Young Embryologist Network

# YEN 2023

Conference booklet

22nd May 2023

# WELCOME TO YEN 2023!

## VENUE

Francis Crick Institute 1
Midland Rd, NW1 1AT
London

**Details:**
Find out more at
youngembryologists.org

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## ABOUT YEN

The Young Embryologist Network conference 2023 (YEN 2023) that will be hosted in person and online on **Monday the 22nd of May 2023,** at **The Francis Crick Institute** in London is the 15th iteration of the network's successful yearly developmental biology meeting. The event has a broad scope, integral to which is networking and strengthening collaborative work between early-career-stage researchers.

As in previous years, PhD students and junior postdocs will have the opportunity to submit abstracts for the chance to present their research in one of the three short talk sessions, or at one of our two poster sessions. Alongside short talks and posters, we have three confirmed invited speakers: **Dr. Andrea Pauli** (IMP, Vienna), **Prof. Katsuhiko Hayashi** (Osaka & Kyushu University, Japan) and **Dr. Laura Pellegrini** (Lancatser Lab, MRC LMB). As a keynote speaker, we are thrilled to have **Prof. Henrik Kaessmann** (ZMBH, Germany) deliver the "Sammy Lee Memorial Lecture". This keynote address is given in honour of the late Sammy Lee, a visiting Professor in Cell and Developmental Biology at UCL and early proponent of YEN, who passed away in 2012, aged 54.

We are proud to be hosting the first YEN **'Perspectives on Equality Diversity and Inclusion (EDI) in Science'** panel discussion with a focus on socioeconomic and intersectional diversity. The scientific research sector is predominantly populated with people from higher socioeconomic backgrounds, and there are still significant barriers to pursuing a career in the life sciences for people from lower socio-economic backgrounds. Our communities and particularly science and research could be much better equipped in ensuring equal opportunities and access for people from all backgrounds. We are therefore inviting **Alison Forbes**, Head of EDI, The Francis Crick Institute and **Dr. Rafael Galupa**, Scientist at CBI France and Social Entrepreneur with substantial work on EDI to spotlight their knowledge on this underdiscussed topic, in the hope that this will push the next generation of leaders to shape more inclusive communities.

In conclusion, our line-up of keynote and invited speakers will showcase outstanding science. The event will also provide an excellent networking opportunity for early career scientists. Furthermore, with our perspective session, we are hoping that our platform will better serve the community by bringing attention to important minority groups within it and across the scientific and social spectrum.

YEN 2023 will be a hybrid event to ensure inclusivity and maximise the reach of the conference whilst reducing our carbon footprint. As always, YEN 2023 Conference will be free-of-charge to attendees and will take place at The Francis Crick Institute, London.

## ACKNOWLEDGEMENTS

We would like to thank all the sponsors, speakers, talk and poster presenters, judges, Karen Lee and guests, attendees and people involved in the organisation of the YEN conference.

**Committee**
Jeremie Subrini, Foteini Papaleonidopoulou

Chantelle Spiteri, Christos Kalaitzis, Cláudia Belém, Ferran Garcia Llagostera Irina Ballaguer Basells, Jack Morgan, Jesus Cantoral Rebordinos, Luca Zanieri, Matyas Bubna-Litic, Michelle Neumann, Mint Htun, Oliver Bower, Olivia Dinwoodie, Ollie Inge, Sergio Menchero

**Judges**
Karen Lee, Dr Giulia Boezio, Dr Scott Wilcockson, Dr Aurélien Courtois and Dr Ignacio Rodriguez.

**Sponsors**
Hosting YEN each year would not be possible without the generosity of our sponsors and supporters. We kindly thank the following societies and institutions: *The Company of Biologists, REGEN, Imperial College London, The Crick Partner Networking Fund, The Genetics Society, The Biochemical Society and The Anatomical Society.*

We also thank our industrial sponsors: *Promega, Bio-Techne and New England BioLabs.*

**We encourage all attendees to visit the associated stands during the refreshment breaks.**

# YEN Conference – Programme

22nd May 2023 | Francis Crick Institute

**Zoom link:** https://crick.zoom.us/j/66176942898?pwd=VmRHd0cvbExHR2J3YnZhVUxIeWRGZz09
Passcode: 161617

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|---|---|
| 8:15 | REGISTRATION |
| 9:00 | WELCOME ADDRESS |
| 9:15 | **Prof Katsuhiko Hayashi** (Osaka University, Japan)<br>TBD (On-line) |
| 9:45 | SELECTED TALKS 1 **(15 min)**<br>**Clara Munger** (University of Cambridge, UK)<br>Microgel culture and spatial identity mapping elucidate the signalling requirements for primate epiblast and amnion formation<br>**Dr Johanna Gassler** (Max-Planck-Institute for Biochemistry, Germany)<br>Orphan nuclear receptors are required for major zygotic genome activation in murine embryos<br>**Dr Ashley Libby** (The Francis Crick Institute, UK)<br>Examining progenitor population dynamics and gene regulatory networks from the caudal epiblast to the neural tube<br>**Dr Lessly Sepulveda-Rincon** (Imperial College London, UK)<br>Interrogating the potency of primordial germ cells by injection into early mouse embryos |

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| 10:45 | **BREAK**<br>Poster session 1 (even numbers only) |
| 11:30 | **SELECTED TALKS 2 (15 min)**<br>**Chloe He** (University College London, UK)<br>Non-invasive 3D Imaging of Cell Arrangement in Preimplantation Human Embryos<br>**Dr Michael Emmerson** (Queen Mary University of London, UK)<br>Parent heat call exposure downregulates transcript expression of protein catabolising genes in the liver of embryonic zebra finches<br>**Gloria Jansen** (University of Cambridge, UK)<br>Dissecting DNA damage tolerance during Drosophila germline development |
| 12:15 | **SCIENTIFIC PERSPECTIVES 1**<br>**Alison Forbes**, Head of EDI, The Francis Crick Institute, UK<br>On Equality, Diversity and Inclusion |
| 12:30 | **Dr Andrea Pauli** (Institute of Molecular Pathology, Austria)<br>Fundamental principles during the egg-to-embryo transition |
| 13:00 | **LUNCH BREAK** |
| 13:45 | **SELECTED TALKS 3 (15 min)**<br>**Dr Allan Carrillo-Baltodano** (Queen Mary University of London, UK)<br>Ancestral synergy of TGF-beta and MAPK during dorsal-ventral specification in Bilateria<br>**Agatha Ribeiro da Silva** (Max Planck Institute for Heart and Lung Research, Germany) |

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| | Egr3 promotes endothelial cell migration during atrioventricular valve formation |
| | **Zukai Liu** (The Jackson Laboratory for Genomic Medicine, US)<br>The evolution of extra-embryonic mesenchymal cells: Origin, mechanism and function |
| 14:30 | **Dr Laura Pellegrini** (Lancaster lab, LMB Cambridge, UK)<br>Temporal development and maturation of choroid plexus organoids |
| 15:00 | SCIENTIFIC PERSPECTIVES 2<br>**Dr Rafael Galupa**, Scientist (CBI France) & Social Entrepreneur<br>Is science only for the rich? Considering socioeconomic background in science |
| 15:15 | BREAK<br>Poster session 2 (odd numbers only) |
| 16:00 | **Prof Henrik Kaessmann** (ZMBH Germany)<br>**Sammy Lee Keynote Lecture**<br>Origins and functional evolution of amniote sex chromosomes |
| 17:00 | PERSPECTIVES: PANEL DISCUSSION<br>Equality, Diversity and Inclusion in Science with **Alison Forbes** and **Dr. Rafael Galupa** |
| 17:30 | PRIZES AND CLOSING ADDRESS |
| 18:00 | DRINKS RECEPTION AND NETWORKING |

# SELECTED TALKS ABSTRACTS

Sorted alphabetically:

**Egr3 promotes endothelial cell migration during atrioventricular valve formation**
By Agatha Ribeiro da Silva (Max Planck Institute for Heart and Lung Research)

Atrioventricular (AV) valves are essential for heart function as they guarantee unidirectional blood flow by opening and closing the AV canal. Their morphogenesis is triggered by high shear forces in the AV canal that activate the AV promoting transcriptional program and signaling pathways to guide cell migration, endothelial-to-mesenchymal transition, and proliferation. Here, we identify the transcription factor Egr3 as a master regulator of AV valve formation in zebrafish, downstream of mechanical forces. Using live confocal imaging, we find that egr3 mutants display AV cell migration defects, resulting in the loss of valve leaflets that leads to severe blood regurgitation and consequent cardiac failure. We show that egr3 is expressed in AV canal cells and that its depletion in endothelial cells is sufficient to recapitulate the egr3 mutant phenotype. Furthermore, we identify the nuclear receptor Nr4a2b and the ECM protein Spp1/Osteopontin as effectors of Egr3 function in AV valve formation. Altogether, our findings reveal that Egr3, through the action of Nr4a2b and Spp1, is essential for AV cell migration and valve formation, thereby uncovering new candidate genes for the investigation and treatment of valve-related congenital heart diseases.

