, Risa
Wang, Han
Weber, Michael
Weidinger, Gilbert
Weinstein, Brant
Weiss, Kurt
Werner, Carsten
Westberg, Lars
White, Andrew
Whitlock, Kathleen
Wiegertjes, Geert
Wiggenhauser, Lucas
Wilhelm, Anke
Willie, Bettina
Wilson, Steve
Wittamer, Valerie
Wittbrodt, Jochen
Wohlmann, Jens
Wright, Gavin J.
Wrighton, Paul
Xu, Bing
Xu, Can
Yamamoto, Yasuhiro
Yanicostas, Constantin
Yates, Christian
Yekelchyk, Michail
Yin, Na
Zaouter, Charlotte
Zempo, Buntaro
Zenagui, Sarra
Zhang, Yixin
Zhao, Yuguang
Zöller, Daniela
Zong, Hui

List of Poster Co-Authors

Abdel-Salam, Ghada
Abu Nahia, Karim
Acker-Palmer, Amparo
Ács, András
Adamson, Peter
Adriana, Hotz
Affolter, Markus
Agalou, Adamantia
Agazzi, Paola
Aghaallaei, Narges
Aherrahrou, Zouhair
Ahn, Jin Hee
Alaerts, Maaike
Alan, Brooke
Albert, Silvia
Alexandre-Moreno, Susana
Allalou, Amin
Allan, Stuart
Almeida, Adriana Mortara
Almodóvar Payá, Aitana
Altenburger, Rolf
Alvarez-Dios, Jose Antonio
Amar Bencheikh, Bouchra Ouled
Amorena, Michele
Amores, Angel
Amorim, João
Andrade-Barros, Aline Ingrid
Andreazzoli, Massimiliano
Andries, Lien
Annaert, Pieter
Annus, Tamás
Anticevic, Ivan
Antinucci, Paride
Aoki, Kanae
Aoki, Ryo
Argenton, Francesco
Armstrong, James P. K.
Arnold, Hannah
Aroca-Aguilar, Jose-Daniel
Arratia, Gloria
Atienzar-Aroca, Raquel
Aukrust, Ingvild
Austad, Eirik
Babin, Patrick J.
Babu, Swathy
Bae, Myung Ae
Bagnat, Michel
Bajoghli, Baubak
Bakkers, Jeroen
Bakos, Katalin
Balan-Lima, Leticia
Balistreri, Francesca
Balogh, Eszter
Balwierz, Piotr
Baranasic, Damir
Barker, Alison
Barrett-Jolley, Richard
Bartůněk, Petr
Baruffini, Enrico
Barwood, Paul
Basnet, Ram
Basso, Cristina
Batista-Filho, Joao
Bazel, Peter
Beatriz, Domingo
Bebianno, Maria J.
Becker, Catherina
Becker, Thomas
Beckers, An
Bednarz, Kinga
Beffagna, Giorgia
Behra, Martine
Beis, Dimitris
Beitsch, Mya
Bek, Jan Willem
Bellucci, Arianna
Belting, Heinz-Georg
Benedetti, Elisabetta
Benjaminsen, Joergen
Bergholt, Mads S.
Bergmans, Steven
Bernardo, Jefferson Thiago Gonçalves
Berta, Izabella
Bertotto, Luísa
Bertrand, Julien Y.
Bessa, Jose
Betz, Timo
Bianco, Isaac H
Bierau, Jörgen
Biga, Veronica
Bobe, Julien
Bock, Illés
Bodart, Hadelin
Boel, Annekatrien
Böhm, Benjamin
Bok, Levinus
Bollati, Valentina
Bonet-Fernández, Juan-Manuel
Bongiovanni, Alberto
Bonkohara, Yuki
Boos, Alena
Borbély, Noémi
Bordeira-Carriço, Renata
Borel, Nadine
Börries, Melanie
Borsani, Giuseppe
Boskovic, Srdjan
Boswell, Curtis
Bothma, Jacques
Bourcier, Laure M.
Branas Casas, Raquel
Brand, Michael
Branthonne, Adèle
Branzei, Dana
Breitzler, Lukas
Brenet, Alexandre
Bretaud, Sandrine
Breuer, Maximilian
Brion, Alice
Brion, Francois
Broccoli, Vania
Broekman, Sanne
Brombacher, Eline C.
Brombin, Alessandro
Brown, Ben
Brunner, Han
Brunt, Lucy
Buenhombre, Jhon
Bueno Marinas, Maria
Bugeon, Jérôme
Bugeon, Laurence
Bugiani, Marianna
Bühler, Anja
Burger, Martin
Burgess, Shawn
Burgos, Miguel
Burlakov, Alexander
Burrows, Dominic R. W.