**Ancestral synergy of TGF-beta and MAPK during dorsal-ventral specification in Bilateria**
By Allan Carrillo-Baltodano (Queen Mary University of London)

Animals with bilateral symmetry (i.e., Bilateria) show the highest diversification of body plans. Bilateral symmetry is stablished early in the embryo, when ancestral cell signalling pathways (e.g. TGF-beta, MAPK, Wnt) work together to set up the initial body axes. However, the roles of MAPK and TGF-beta signalling remain elusive in Spiralia, one of the three major clades within Bilateria. Here, we use the spiralian annelid Owenia fusiformis to reveal an interplay between MAPK and TGF-beta (e.g. BMP, nodal/activin) to establish dorsoposterior fates. Blocking MAPK results in the loss of dorsoposterior tissue (ventralization), while blocking TGF-beta reduces the dorsoposterior tissue, but maintaining the bilateral symmetry. On the contrary, overactivating TGF-beta results in the specification of extra dorsoposterior tissue (dorsalization). Moreover, overactivation of TGF-beta in conjunction with inhibition of MAPK does not rescue the dorsoposterior tissue, proving that MAPK is upstream of the effect of TGF-beta in dorsoposterior specification. Transcriptomic analyses between the different loss and gain of function experiments enable us to elucidate a gene regulatory network that could be responsible for specifying dorsal-ventral (DV) axis. Our results in combination with those from certain molluscan lineages (Patellogastropods) support these pathways are ancestral mechanisms for dorsal-ventral (DV) axis specification in Spiralia, while the solely use of nodal/activin for DV specification has evolved independently in some lineages of annelids.

**Examining progenitor population dynamics and gene regulatory networks from the caudal epiblast to the neural tube**
By Ashley Libby (The Francis Crick Institute)

Embryonic development requires the coordinated emergence of multiple cell populations that form the basis of future organs. To generate the spinal cord, axial progenitors located in the caudal epiblast (CE) move to the preneural tube and then continue to stratify into the progenitor domains of the neural tube. Despite an overall morphogenic understanding of this process, the molecular mechanisms that regulate the specification of cell identity and coordinate the transitions between the CE, preneural tube, and

neural tube remain ill-defined. Here we investigate this process by pairing single-cell imaging and genetic techniques. In ovo, multiphoton imaging of chick embryos revealed a gradient of decreasing lateral cell movement as progenitors progress from the CE to the neural tube. Cellular neighbourhoods showed region specific progenitor movement dynamics suggesting regional movement variations as progenitors become more defined in fate. A single-cell transcriptome dataset identified genes associated with fate transitions have an abundance of genes associated with tissue architecture and cell surface receptors: CLDN1, CMTM8, F2RL1, FGFR1. To coordinate changes in architecture and examine progenitor genetic plasticity an in vivo CRISPR screen was conducted to test the function of selected genes. Several genes were identified to modulate the proportions of cell fates within the preneural tube: CMTM8, MLLT3, and TBXT. With these data we aim to pinpoint key regulators of progenitor specification and movement in the CE that affect the emergence of the stereotypical stratified progenitor domains within the neural tube.

**Non-invasive 3D Imaging of Cell Arrangement in Preimplantation Human Embryos**
By Chloe He (UCL)

Cell arrangement plays a crucial role in early embryonic development, influencing cell signalling, polarity, and early cell fate decisions in the preimplantation human embryo. However, studying these aspects in clinical human embryos is challenging due to the cost and safety concerns associated with traditional imaging methods such as confocal microscopy. To address these issues, we introduce a novel, non-invasive 3D imaging system based on the Hoffman modulation contrast microscopes commonly used in IVF clinics. Our system uses deep learning to detect and reconstruct cells from focal stacks of unstained cleavage stage embryos. We demonstrate the utility of our system in a clinical setting, using it to investigate networks of cell contact in embryos from IVF cycles. Our findings replicate previous studies showing that increased intercellular contact is linked to greater blastocyst formation and quality in 4-cell human embryos. In addition, we present new insights on 8-cell embryos, providing evidence that higher levels of intercellular contact are associated with greater blastocyst formation and quality, as well as increased rates of pregnancy and live birth. These preliminary findings highlight the importance of cell arrangement and intercellular contact in early embryonic development and suggest that our imaging system may be a valuable tool for understanding preimplantation embryo development.

**Microgel culture and spatial identity mapping elucidate the signalling requirements for primate epiblast and amnion formation**
By Clara Munger (University of Cambridge)

The early specification and rapid growth of extraembryonic membranes are distinctive hallmarks of primate embryogenesis. These complex tasks are resolved through an intricate combination of signals controlling the induction of extraembryonic lineages and, at the same time, safeguarding the pluripotent epiblast. Here, we delineate the signals orchestrating primate epiblast and amnion identity. We encapsulated marmoset pluripotent stem cells into agarose microgels and identified culture conditions for the development of epiblast- and amnion-spheroids. Spatial identity mapping authenticated spheroids generated in vitro by comparison with marmoset embryos in vivo. We leveraged the microgel system to functionally interrogate the signalling environment of the post-implantation primate embryo. Single-cell profiling of the resulting spheroids demonstrated that activin/nodal signalling is required for embryonic lineage identity. BMP4 promoted amnion formation and maturation, which was counteracted by FGF signalling. Our combination of microgel culture, single-cell profiling and spatial identity mapping provides a powerful approach to decipher the essential cues for embryonic and extraembryonic lineage formation in primate embryogenesis.

**Dissecting DNA damage tolerance during Drosophila germline development**

By Gloria Jansen (Cambridge)

Transposable elements (TEs) harbour a selfish drive to increase in copy number in host genomes. Due to its role in inheritance, TEs use the germline as a platform to maximize their influence over genetic information passed between generations. Regardless of the mechanism used by TEs, this process inflicts damage on the genome and may impair the functionality and viability of germ cells. A textbook example of TE-induced damage is P-element hybrid dysgenesis, a syndrome characterized by germ cell loss during development leading to animal sterility. We used this system to understand how germ cells sense and respond to DNA damage. Our analyses identified cell cycle arrest as the earliest germ cell response to accumulating DNA double strand breaks (DSBs) resulting from P-element activity. We further established a CRISPR-Cas9 system to mimic DSBs caused by P-element activity in the germline. In the presence of P-elements but absence of endogenous P-element activity, Cas9-induced DSBs were sufficient to induce germ cell death during development and adult sterility at the same, full penetrance observed in dysgenesis. We then leveraged further on the Cas9 system to demarcate the DNA damage tolerance threshold of germ cells. Our analysis indicated that in contrast to somatic cells, few DSBs are sufficient to induce germ cell death and full adult sterility. Our work highlights the importance of cellular responses to DNA damage in driving decisions to repair or die in the germline.

**Orphan nuclear receptors are required for major zygotic genome activation in murine embryos**
By Johanna Gassler (Max-Planck-Institute for Biochemistry)

The formation of the totipotent embryo is initiated by the fusion of two terminally differentiated germ cells, the egg and the sperm. This transition from oocyte to embryo is characterized by a switch from maternal to zygotic control, which occurs through the activation of the initially silent embryonic genome during zygotic genome activation (ZGA). Despite its importance, the key regulators of ZGA in mammals remain largely unknown. Upon investigation of upstream cis-regulatory regions of major ZGA genes, we identified a motif array that has 90% sequence identity with a subtype of SINE B1/Alu transposable elements. One motif in the array is a putative binding site of the orphan nuclear receptor Nr5a2, implying that it might have a function in regulating ZGA. Indeed, perturbation of Nr5a2 by knockdown, protein degradation or chemical inhibition revealed Nr5a2, and potentially other orphan nuclear receptors, are the main activators of up to 72% of major ZGA genes in mouse embryos. Moreover, they are required for timely development beyond the two-cell stage, highlighting their importance in this process. Further biochemical characterization revealed that Nr5a2 acts as a pioneer factor, promoting chromatin accessibility during ZGA and binding to entry/exit sites of nucleosomal DNA in vitro. Taken together, we identified novel regulators with critical functions in early development and zygotic genome activation in the mouse embryo.

**Interrogating the potency of primordial germ cells by injection into early mouse embryos**
By Lessly Sepulveda-Rincon (Imperial College London)

Primordial germ cells (PGCs) are the earliest precursors of gametes. During normal development PGCs only give rise to oocytes or spermatozoa. However, PGCs can acquire pluripotency in vitro by forming embryonic germ cells and in vivo during teratocarcinogenesis. Classic embryological experiments directly assessed the potency of PGCs by injection into the pre-implantation embryo. As no contribution to embryos or adult mice was observed, PGCs have been described as unipotent. Here we demonstrate that PGCs injected into 8-cell embryos can initially survive, divide and contribute to the developing inner cell mass. Apoptosis-deficient PGCs exhibit improved survival in isolated epiblsts, and can form naive pluripotent embryonic stem cell lines. However, contribution to the post-implantation embryo is limited, with no functional incorporation observed. In contrast, PGC-like cells show extensive contribution to mid-gestation chimaeras. We thus propose that PGC formation in vivo establishes a latent form of pluripotency that prevents chimaera contribution.

**Parent heat call exposure downregulates transcript expression of protein catabolising genes in the liver of embryonic zebra finches**
By Michael Emmerson (Queen Mary University of London)

Responsiveness to sensory stimuli emerges during embryonic development, with embryos in many birds becoming responsive to sound. Parent vocalisations shape both immediate and later-life avian phenotypes, with evidence emerging for adaptive phenotypic calibration by some vocalisations. In zebra finches, for example, embryo exposure to parent heat vocalisations (produced at ambient temperatures above 26°C) results in lower post-hatch growth rates with increasing temperature compared to control vocalisation exposure. Such a growth trajectory is associated with higher reproductive success, suggesting adaptive calibration of development by heat calls. Lower expression of metabolism-linked genes in tissues regulating metabolism may underpin this, but whether sounds shape gene expression outside the brain is unknown. Livers function to store glycogen and influence carbohydrate metabolism, so this study tested if heat calls downregulate metabolism regulating gene expression in embryonic liver. Zebra finch eggs (n = 25) were exposed to parent heat or control vocalisations during incubation, and one day before hatching livers were taken for genome transcript abundance quantification via QuantSeq. Transcript expression of 15431 genes were detected, with 8 lower in heat versus control call exposure. Over-representation analysis and predicted protein interaction networks show the genes were disproportionately present in functionally connected protein catabolism gene sets. Heat calls may therefore bias birds toward post-hatch growth associated with greater reproductive success by downregulating a network of protein catabolising genes in the embryo liver. Our results are the first to demonstrate sounds shape the embryonic liver transcriptome and highlight the importance of emerging sensory systems to embryo phenotype construction.

**The evolution of extra-embryonic mesenchymal cells: Origin, mechanism and function**
By Zukai Liu (The Jackson Laboratory for Genomic Medicine, US)

Mammalian early development differs significantly between species, the origins of extra-embryonic mesenchyme (ExMC) is a prime example. Derived from definitive mesoderm in mouse, the cellular origins of human ExMC remain unknown. In a human induced pluripotent stem cell model, we report that ExMC initially arises from the trophoblast. We define HAND1 as an essential regulator of ExMC specification, with null cells remaining in the trophoblast lineage. Through analysis of bound genomic loci and primary target genes of HAND1, we reveal ape-specific, endogenous retrovirus-derived LTR2B contributes to unique features of this cell lineage. In addition, we show the ExMC provides a microenvironmental niche to support pluripotent stem cell maintenance, perhaps reflecting its role in maintaining epiblast pluripotency through peri-implantation development. Our data emphasize the nascent evolutionary innovation in human early development, provides a cellular system to study this, and offers an efficient model to study genetic variants associated with connective tissue disorders.

## POSTER ABSTRACTS

Sorted alphabetically:

**Poster 1. Heterogeneity in the timing of embryo polarization biases the first lineage specification in mice**

By Adiyant Lamba (University of Cambridge)

The first lineage allocation of the mouse embryo separates embryonic inner cell mass (ICM) from extra-embryonic trophectoderm (TE). Segregation of these lineages is classically attributed to polarization of the late 8-cell stage embryo - the process by which each cell gains an apical domain - as well as subsequent cell divisions, in which the apical domain is differentially inherited. After division, those cells which remain polarized are specified as TE, whilst apolar cells are specified as ICM. Recent evidence has shown that heterogeneities between blastomeres at the 4-cell stage also bias first lineage allocation, but the mechanisms linking polarization and early heterogeneities have remained unclear. Through live imaging, we show that heterogeneity in the timing of polarization exists at the 8-cell stage, with a proportion of cells polarizing early. These 'early polarizing' cells follow the canonical polarization pathway, although they have distinct cellular properties such as a shorter apical-nucleus distance. Importantly, lineage tracing has shown that early polarizing cells are biased towards TE fate. This is consistent with the fact that early polarizing cells have a wider geometry and higher expression of the TE fate specifier Cdx2. Moreover, inhibition of the arginine methyltransferase CARM1, whose heterogeneous activity at the 4-cell stage influences cell fate, increases the frequency of early polarization, as does overexpression of its downstream substrate BAF155. We infer that early heterogeneities influence cell fate by altering the timing of polarization. Our findings have exciting implications for embryos of other mammals, including humans, with much more heterogeneous polarization timing.

**Poster 2. The role of non-muscular myosin II in mesodermal collective cell migration in the zebrafish embryo**
By Amélie ELOUIN (Laboratory for Optics & Biosciences, Ecole Polytechnique, Route de Saclay, 91120 Palaiseau, FRANCE)

Collective cell migration is crucial for many developmental processes. It is also involved in pathological situations such as cancer cell invasion. During zebrafish gastrulation, extension of the embryonic axis is led by the mesendodermal polster that migrates towards the animal pole. Years ago, we established that migration of the polster is a collective process as each cell requires contact with neighbours to orient its migration in the right direction. More recently, we established that each cell is guided by its immediate followers, and that this guidance is relying on mechanotransduction through α-catenin. We proposed a model in which each migrating cell exerts tension on the cell in front via its protrusion. This tension is perceived through α-catenin and used as a directional information. How tension is created at cell-cell contacts is still unknown. I investigate the potential role of non-muscular myosin II (NMMII). Using a transgenic line expressing a fluorescent myosin II, I observed that NMMII is located both at the cortex with a posterior bias and at the basis of protrusions in polster cells. Using a dominant-negative form of myosin light chain kinase, advanced cell transplantations, 4D time-lapse and laser ablations, I obtained results showing a non-autonomous role of NMMII in orienting polster cells and an autnomous role of NMMII regarding protrusion contractility. These results are consistent with the idea that one cell orients the cell in front by pulling on it, and make myosin II a good candidate for generating the pulling force.