Busch, Wibke
Busch-Nentwich, Elisabeth
Butti, Zoe
C. Ranawakage, Deshani
Cabezas-Sainz, Pablo
Cabooter, Deirdre
Cabrita, Elsa
Cacialli, Pietro
Cafora, Marco
Caioni, Giulia
Calpona, Sebastiano
Camelo, Carolina
Campos Costa, João
Cancela, M. Leonor
Cantres-Velez, Juan
Cao, Yihai
Cardona, Emilie
Carine, Giovannangeli
Carl, Matthias
Carletti, Alessio
Carnevali, Oliana
Carra, Silvia
Carrara, Mathieu
Carrillo, Estefania
Carswell, Gleta
Casarosa, Simona
Cason, Marco
Castaldi, Matthew
Castellini, Maria Elena
Castranova, Daniel
Castro, Daniela
Cavalieri, Vincenzo
Celeghin, Rudy
Certal, Ana Catarina
Cimini, Annamaria
Ciruna, Brian
Cocchi, Claudia
Colon-Cruz, Luis
Colucci, Patrizia
Constantin, Lena
Coon, Steven L.
Cordelières, Fabrice P.
Cossette, Patrick
Costa, Ilaria
Costa, Mariana
Costagliola, Sabine
Cotelli, Franco
Coucke, Paul
Crawford, Bryan
Crilly, Siobhan
Crim, Marcus
Crowell, Helena
Csenki, Zsolt
Cserháti, Mátyás
Cudak, Nicole
Cumplido, Nicolás
Cunha, Viviane Tomazia
Cutignano, Adele
Czimer, Dávid
Czimmerer, Zsolt
Daczewska, Małgorzata
Daetwyler, Stephan
Dale, Ryan
Dalla Valle, Luisa
Dalle Nogare, Damian
Dallman, Margaret J.
Damora, Marta
D'Angelo, Livia
Dassy, Benjamin
Daub, Carsten
Davis, Andrew E.
Daza-Cardona, Erika
De Angelis, Francesco
De Cesari, Chiara
De Clercq, Adelbert
De Corzé, Noémie
De Girolamo, Paolo
De Groef, Lies
De Saffel, Hanna
de Sena, Carmen
De Seni, Ana Carolina
De Sonneville, Jan
De Vita, Alessandro
De Vrieze, Erik
de Witte, Peter
Debier, Cathy
Decker, Nathalie
Deflorian, Gianluca
del Pino, Victória
Delahodde, Agnes
Delbue, Serena
Deleye, Lieselot
Della Barbera, Mila
Den Broeder, Marjo
Deng, Qing
Denker, Elsa
Dennis, Harvey
Dente, Luciana
Dhakal, Susov
Di Bella, Daniela
Di Franco, Gregorio
Di Lauro, Caterina
Di Menna, Giandomenico
Dick, Advaita M.
Dicks, Severin
Digregorio, Matteo
Dinarello, Alberto
Diofano, Federica
Diogo, Patrícia
Disner, Geonildo
Distel, Martin
Diz-Muñoz, Alba
Dobiasovska, Ivana
Dolci, Maria
Doll, Larissa
Domingo, Beatriz
Dona, Margo
Dooley, Christopher
d'Orazio, Fabio
Dormal, Elise
Dosch, Roland
Dotti, Silvia
Doyle, James
Dragojević, Jelena
Dreosti, Elena
Dubrana, Leslie E.
Dulla, Kalyan
Duvernois-Berthet, Evelyne
Dwyer, Toni
Dye, Louis E.
Eckert, Cornelia
Edina, Garai
Eggert, Angelika
Eghan, Kojo
Ekehorn Gimdal, Adrian
Ekker, Marc
Elbialy, Abdalla
Elias, Magdalena