**Poster 3. Cfl1-dependent Cytoskeletal Remodelling in Cranial Morphogenesis**
By Andrea Krstevski (ICH GOSH, UCL)

Cofilin (Cfl1) is an actin regulatory protein that severs actin filaments, thereby depolymerizing F-actin. F-actin depolymerisation and turnover are required for cell migration and Cfl1 activity facilitates migration of cell lineages arising from the neural ectoderm as well as the paraxial mesoderm1. Global loss of Cfl1 in mice has been shown to cause failure of cranial neural tube closure (exencephaly) and early lethality1. We employed Mesp1Cre deletion of Cfl1 to test its involvement in migration of anterior

mesoderm, including the progenitors of the cranial and cardiac mesoderm. Homozygous conditional deletion of Cfl1 results in pericardial oedema in 39% of embryos by embryonic day (E)9.5. At this timepoint, conditional Cfl1 deletion reduces head size, without impacting overall body size. Mesp1Cre lineage-traced cranial mesoderm expands rostrally and dorsally to encompass the midbrain shortly following completion of neural tube closure. Dorsal mesoderm expansion is achieved despite conditional Cfl1 deletion. However, quantitative image analysis shows loss of Cfl1 favours longer filopodia-like protrusions in migrating cells at the leading edge of the dorsal cranial mesoderm at E9.5. By E10.5, orthogonal sections show conditional loss of Cfl1 diminishes cranial mesodermal confluence, producing apparent gaps within the normally homogenous mesoderm layer between the neuroepithelium and surface ectoderm. Thus, Cfl1 promotes coherence of migrating cranial mesoderm cells at early stages of cranial expansion.

Reference
1.Gurniak, Christine B et al. "The actin depolymerizing factor n-cofilin is essential for neural tube morphogenesis and neural crest cell migration." Developmental biology vol. 278,1 (2005): 231-41.

**Poster 4. Cellular regulation of pacemaker activity in zebrafish**
By Annika Nürnberger (Max Planck Institute for Heart and Lung Research)

Located at the cardiac sinoatrial region, the primary pacemaker rhythmically depolarizes the surrounding working myocardium to initiate the heartbeat. Malfunction of this complex machinery results in cardiac defects like arrhythmias, potentially leading to heart failure. Here, we aim to uncover the formation and functional role of cells interacting with the pacemaker. Focusing on pacemaker insulation and innervation, we investigate fibroblast insulation and neuronal innervation. Using live 3D confocal imaging in zebrafish embryos as early as 48 hours post fertilization we discovered a cluster of epicardial-derived cells (EPDC) that is localized at the pacemaker region and follows the moving pacemaker over time. Since EPDCs can differentiate into fibroblasts, we hypothesize that this cluster will form the pacemaker insulation once differentiated. In terms of innervation, we could identify axons extending to the heart at 6 days post fertilization by tracking parasympathetic gene expression of choline O-acetyltransferase a. Intriguingly, the EPDCs and axons co-localize, suggesting a potential interaction between these cell types at the pacemaker region. Sympathetic marker gene expression of tyrosine hydroxylase on the other hand was first visible later in development. Altogether, these data confirm the presence of EPDCs as well as axons in proximity to the zebrafish pacemaker in early development. We aim to strengthen these observations by identifying the role of these cell types during pacemaker development using transcriptomic and functional studies.

**Poster 5. Neurocrestoids on-a-chip: Modeling human neural crest development in 3D using a bioengineered device**
By Carmen Moreno Gonzalez (The Francis Crick Institute)

The neural crest is a transient embryonic cell population that arises in the dorsal neural tube during neural system development and contributes to multiple tissues in an axial position-dependent manner. The differentiation potential of neural crest cells is dependent on their axial identity, time of delamination, and environmental cues during migration. Neural crest development is a highly complex process involving spatiotemporal regulation of mechanobiological forces and signaling gradients originating from the surrounding ectoderm and underlying mesoderm, which remain largely unknown in humans. Due to the complexity and transient nature of neural crest cells, it is highly challenging to model in vitro, necessitating the development of novel complex humanized models. While recent advances in hPSC differentiation protocols have demonstrated the efficient generation of neural crest cells in 2D, these models lack cellular and structural complexity and are unable to recapitulate the

changes in mechanical and durotactic cues that occur during neural crest development and are integral to the differentiation potential of these cells. In this project, we aim to create a high-throughput platform combining hPSCs, advanced 3D cultures and microfabrication to study early human neural crest development in health and in disease. We have described a novel pipeline to generating neurocrestoids on-a-chip: a bioengineered platform featuring 3D neuroepithelial organoids capable of producing and guide migration of hiPSC-derived neural crest cells.

**Poster 6. Mechanochemical feedback loops regulate endocardial cell volume in zebrafish early heart valve development**
By Christina Vagena-Pantoula (Imperial College London)

Heart valve morphogenesis requires endocardial cell rearrangements coordinated by intricate mechanochemical feedback loops. Endocardial cell volume decrease is essential for zebrafish heart valve formation and depends on blood flow-generated mechanical forces. However, the cellular and molecular mechanisms underlying endocardial cell volume changes remain unknown. Here, we demonstrate that the interplay of piezo mechanosensitive ion channels, the genetic factor notch1b and aquaporin (aqp) water channels controls endocardial cell volume reduction. We show that aqp8a.1 expression is regulated by notch signalling. Moreover, the overexpression of piezo1, using the genetically encoded piezo1 reporter GenEPi, leads to downregulation of notch1b and subsequently aqp8a.1, resulting in increased endocardial cell volume. Aqp8a.1 mutant larvae display heart valve defects, altered cell polarity and absence of F-actin remodelling, required for endocardial cell volume regulation. Altogether, this work identifies new modulators of endocardial cell volume changes and heart morphogenesis, thereby increasing our fundamental knowledge on early organogenesis.

**Poster 7. Characterising a novel contractile cytoskeleton in epithelial morphogenesis**
By Courtney Lancaster (University College London, Lab for Molecular Cell Biology)

During development, tissues undergo shape changes to reach their final form. Such morphogenetic changes include tissue elongation, bending, and folding, which are vital for generating functional organs. Tissue remodelling can occur through multiple processes such as cell division, cell movement, extracellular matrix regulation, and cell shape changes. My work aims to understand how cells coordinate shape changes to induce 3D tissue organisation. Epithelia have two distinct surfaces, apical (top) and basal (bottom). Although we have a good understanding of how cells remodel their apical geometry, by creating or shrinking apical-lateral adherens junctions, our knowledge of the mechanisms that induce basal geometry remodelling is limited.
At the basal surface, cells do not share adherens junctions. Instead, they are attached to a specialised extracellular matrix (ECM) called the basement membrane (BM). Therefore, the mechanisms of apical and basal cell shape changes are likely to be different, requiring different forces and modes of coordination.
I am using the genetically tractable Drosophila retina to investigate the process of cell basal constriction which is essential for shaping the compound eye. I have found that F-actin organisation at the basal surface of retinal cells requires muscle related actin regulators. This includes tropomyosin which is well known for its role in muscle contraction. I hypothesise that basal constriction requires a robust contractile cytoskeleton to counteract basal attachment to the BM. Consistent with this idea, my work shows that basal surface contraction is accompanied by BM remodelling. Altogether, my work demonstrates that there is an unappreciated cytoskeletal machinery required for basal surface actin organisation which also coincides with BM remodelling.

**Poster 8. Integrated cellular, molecular, and mathematical investigation of the evolution of early telencephalon organisation**
By Dana Fakhreddine (King's College London/Francis Crick Institute)

Forebrain evolution has led to the emergence of a rich variety of behaviours, abilities, and cognitive functions across vertebrate species. Contrasting with this variability, forebrain developmental processes are mostly conserved. In the developing telencephalon , the anterior-most part of the forebrain, Shh and Wnt/BMP signals produced at opposing ends of the dorsoventral axis induce the expression of region-specific transcription factors that demarcate its subpallial and pallial progenitor zones. The spatial organisation of these progenitor populations shapes the distinct adult forebrain compartments. While signalling processes are conserved, spatiotemporal modulation of signals and their downstream effectors are thought to drive the evolution of brain complexity. Yet, the molecular mechanisms controlling this modulation remain poorly understood. We aim to identify the molecular mechanisms underlying early telencephalon patterning and the cross-species differences that explain its evolutionary diversity. To achieve this, we quantify the dynamics of morphogen signalling activity along the dorsoventral axis in human, mouse, chick, zebrafish, and shark telencephalon by performing high-resolution in-situ staining using hybridisation chain reaction on whole embryonic heads at different developmental stages. We use the 3D-rendered images to quantify the concentration gradients of morphogens and the temporal change in shape and size of the telencephalic regions affected by them. We also analyse single-cell transcriptomic data to capture the dynamics of gene expression and infer gene regulatory networks at play during telencephalon development. We plan to use data generated from imaging and scRNAseq to construct a mathematical model of telencephalic spatiotemporal organisation that elucidates differences in telencephalon patterning across species.

**Poster 9. Fate mapping the hindgut endoderm during mouse axial elongation**
By Danielle Liptrot (UCL)

Development of the caudal embryonic region takes place in a rostral-caudal fashion through the coordinated effort of axial progenitors. Neuromesodermal progenitors (NMP) are a bipotent cell population residing in the mouse tailbud at E9.5, and giving rise to the caudal trunk mesoderm and neural tube. Following gastrulation, However, we and others find that dorsal hindgut cells and the NMP-derived neural tube and paraxial mesoderm have overlapping genetic lineage profiles, as seen through use of Sox2, Brachury and Cdx2 to drive Cre recombinase. It has been hypothesised that the hindgut lineage is derived from a separate, pre-specified progenitor population established during gastrulation. However, the contributions of progenitor populations to the hindgut are yet to be fully delineated. Using Cre-based lineage tracing and lipophilic dye microinjections, we are fate mapping gut endoderm progenitors from the hindgut invagination at E8.5 to the caudal hindgut at E9.5. These results currently support the existence of two distinct progenitor regions, both rostrally and caudally located, for the hindgut.

**Poster 10. Modelling human Purkinje cell development using iPSC-derived cerebellar organoids**
By Elizabeth Apsley (University of Oxford)

The cerebellum is a critical brain region for coordination of motor function, as well as contributing to a range of higher cognitive functions, including language. Our understanding of cerebellar development largely comes from animal models. However, recent studies have highlighted human specific features of cerebellar development and demonstrated the need for human models. Differentiation of induced-pluripotent stem cells (iPSCs) into cerebellar organoids provides an accessible model for studying early stages of human cerebellar development. However, there are still limitations, including the relative immaturity of neurons produced and difficulty tracking cell populations.

To address these challenges, we generated a FOXP2-P2A-mNeonGreen iPSC line to monitor Purkinje cells in cerebellar organoids. Forkhead transcription factor FOXP2, is widely expressed in Purkinje cells from early in cerebellar development, making it a useful marker for the emerging Purkinje cell

population. The FOXP2-P2A-mNeonGreen reporter line enabled live and accurate visualisation of endogenous FOXP2 expression. Moreover, it facilitated screening of conditions to improve Purkinje cell production.

In addition, the FOXP2-P2A-mNeonGreen reporter line provided a tool to study the role of FOXP2 in early human Purkinje cells. Despite being one of the first genes associated with speech and language development to be identified, the function of FOXP2 is not yet fully understood. To characterise the FOXP2 expressing cells in cerebellar organoids, we isolated mNeon positive and negative populations and conducted RNA-sequencing. Transcriptomic analysis confirmed the enrichment of FOXP2 in the mNeon+ population, which also showed expression of other Purkinje cell marker genes.

**Poster 11. Exploring CHD7 and SEMA interactions to identify novel pharmacological targets for the treatment of CHARGE syndrome**
By Federica Amoruso (University of Milan, Italy)

CHARGE syndrome (CS) is a rare disease characterized by several neuronal dysfunctions, for which no pharmacological treatments are available. Most CS patients carry mutations in CHD7 gene, which encodes for a chromatin remodeler. CS high phenotypic variability could be explained by mutations in genes with an epistatic relationship to CHD7, that may alter the expressivity of disease traits. Interestingly, recent evidence revealed that genes belonging to the semaphorin (SEMA) family might be good candidates as CHD7 genetic interactors. Thus, we combined in vivo and in vitro CS models to explore the genetic interaction among these genes and to identify novel pharmacological targets for CS treatment. In vivo, several developmental phenotypes of Caenorhabditis elegans chd-7 deletion mutant were evaluated to set-up a drug screening, that preliminary revealed compounds able to rescue the aberrant reproductive capacity of mutant worms. Candidate hits will be tested to identify molecules able to restore physiological levels of Sema-genes expression, altered in mutants. In parallel, as in vitro model of CS, nine Chd7 gene knock-out (KO) neuronal cell clones were generated applying CRISPR/Cas9 technique. Preliminary analyses revealed a significant reduction of CHD7 protein levels, viability, proliferation, and G1 cell cycle phase arrest for clone 2, 4 and 51. We next plan to perform gene expression studies on KO clones, as well as to test promising compounds identified in vivo for their ability to rescue CHD7 deficiency also in vitro. This knowledge will highlight molecules potentially able to target deregulated downstream genes in CS patients, by-passing CHD7 mutations.

**Poster 12. Telling the time inside the embryo: how epigenetic timing explains animal life cycle evolution**
By Francisco Manuel Martin Zamora (Queen Mary University of London)

Indirect development with an intermediate larva exists in all major animal lineages, which makes larvae central to most scenarios of animal evolution. Yet how larvae evolved remains disputed. Here we show that temporal shifts (that is, heterochronies) in trunk formation underpin the diversification of larvae and bilaterian life cycles. We performed chromosome-scale genome sequencing in the annelid Owenia fusiformis with transcriptomic and epigenomic profiling during the life cycles of this and two other annelids. We found that trunk development is deferred to pre-metamorphic stages in the feeding larva of O. fusiformis but starts after gastrulation in the non-feeding larva with gradual metamorphosis of Capitella teleta and the direct developing embryo of Dimorphilus gyrociliatus. Accordingly, the embryos of O. fusiformis develop first into an enlarged anterior domain that forms larval tissues and the adult head. Notably, this also occurs in the so-called 'head larvae' of other bilaterians, with which the O. fusiformis larva shows extensive transcriptomic similarities. Furthermore, we propose a histone-based regulation to be one of the main underlying mechanisms upstream of the heterochronies. For that matter, we perform here the first description of the histone-related complement in spiralian species, describe the landscape of histone modifications, and carry out a developmental time-course of genome-

wide profiling of histone modifications. Together, our findings suggest that the temporal decoupling of head and trunk formation, as maximally observed in head larvae, facilitated larval evolution in Bilateria. This diverges from prevailing scenarios that propose either co-option or innovation of gene regulatory programmes to explain larva and adult origins.