Elisabetta, Giorgini
Ellingsen, Ståle
Elsayed, Randa
Emilsson, Lina
Englert, Christoph
Erdmann, Jeanette
Escribano, Julio
Eski, Sema Elif
Eskova, Anastasia
Facchinello, Nicola
Faisal, Zelma
Falcao, Maria Alice Pimentel
Faustini, Gaia
Favre-Bulle, Itia
Felline, Serena
Fernandez, Joseph
Ferrari, Luca
Ferre-Fernández, Jesús-José
Ferreira, Jorge M
Ferris, Wyndham
Filipek-Gorniok, Beata
Finazzi, Dario
Fiolka, Reto
Fior, Rita
Fitzgerald, Jennifer
Fonseca, Paulo J.
Fornetto, Chiara
Fortier, Gabrielle
Frisk, Jun Mei
Frohnhöfer, Hans-Georg
Fujiwara, Mamoru
Fülöp, Krisztina
Funel, Niccola
Furones Cuadrado, Beatriz
Fürthauer, Maximilian
Gaballah, Shaza
Gabellini, Chiara
Gacquer, David
Gaede, Fjedor
Gaillard, Anne-Laure
Galhardo, Mafalda
Gali-Macedo, Ana
Garai, Edina
Garteizgogeascoa Suner, Ines
Gauert, Anton
Gavaia, Paulo J.
Gay, Stéphanie
Gazsi, Gyöngyi
Gehrig, Jochen
Geisler, Robert
Gellera, Cinzia
Geurtzen, Karina
Ghosh, Marcus
Giacomotto, Jean
Gillotay, Pierre
Gioacchini, Giorgia
Giusti, Arianna
Gloger, Marleen
Gober, Ryan D.
Gomes, Kamila
Goncalves, Claudia
Gontarz, Paul
Gonzalez-Cuevas, Gustavo
Gordienko, Kirill
Gordillo-Martínez, Flora
Goudmaeker, Apolline
Gouveia Jr, Amauri
Grissenberger, Sarah
Gross-Thebing, Sargon
Guarin, Marlly
Gubó, Eduárd
Guck, Jochen
Guglielmi, Luca
Gupta, Mansi
Gursky, Eduard
Gyüre, Zsolt
Habraeken, Nikki
Haerlingen, Benoit
Haghebaert, Marie
Haitina, Tatjana
Hall, Thomas E.
Hamar, Renáta
Hammer, Juliane
Harami, Gábor
Harrington, Kyle
Harris, Matthew
Hartmann, Jonas
Hason, Martina
Hassan-Abdi, Rahma
Hearn, Timothy
Heeren-Hagemann, Anja
Henke, Katrin
Hertafeld, Sydney
Heyne, Ronja
Hill, Caroline
Hinits, Yaniv
Hipolito, Lucas
Hoertenhuber, Matthias
Hoffman, Ellen
Hoffmann, Georg
Hogset, Hakon
Honkoop, Hessel
Hopfenmüller, Vera
Horgan, Conor C.
Houart, Corinne
Howey, Xia Meng
Hoxha, Mirjam
Hsu, Alan
Hsu, Alan Y.
Hu, Bo
Hua, Khang
Hughes, Simon M.
Huisken, Jan
Hüvel, Jan
Huveneers, Stephan
Hykl, Martin
Chan, Hee Lam
Chandrasekaran, Gopalakrishnan
Chatani, Masahiro
Chavez, Myra
Chen, Qu
Chen, Zelin
Chitnis, Ajay
Cho, Jae-Woo
Choi, Eun-Young
Choi, Seok-Yong
Chondrogianni, Niki
Chorley, Brian
Christophe, Armelle
Chun, Hang-Suk
Iannelli, Fabio
Iben, James
Ibrahim, Toni
Imai, Katunori
Inbal, Adi
Ingham, Philip
Ioannou, Efstathia
Iodice, Simona

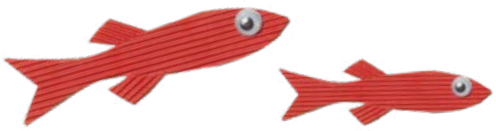
Irion, Uwe
Islam, Tanvir
Iturria, Iñaki
Iványovics, Bence
Izabella, Pena
Jakobs, Gianina
Jamadagni, Priyanka
Janardhana Kurup, Ahshai
Jasnic, Jovana
Jean-Paul, Concordet
Jeffrey, Emma
Jeffries, Jacob
Jensen, Lasse
Jezsó, Bálint
Jimenez Gonzalez, Ada
Jindrich, Jindrich
Johansson, Stefan
Jorge, Sara
Jovičić, Jovana
Junker, Jan Philipp
Jurisch-Yaksi, Nathalie
Jussep, Salgado-Almario
Jussila, Maria
Just, Steffen
Juul Belling, Helene
K. Menyhárd, Dóra
Kaba, Sabine
Kabitskaya, Olga
Kakinuma, Hisaya
Kalyviotis, Konstantinos
Kamminga, Leonie
Kamsteeg, Erik-Jan
Kamstra, Jorke
Kánainé S., Dóra
Karasik, David
Kasher, Paul
Kassa, Betelhem
Kawakami, Atsushi
Kazzazi, Latifa
Keane, Timothy J.
Keatinge, Marcus
Keil, Sebastian
Kerekes, Flora
Kesavan, Gokul
Kettunen, Petronella
Khamseh, Ava
Khokhlov, Demid
Killen, Shaun
Kim, Myeong-Kyu
Kim, Woo-Keun
Kirchmaier, Bettina
Kislyuk, Stanislav
Kit-Yi, Leung
Klingström, Tiffany
Klüver, Nils
Knapen, Dries
Knappskog, Per Morten
Knight, Olivia
Knoll-Gellida, Anja
Knopf, Franziska
Kobayashi, Shunsuke
Kojic, Snezana
Kolar, Michal
Kolb, Julia
Kölker, Stefan
Koltowska, Kaska
Koltowska, Katarzyna
Könemann, Sarah
Kongsted, Marie
Korzh, Vladimir
Kotini, Maria
Kourpa, Aikaterini
Kovács, Mihály
Kovar, Heinrich
Kovář, Martin
Kozár-Risa, Anita
Kremer, Hannie
Kreutz, Reinhold
Krezel, Andrzej
Kriszt, Balázs
Kroll, Francois
Kuan, Yung-Shu
Kula-Alwar, Duvaraka
Kulcsár, Kornélia
Kumari, Pooja
Kundap, Uday
Kutsia, Manana
Kyriakie, Sarafoglou
Labbaf, Zahra
Laizé, Vincent
Lake, Elizabeth
Lalla, Laetitia
Landi, Elena
Lange, Christian
Lange, Pia
Łapiński, Maciej
Laquatra, Claudio
Larondelle, Yvan
Lau, Ieng Hou
Lavró, Viktória
Lea, Robert
Ledin, Johan
Lee, Sangwoo
Lee, So-Hyun
Legler, Juliette
Légrádi, Regina
Leite-Santos, Natalia Vitorino
Lelek, Sara
Lenhard, Boris
Léonard, Marc
Lesage, Manon
Lesche, Matthias
Leuthold, David
Lewicka, Anna
Li, Jennifer
Liang, Ting
Liao, Meijiang
Lim, Sumi
Lima, Carla
Lisa, Schimmenti
Liu, Sheng
Liu, Yuanyuan
Liverani, Chiara
Llopis, Juan
Lo, Harriet
Locatello, Lisa
Lodi, Tiziana
Loeys, Bart
Loncar, Jovica
Lončar, Jovica
Longhena, Francesca
Lopes-Ferreira, Monica
Lopes-Ferreira, Mônica
Lu, Hao
Lucini, Carla
Lukicheva, Nadezhda
Lundegaard, Pia Rengtved
Lupi, Eleonora