**Poster 13. Dissecting the function of Wnt signalling in Neural Crest Development**
By Gemma Sutton (Living System Institute, University of Exeter)

In vertebrate embryos the neural crest is a multipotent cell population with extraordinary migratory capacity. The neural crest forms a variety of cell derivatives including pigment cells, neurons and glia of the peripheral nervous system, cardiomyocytes and ectomesenchymal cells in craniofacial tissue. The Wnt/β-catenin signalling pathway has an ongoing role in neural crest development. In the process of neural crest fate restriction, Wnt/β-catenin signalling promotes the specification of the melanocyte cell lineage at the expense of neuronal derivatives. However, little is known about the source of Wnt signals and their mechanism of transport to the neural crest. We hypothesise that canonical Wnt ligands expressed in the dorsal roof plate of the neural tube and midbrain-hindbrain boundary are crucial for neural crest specification. Furthermore, as Wnt proteins are hydrophobic, we hypothesise that these signalling ligands are transported between cells via specialised signalling filopodia, known as cytonemes. To investigate this process, we are using Tol2 transgenesis in zebrafish to label Wnt-producing cells of the dorsal roof plate and midbrain-hindbrain boundary. Using transgenic lines and live-imaging, we can assess the mechanism of Wnt ligand transport from the cells of the neural tube to the neural crest. Furthermore, using tissue-specific promoters, we are developing transgenic tools to regulate levels of Wnt produced in the neural tube, and the level of Wnt signalling in the neural crest.

**Poster 14. Investigating the regulatory logic controlling CDX2 during epiblast regionalization**
By Irene Amblard (Imperial College - London Institute of Medical Sciences)

A long-held question in developmental biology is how extrinsic signals can be interpreted by cells to produce a wide variety of cell types in individual tissues. In the post-implantation epiblast, a crucial distinction is made between anterior versus posterior progenitors that will later contribute to cranial versus trunk derivatives in the embryo. The transcription factor CDX2 plays a central role in this process, by repressing cranial, and promoting caudal, identities in epiblast cells. However, cells display a limited time window in which they are competent to express CDX2, in response to WNT and FGF signalling. This time-restricted competence limits the production of caudal tissues, such as the spinal cord and paraxial mesoderm, to a specific developmental window. This raises the question, how do cells alter their response to the same extrinsic cues during development, to ensure the production of diverse cell types? Using an in vitro model to differentiate caudal epiblast progenitors into spinal cord versus paraxial mesoderm, we provide evidence that the competence to express CDX2 depends on tissue-specific regulatory mechanisms. Through the generation of CRISPR/Cas9 enhancer deletions, we are systematically testing the role of individual and combined candidate regions in the control of CDX2 expression dynamics. This strategy will shed light on the regulatory logic that defines the developmental window in which cells are competent to generate spinal cord and presomitic mesoderm, and provides an opportunity to control the window of competence through the manipulation of regulatory elements.

**Poster 15. The mechanical regulation of Eph/ephrin signalling in the developing Xenopus brain**
By Jana Sipkova (University of Cambridge)

Eph receptors and their membrane-bound ligands, ephrins, provide key signals in many developmental processes including neuronal guidance. However, despite immense progress in our understanding of Eph/ephrin signalling, discrepancies between in vitro and in vivo work remain. As

axon pathfinding is regulated by chemical and mechanical signals, and the mechanical regulation of Eph/ephrin signalling is currently poorly understood, we here investigated the role of mechanical cues in this signalling pathway. Xenopus retinal ganglion cell axons cultured on soft substrates mechanically resembling brain tissue had the opposite response to ephrinB1 compared to those cultured on glass. Furthermore, in vivo atomic force microscopy data showed that the Xenopus visual area of the brain, the optic tectum, becomes mechanically heterogenous as the axons of retinal ganglion cells approach the diencephalon-tectum boundary and begin to innervate it. The stiffness gradient which develops correlates with both a cell density gradient and a concentration gradient of EphB expression. Since EphB/ephrinB signalling in Xenopus retinal ganglion cells is affected by substrate stiffness in vitro, and a stiffness gradient develops across the optic tectum at the time of innervation, our data suggest that mechanical cues could be important in tuning retinotectal mapping through the regulation of chemical signalling. A similar regulation of chemical signalling through tissue mechanics is likely to be important across multiple aspects of neural development, as well as in other organ systems.

**Poster 16. Interplay between Shh and Wnt pathways in tooth regeneration**
By Jiayun Xu (King's College London)

Teeth are essential for processing food as well as for flashing an attractive smile. Mammals show an extensive variation in tooth number and, consequently, tooth regeneration capacity. Two important pathways—Sonic Hedgehog (Shh) and canonical Wingless/Integrated (Wnt)—control tooth number and spatial patterning through a delicate balance of activation and inhibition. It has been demonstrated that a single dose of the Shh inhibitor, Vismodegib, given to mouse embryos at e15.5, resulting in the production of an additional molar, lingual to the first molar (M1), almost like a replacement molar. To better understand the interactions between Wnt and Shh, and their roles in tooth production, including of the replacement dentition, we tested Vismodegib in both in-vivo and in-vitro settings. We added Vismodegib to molar placode cultures, commenced at embryonic stages e13.5-e17.5, and also administered Vismodegib to mice between e15-p7. We found a mix of resulting phenotypes, including both supernumerary "replacement" molars, but also inhibition of some primary molars. To confirm the identity of the supernumerary molars, we stained the sections with trichrome. To ascertain the interplay of Shh and Wnt expression, we used immunofluorescence assay targeting Lef1, and RNAScope on Gli1. Through gaining a better understanding of the molecular mechanisms behind tooth regeneration, we can develop effective treatments for both hypodontia (lack of teeth) and hyperdontia (excess teeth) in humans.

**Poster 17. Ribosomal protein paralogues in germline development**
By Katarina Grobicki (University of Cambridge)

Ribosomal proteins, together with ribosomal RNAs, form the backbone of the cellular machinery responsible for catalysing protein synthesis. Many ribosomal protein genes have been duplicated during evolution, leading to co-existing paralogous genes within species, the implications of which remains to be determined. In Drosophila, one paralogous ribosomal protein gene is usually expressed ubiquitously and is essential for translation; mutations in these genes are homozygous lethal and heterozygotes usually display the minute phenotype. Less is known about their paralogues, however a number show tissue-specificity and many have enriched expression in the germline. We performed systematic, site-specific mutagenesis and showed that ribosomal protein paralogues that are expressed in a tissue-specific manner are generally not required for viability or fertility, while identifying one which is essential for female fertility, RpS5b.
By studying the phenotype associated with the loss of RpS5b, we established that ribosome biogenesis stress in germ cells is sufficient to trigger strong activation of the Tor pathway, leading to remodelling of germline metabolism. In addition, this germline stress response is transduced to the neighbouring somatic epithelium, leading to overgrowth, disorganisation, incomplete Notch activation, and non-

autonomous activation of Tor kinase. In conjunction with the metabolic changes, this halts oogenesis and causes complete female sterility. Altogether, our analyses uncovered an essential inter-tissue mechanism coordinating the control of growth, metabolism and development in soma and germ cells.

**Poster 18. Diverse DNA Methylation landscapes in Annelida reveal the Evolution and Role of this Epigenetic mark in Transcriptional Regulation**
By Kero Guynes (Queen Mary, University of London)

Cytosine DNA methylation (5mC) is a conserved epigenetic mark found across eukaryotes known to be involved in diverse genome regulatory processes, from the control of gene expression to repression of transposable elements (TEs). Extensive research in vertebrates has revealed the importance of 5mC in silencing TEs and notably in the embryonic development of mammals. However, the presence or functional implications of this mechanism in invertebrates remain unclear. To address this knowledge gap, we studied DNA methylation in Annelida, one of the largest and more diverse groups of animals that are still understudied. We performed whole-genome bisulphite sequencing (WGBS) on three annelid species—Owenia fusiformis, Capitella teleta, and Dimorphilus gyrociliatus—at embryonic and adult stages. They each have varying genome sizes, gene repertoires and TE landscapes. Our analysis revealed substantial methylation in the gene bodies, and specific targeting of certain TE classes in O. fusiformis and C. teleta—interestingly, the latter is not a characteristic feature in most invertebrate genomes studied to date. D. gyrociliatus, on the other hand, seem to have lost 5mC likely due to extreme genome compaction and the subsequent loss of some DNA methylation repertoire. Most importantly, we found dynamic changes of 5mC levels during embryogenesis in O. fusiformis, and using drugs to disrupt DNA methylation results in morphological defects. Together, our work reveals an array of 5mC landscapes in Annelida, which correlates with TE abundance, and suggests that the late developmental methylation reprogramming is a common feature of bilaterian animals.