Luschnig, Stefan
M. Burgess, Shawn
Mabey, Matthew
Maciąg, Monika
Mackenzie, Åsa
Magri, Stefania
Magyari, Dorottya
Mahé, Fabrice
Machikhin, Alexander
Machoň, Ondřej
Machonova, Olga
Machoňová, Olga
Maier, Paul
Maios, Claudia
Maj, Maciej
Maleski, Adolfo Luis Almeida
Manuel, Vicente
Maradonna, Francesca
Marber, Michael S.
Marcelino, Hugo
Marcolino-Souza, Milena
Marić, Petra
Marie, Bruno
Marini, Silvia
Marín-Juez, Rubén
Marozzi, Anna
Marques, Joana
Martell, Nick
Martin, Patricia
Martinez-Agosto, Julian A.
Martini, Davide
Martins, Gil
Martorano, Laura
Masai, Ichiro
Masin, Luca
Mastrodonato, Valeria
Mata, Leonardo
Mathers, Marie E.
Matis, Maja
Matosevic, Sandro
Mazzola, Mara
McAdow, Ryan
McMahon, Kerrie-Ann
Memo, Maurizio
Méndez Acevedo, Kevin Manuel
Mercader, Nadia
Mercatali, Laura
Merola, Carmine
Messina, Andrea
Metz, Juriaan
Meyer, Martin P.
Meyer, Alexander
Meyer, Stéphanie
Miao, Jiayi
Migliaccio, Enrica
Mignani, Luca
Mignot, Mélanie
Migocka-Patrzałek, Marta
Miguel, Graça
Mihaljević, Ivan
Mikulasova, Tereza
Milosevic, Emilija
Minhas, Rashid
Miserocchi, Giacomo
Misra, Tvisha
Modes, Carl
Moers, Carine
Moiche, Visila
Molica Colella, Erika
Möllmert, Stephanie
Mollo, Ernesto
Monteiro, Joana
Montesanti, Ida E. R.
Montfort, Jerome
Moons, Lieve
Morelli, Luca
Morelli, Luisa
Morgan, Rhiannon
Moro, Enrico
Mosimann, Christian
Mostowy, Serge
Mowry, Bryan
Mueller, Ferenc
Mui, Chun-Long
Müller, Dalma
Muriana, Arantza
Murrakam, Kadir Abdul
Muscò, Alessia
Myren-Svelstad, Sverre
Nagy, Kinga
Naranjo, Silvia
Natesh, Naveen
Nathalie, Jurisch-Yaksi
Nathalie, Lepage
Navalho, João
Nelson, Andrew
Nemoz-Billet, Laurie
Neuhauss, Stephan
Neuhauss, Stephan CF
Nguyen, Mai Thu
Nguyen, Phong
Nguyen, Xuan-Bac
Nicolaï, Johan
Nicolas, Greene
Nieuwenhuize, Susan
Niklaus, Stephanie
Nishiwaki, Yuko
Njegic, Alexandra
Nóbrega, Rafael Henrique
Noël, Emily
Noël, Emily S.
Noor, Suzita Mohd
Norazit, Anwar
Norton, Will
Norton, William
Novkovic, Mirjana
Nüsslein-Volhard, Christiane
Ny, Annelii
O' Driscoll, Mark
O. Vasconcelos, Raquel
Ober, Elke
Obrebski, Tomasz
Ohnesorge, Nils
Okada, Keita
Okamoto, Hitoshi
Okun, Jürgen
Olesen, Morten Salling
Oliver, Griesbeck
Olk, Nadine
Olsson, I Anna S
Oltová, Jana
Onderisin, Chris
Ono, Yosuke
Oorts, Marlies
Oosterhof, Nynke
Ott, Sascha
Otten, Cecile
Özcan, Ismail

Pakdaman, Yasaman
Pal Singh, Sumeet
Panáková, Daniela
Panara, Virginia
Pandard, Pascal
Panella, Gloria
Pantazis, Periklis
Papalopulu, Nancy
Paridaen, Judith
Park, Deung-Dae
Park, Jong-Su
Parker, Alex
Parry-Jones, Adrian
Parton, Robert G.
Patra, Chinmoy
Patten, Kessen
Patten, Kessen (Shunmoogum)
Patton, E. Elizabeth
Peffers, Mandy
Peirce, Judy
Pelicci, Pier Giuseppe
Penc, Mirjam
Peravali, Ravindra
Pereira, Hugo
Perey-Simon, Viktória
Periyasamy, Sathish
Perner, Birgit
Perrod, Chiara
Perugini, Monia
Peskin, Brianna
Peters, Theo
Pézeron, Guillaume
Pezzotta, Alex
Phelan, Marie
Phillips, Jennifer B.
Pierre, Drapeau
Pierre, Vincent
Piferrer, Francesc
Pilichou, Kalliopi
Pimentel-Gutiérrez, Helia
Pistocchi, Anna
Piwocka, Katarzyna
Płazińska, Anita
Plutzer, Judit
Podobnik, Marco
Poetsch, Anna
Poggi, Lucia
Polès, Sophie
Pollina, Luca E.
Pollitt, Eric
Ponting, Chris P.
Poór, Miklós
Popovic, Marta
Popović, Marta
Popp, Hans-Peter
Porok, Klaudia
Poss, Ken
Posset, Roland
Postlethwait, John
Postlethwait, John H.
Poulain, Morgane
Poulsen, Rebecca
Powell, Gareth
Pozo Morales, Macarena
Prevedel, Robert
Préville, Marilou
Priami, Chiara
Probst, Joris
Pronobis, Mira
Prummel, Karin D.
Psutková, Viktorie
Pucci, Cecilia
Puertas, Ana
Rácz, Anita
Radojkovic, Dragica
Raffa, Vittoria
Rajendra Kumar, Amrish
Ramírez, Ana-Isabel
Ranieri, Cecilia
Raphaëlle, Riché
Rasola, Andrea
Rasotto, Maria Berica
Rayrikar, Amey
Raz, Erez
Rebelo De Almeida, Cátia
Reichman-Fried, Michal
Reina, Chiara
Reining, Márta
Renaud, Olivier
Rengtved Lundegaard, Pia
Reuter, Hanna
Ribas, Laia
Ribeiro, Ana
Rihel, Jason
Risato, Giovanni
Rivero-Gil, Solangel
Robert, Kensler
Roberto, Vânia
Robinson, Kate
Robinson, Mark D.
Robson, Drew
Rodenburg, Frans
Rodrigues, Alexandre
Rodrigues, Maira da Silva
Rodriguez-Morales, Roberto
Romitti, Mirian
Rosa, Joana T.
Rosch, Richard E.
Rosso, Gonzalo
Rota, Federica
Rotem-Bamberger, Shahar
Rottbauer, Wolfgang
Roussis, Vassilios
Roy, Sudipto
Ruggiero, Florence
Ruscitto, Federica
Rusinek, Jakub
Ryu, Hyunjin
Sabban, Meyrav
Salazar, Juan-José
Salgado-Almario, Jussep
Salling Olesen, Morten
Salman, Huseyin Enes
Salsano, Ettore
Samanta, Palas
Samarut, Eric
Samarut, Éric
Sampaio, Mariana
Sampath, Karuna
Sampieri, Maria
Sanchez-Iranzo, Hector
Sánchez-Iranzo, Héctor
Sanchez-Posada, Juliana
Santana-Cruz, Alexis
Santos, Bruno
Santos, Tamára
Saraswathy, Vishnu Muraleedharan
Sasse, Ramona

Saúde, Leonor
Saxena, Vishal
Scott, Ethan
Sealy, Ian M
Seni-Silva, Ana Carolina
Sessa, Alessandro
Shankar, Meghna
Sharma, Priyanka
Sheffield, Nathan
Shin, Jimann
Shirokov, Sergey
Shochat Carvalho, Chen
Shu, Xinhua
Schepers, Dorien
Schiano, Valentina
Schneider, Isabelle
Scholpp, Steffen
Scholz, Leandro
Scholz, Stefan
Schuster, Bjoern
Schüttler, Andreas
Schweiger, Nicole
Schweitzer, Christine
Sidoti, Diletta
Sieliwonczyk, Ewa
Singh, Amrita
Singh, Sumeet
Skoczylas, Renae
Skuta, Ctibor
Slijkerman, Ralph
Smital, Tvrtko
Snyders, Dirk
Soares, Amanda Beatriz Silva
Sojan, Jerry Maria
Solis, Juan Carlos
Solnica-Krezel, Lilianna
Somkhit, Julie
Soneson, Charlotte
Song, Jeong-ah
Soriano, Joaquim
Soto, Ximena
Sousa, Pêssi
Soussi-Yanicostas, Nadia
Souza, Wilton Queiroz
Spadazzi, Chiara
Specher, Alessandra
Spreafico, Marco
Stainier, Didier YR
Stamenkovic, Nemanja
Stednitz, Sarah
Stenkamp, Deborah
Stevens, Molly M.
Straehle, Uwe
Stumpel, Constance
Sulej, Agata
Supina, Christine
Surányi, Éva
Svatonova, Petra
Svoboda, Ondrej
Svoboda, Ondřej
Syahirah, Ramizah
Sykiotis, Gerasimos
Szente, Lajos
Tabei, Teruyuki
Takada, Takuya
Takaki, Takashi
Takami, Masamichi
Takeuchi, Yuki
Tal, Tamara
Tanimoto, Yuki
Tantussi, Francesco
Tarantini, Letizia
Tarasco, Marco
Taroni, Franco
Teixeira, Joana
Tenbrinck, Daniel
Terlizzi, Antonio
Thermes, Violette
Thestrup, Ilcim
Thibaut, Morgane
Thiene, Gaetano
Thomas, Manon
Tchivelekete, Gabriel Mbuta
Tiso, Natascia
Torigoe, Makio
Torraca, Vincenzo
Torrado-Tapias, Aranza
Tory, Kálmán
Tostivint, Hervé
Tralle, Eugeniusz
Traver, David
Travnickova, Jana
Treaster, Stephen
Tromp, Alisha
Trubiroha, Achim
Truszkowski, Lukasz
Tsarouchas, Themistoklis
Tsata, Vasiliki
Tsingos, Erika
Tunbak, Hande
Tuschl, Karin
Umulis, David
Urbányi, Béla
Usai, Alice
Vaganay, Elisabeth
Valdivia, Leonardo E
Valdivieso, Alejandro
Valentim, Ana
van den Wijngaard, Arthur
van der Knaap, Marjo
van der Kolk, Kees-Jan
van der Stoel, Miesje M.
van Diepen, Hester
Van Dyck, Annelies
Van Gelderen, Tosca
Van Niel, Guillaume
van Wijk, Erwin
Vandendriessche, Bert
Vanhunsel, Sophie
Vanoevelen, Jo
Vanwalleghem, Gilles
Vanzi, Francesco
Váradi, András
Varela, João
Varela, Tatiana
Varga, Emese
Varga, Máté
Varshney, Gaurav
Vásárhelyi, Erna
Vasconcelos, Raquel O.
Vasile, Enrico
Vassilieva, Galina
Vedder, Viviana
Venero Galanternik, Marina
Venselaar, Hanka
Vergauwen, Lucia
Vértessy, Beáta
Verweij, Frederik