**Poster 19. Highly specific and non-invasive imaging of Piezo1-dependent activity across scales using GenEPi**
By Konstantinos Kalyviotis (Imperial College London)

Mechanosensing is a ubiquitous process to translate external mechanical stimuli into biological responses. Piezo1 ion channels are directly gated by mechanical forces and play an essential role in cellular mechanotransduction. However, readouts of Piezo1 activity have been mainly examined by invasive or indirect techniques, such as electrophysiological analyses and cytosolic calcium imaging. Here, we developed GenEPi, a genetically-encoded fluorescent reporter for non-invasive optical monitoring of Piezo1-dependent activity. We demonstrate that GenEPi has high spatiotemporal resolution for Piezo1-dependent stimuli from the single-cell level to that of the entire organism. GenEPi revealed transient, local mechanical stimuli in the plasma membrane of single cells, resolved repetitive contraction-triggered stimulation of beating cardiomyocytes within microtissues, and allowed for robust and reliable monitoring of Piezo1-dependent activity in vivo. GenEPi will enable non-invasive optical monitoring of Piezo1 activity in mechanochemical feedback loops during development, homeostatic regulation, and disease.

**Poster 20. Temporal Notch signaling regulates mucociliary cell fates through Hes-mediated competitive de-repression**
By Magdalena Engelhardt (University Freiburg Medical Center)

Tissue functions are determined by the types and ratios of cells present, but little is known about self-organizing principles establishing correct cell type compositions. Mucociliary airway clearance relies on the correct balance between secretory and ciliated cells, which is regulated by Notch signaling across mucociliary systems. Using the airway-like Xenopus epidermis, we investigate how cell fates

depend on signaling, how signaling levels are controlled, and how Hes transcription factors regulate cell fates. We show that four mucociliary cell types each require different Notch levels and that their specification is initiated sequentially by a temporal Notch gradient. We describe a novel role for Foxi1 in the generation of Delta-expressing multipotent progenitors through Hes7.1. Hes7.1 is a weak repressor of mucociliary genes and overcomes maternal repression by the strong repressor Hes2 to initiate mucociliary development. Increasing Notch signaling then inhibits Hes7.1 and activates first Hes4, then Hes5.10, which selectively repress cell fates. We have uncovered a self-organizing mechanism of mucociliary cell type composition by competitive de-repression of cell fates by a set of differentially acting repressors. Furthermore, we present an in silico model of this process with predictive abilities.

**Poster 21. Inhibition of DNA methylation impairs zygotic genome activation in the amphipod Parhyale hawaiensis**
By Manuel Jara-Espejo (University of Oxford)

A defining feature of early embryogenesis is the transition from maternal to zygotic control (MZT), which requires embryonic genome activation (ZGA). We are analysing the timing of and the molecular factors potentially controlling ZGA in Parhyale hawaiensis embryos. We analysed MZT transcriptional dynamics using PolyA and Total RNA-seq data spanning stages from fertilization to the onset of gastrulation. The maternal contribution was estimated to be ~11000 mRNAs, including several transcription factors (TF), as well as transcripts encoding genes involved in mRNA degradation, translational regulation and cell cycle control. We determined that ZGA starts at 12hrs and occurs in two waves; the first zygotic transcripts are short, intron-poor and evolutionarily Parhyale/amphipod-specific genes. We also profiled the embryonic DNA methylation landscape before and after ZGA using Methyl-seq. CpG methylation is enriched in non-coding intergenic and repetitive regions, while gene-related methylation was abundant at the beginning of genes and temporally and spatially dynamic. Gene expression and methylation are positively correlated, with non-methylated genes forming the majority of low/non-expressed genes. Genes activated zygotically during the minor wave of ZGA were more methylated than those maternally supplied. We found that inhibition of DNA methylation using 5-Aza-C impaired Parhyale embryogenesis and-treated embryos were delayed and die before hatching. 5-AzadC treatment strongly affected the early embryonic transcriptome leading to down-regulation of ~2000 genes. Among these methylated maternal-zygotic genes are overrepresented suggesting methylation may play a role in the establishment of MZT. In summary, studying the transcriptional dynamics controlling MZT in Parhyale we identified a potential linkage between DNA methylation and gene expression during early embryonic development in crustaceans.

**Poster 22. Evolution of the Vertebrate Cranial Sensory Ganglia**
By Mayur Prag (University of Oxford)

The Cranial Sensory Ganglia (CSG) form the developmental origins of most vertebrate sensory systems, the basis for many species' ability to respond and adapt to its environment and can be seen as an important driver of adaptive fitness throughout evolution. The genes governing the development of the CSG are highly conserved across vertebrates and chordates including many similarities in tunicates, the closest link to ancestral vertebrates. We want to understand how vertebrates developed a complex sensory nervous system from a basic toolkit of genes and regulatory networks. Hmx is a homeobox gene in this network and drives the development of bipolar tail neurons in Ciona intestinalis as well as being involved in the differentiation of cells in vertebrate CSG. A conserved non-coding element (CNE) controls the expression of Hmx across vertebrates and tunicates with the element being tandemly duplicated in the vertebrate lineage. Intriguingly, the duplicated regulatory elements each have a distinctive function in the vertebrate development with uCNE and dCNE both driving expression in the CNS, while uCNE also drives expression in CSG derivatives. I aim to investigate the regulatory network

surrounding Hmx as well as uncovering the evolution of the network across the vertebrate lineage. I will also identify the key elements responsible for the divergent activity of vertebrate u/dCNE to understand how these regulatory elements contributed to the sensory diversification of modern vertebrates.

**Poster 23. Identifying the neural correlates of an aversive response in the Habenulo-Interpeduncular pathway of the zebrafish larvae**
By Nicole Ortiz (Sorbonne Université)

Exposure to threatening and dangerous situations elicit distinct adaptive and defensive responses. The appropriate selection and sequence of these behaviours are crucial for survival. The Habenulo-Interpeduncular Nucleus (Hb-IPN) pathway, evolutionarily conserved across vertebrates, consists of cholinergic and non-cholinergic circuits which play distinct roles in aversive responses. However, the underlying mechanisms for how the Hb-IPN pathway modulates aversion still remain poorly understood. In the zebrafish larvae, these two circuits display negatively correlated activity in their axon terminals at the IPN. We demonstrate in vivo that an aversive stimulus, mild electric shock, activates the cholinergic neurons which inhibit non-cholinergic transmission at the dorsal IPN through retrograde signalling via presynaptic GABAB receptors. Genetic mutation of the GABAB 1a receptor (gabbr1a) in non-cholinergic neurons shows that while cholinergic circuit activity is not perturbed, there is attenuation of inhibition at the dorsal IPN, suggesting that GABAB receptors are necessary for the inhibition of the non-cholinergic circuit. Our preliminary findings demonstrate that gabbr1a mutants display altered locomotor activity post-shock, suggesting that GABAB-mediated inhibition is important for the resumption of locomotion after aversive events. These findings suggest that the interaction between the cholinergic and non-cholinergic circuits is critical for the manifestation of distinct aversion-driven responses. Elucidating the mechanisms underlying aversion encoding will provide the framework to understand the development of psychiatric problems.