Vettori, Andrea
Vicente Ruiz, Manuel
Viegas, Michael
Volkov, Michail
Vom Berg, Colette
Vondráková, Zuzana
Vos, Bart
Waldmann, Laura
Walker, Cristiani Isabel Banderó
Wallmeyer, Bernhard
Walmsley, Gemma
Walton, Eric
Wan, Jun
Wang, Haitao
Wang, Jiao
Wang, Liya
Wang, Tianqi
Wang, Xu
Wang, Yueyang
Wardle, Fiona
Weber, Isabell
Wegner, Jeremy
Wehmas, Leah
Wehner, Daniel
Weinmann, Karolina
Weinstein, Brant
Welling, Maaike
Wen, Shih-Hsian
Westerfield, Monte
Westerich, Kim
Wevers, Ron A
White, Richard J
Whitmore, David
Wilcockson, Scott
Willaert, Andy
Wilson, Carole
Wilson, Catherine
Wilson, Stephen
Wilson, Stephen W
Winata, Cecilia
Wittamer, Valérie
Wittbrodt, Joachim
Wnorowski, Artur
Wojciechowska, Sonia
Wong, Chee Ern David
Wong, Man Ieng
Wood, Carmen
Wu, Chun-Shiu
Wyatt, Rachael
Xiao, Wu
Xiong, Zherui
Yaksi, Emre
Yalcin, Huseyin Cagatay
Yanicostas, Constantin
Yoon, Seokjoo
Yudowski, Guillermo
Yusuke, Kamachi
Zanardi, Federica
Zang, Jingjing
Zaouter, Charlotte
Zaucker, Andreas
Zehnder, Tobias
Zhang, Hanqing
Zhaoping, Li
Zheng, Susan
Zhou, Siyu
Zhou, Wenqing
Zielonka, Matthias
Zippo, Alessio
Ziros, Panos
Zizioli, Daniela
Zoeller, Joe
Zoltán, Bokor



Zebrafish 2020 Sponsors

DIAMOND SPONSOR

TECNIPLAST
innovation through passion

GOLDEN SPONSORS

AQUANEERING INCORPORATED
Because Your Research Depends On It

PLEXX

IWAKI AQUATIC
SYSTEMS AND SERVICES

EXHIBITORS

ACQUIFER

bionomous

daniolab

Noldus
Information Technology

PLANKTOVIE
Innovation for Aquatic Animal Research

PyRAT
animal facility software
aquatic

ViewPoint
Behavior Technology

ZEBRAFEED
by sparos

ZANTIKS
behaviour, simply

SUPPORTERS

AQUA SCHWARZ GmbH
Made in Germany
aquaculture & lab systems

Trigon plus®