**Poster 24. Interactions of mechanical and long-range chemical signalling in the developing Xenopus brain**
By Rachel Mckeown (University of Cambridge)

The extending axons of developing neurons must navigate through their environment and connect with specific targets that may be potentially distant. Previous data support axon pathfinding mediated by biochemical gradients of attractive and repulsive molecules, but this fails to fully explain all in vivo axon guidance phenomena. Recent work has demonstrated that axons are sensitive to mechanical properties of their environment and adjust their behaviour according to substrate stiffness in the physiological range of brain tissue. Using the optic pathway of the African clawed frog Xenopus laevis as an established axon guidance system, our work explores how these signalling modalities interact during neuronal development. Our data suggest that chemical guidance of retinal ganglion cell axons in response to Semaphorin3A, a key guidance molecule found along the Xenopus optic pathway, is regulated by tissue stiffness. The mechanosensitive ion channel Piezo1 is a key mediator in this mechanical regulation of chemical signalling. Such interactions may be important to secure accuracy and reproducibility for axon guidance and hence mature nervous system function, as guidance takes place in a complex and noisy in vivo environment where long-range chemical gradients, reliant on diffusion, are likely to be shallow and variable.

**Poster 25. In vitro expanded stromal cells reconstitute a long-lived phenocopy of the native human thymus ex vivo and in a novel humanised mouse model**
By Roberta Ragazzini (Crick Institute)

The thymus is a primary lympho-epithelial organ where the T cell-mediated immune function develops. Alterations of thymus development or function result in severe immunodeficiency and autoimmunity. Despite its crucial role in immune regulation, the cellular and molecular mechanisms that regulate the generation and maintenance of the human thymus remain largely unknown. Therefore, there has been long-standing interest in developing models to study thymus generation and the potential to manipulate it clinically. Here, we developed the conditions for isolation of human thymic stomal cells capable of extensive in vitro expansion and characterized by an epithelial-mesenchymal phenotype, an unique, cell-intrinsic feature of thymus epithelial stroma that is stably maintained over many passages in culture. Moreover, we accomplished the full reconstitution of a human organ (not an organoid) long-term in vivo thanks to the cooperation of thymic epithelial, interstitial cells and haematopoietic stem cells (HSC) supported within a natural extra-cellular matrix (ECM) obtained by a novel perfusion-decellularization approach. Crucial to demonstrating the functional competence of thymic stroma upon in vitro expansion was its capacity to attract
circulating human HSC, support T cell development and repopulate the periphery of athymic NSG mice. Such a whole human system opens the possibility of addressing immunological questions including human T cells development and the establishment of tolerance. These findings support the feasibility of a multidisciplinary approach to rebuild a functional human organ and establish a basis for studying the crosstalk between stroma, ECM and thymocytes, thus offering practical prospects for treating congenital and acquired immunological disorders.

**Poster 26. Does the mouse secondary neural tube produce neural crest cells?**
By Rosie Marshall (UCL Institute of Child Health)

Neural crest cells (NCCs) are a vertebrate migratory population of multipotent cells which contribute to a wide range of derivatives in the developing embryo. Pre-migratory NCCs are specified at the dorsal neural tube (NT), undergo epithelial-mesenchymal transition, before migrating to their final destination and differentiating into their ultimate derivatives. NCCs arise from four regions in mouse embryos – cranial, vagal, trunk and sacral. Whether NCCs arise from a potential fifth region, the 'tail' containing the secondary NT (SNT), is unknown. This region corresponds to the level at which human spinal lipomas form, making an understanding of SNT development clinically important. In this study, we discovered a novel population of migratory cells emerging from the SNT in vitro. Strikingly, these cells do not express Wnt1, suggesting that they may be distinct from the classical definition of 'neural crest'. In vivo, Wnt1-expressing cells are found in the dorsal SNT, however they do not appear to migrate or express traditional NCC markers. RNA sequencing confirms that the SNT-derived cells do not display a typical NCC gene expression signature. Ongoing analysis of RNA sequencing data may provide answers to the identity and possible function of these cells including a possible association with lipoma-associated malformations.

**Poster 27. Altered Neurogenesis During Fetal Brain Sparing**
By Ruya Abdulsalam (Imperial College London / Francis Crick Instiute)

Intrauterine growth restriction (IUGR) affects approximately 1:7 pregnancies worldwide and results in a restricted nutrient and/or oxygen supply to the fetus. In humans and other mammals, IUGR is often asymmetric, favouring growth of the brain over the body via a process called brain sparing. Brain sparing is an important survival adaption to developmental stress but the underlying mechanisms remain poorly understood. To address this knowledge gap, we are using a maternal low protein model of asymmetric IUGR that displays a significantly elevated brain:body weight ratio at embryonic day 18.5. To determine how cortical neurogenesis is affected by IUGR, we conducted a time course of brain immunostainings using a panel of cell-type specific markers. This revealed that the numbers of neural stem cells and early-born (deep layer) neurons are not affected during asymmetric IUGR. In contrast, we found that the numbers of late-born (upper layer) neurons are depleted. We are currently following

up this layer-specific phenotype using markers for intermediate progenitors as well as neuronal birth-dating assays. This Master's project aims to elucidate the cellular and molecular basis of brain sparing in mice with the long-term goal of explaining the underlying pathophysiology of brain sparing in humans.

**Poster 28. Enemies among friends: Classifying unwanted genetic variants in stem cell populations**
By Samantha Ivings (University of Sheffield)

Human pluripotent stem cells (hPSCs) are greatly promising for regenerative medicine, as they self-renew in vitro and can grow into any cell type in the human body. Therefore, a patient with damaged or lost tissue could be treated with a cell transplant, in which hPSCs are grown into the required cell type. However, hPSCs in culture may acquire unwanted genetic mutations over time, which allow them to out-compete wildtype cells and take over cultures. This happens quickly, so rapid detection of variant cells is important. Unfortunately, these genetic changes are visually subtle, so automatic single-cell detection of variants from images poses challenging. Here, a range of machine learning classification models are trained at the cellular level to distinguish wildtype and variant hPSCs using time-lapse microscopy images. The individual learners are then combined into a novel ensemble super-learner. A substantially reduced feature set is required due to the implementation of forward feature selection, which chooses only the most useful cell features for classification. The goals of this model were to be: 1) quick to deploy, 2) accurate, 3) requiring minimal human intervention, and 4) without disruption to cells. The resultant model accuracy was 73.9% with an F1-score of 0.82, needing only 3 hours of data to achieve this result. This model thereby substantially improves upon existing computational methods for detecting genetic variants in hPSCs populations. As the model does not use pixel intensities for classification, it is hoped that the classifier can be generalised to data obtained across various imaging platforms.

**Poster 29. Deciphering the molecular and cellular trajectories of skeletal muscle specification by PAX3 during development**
By Sarah Chebouti (IMRB)

PAX3 belongs to the paired homeobox family of transcription factors and is an upstream regulator of myogenesis. During development, Pax3 is first expressed in the presomitic mesoderm and somites but later becomes restricted to the dorsal part of the mature somites, the dermomyotome. In the central region of the dermomyote, a population of cells that co-express Pax3 and Pax7 specify the myogenic lineage progenitor cells of the trunk and limb muscles. Despite the functional role of PAX3 in myogenesis, only a few PAX3 direct target genes have been identified and the transcriptional regulation of its gene regulatory network in vivo is yet to be understood. Thus, the main objective of my project is to unveil shared and distinct gene regulatory networks downstream of PAX3 in different populations that derive from PAX3-expressing cells and how this is modified over time and in distinct PAX3 activity levels. To do this, I am using transgenic mouse lines that modulate PAX3 transcriptional activity. In these mouse lines, GFP is used as a reporter gene to trace the Pax3 lineage. The mouse lines include the control Pax3GFP/+embryos; the null Pax3GFP/GFP embryos, where PAX3 activity is abrogated and the Pax3PAX3-FOXO1/GFP genotype that confers PAX3 gain-of-function. I performed scRNA-seq experiments on embryos for the above-mentioned genotypes at different time points: E9.5 and E10.5, representing early phases of the myogenesis establishment. Combining the scRNA-seq data analysis from different alleles at different timepoints provided us a list of putative PAX3 target genes being modulated by changes in PAX3 transcriptional activity. In vivo validation experiments of these putative target genes are ongoing.

**Poster 30. Identifying and following putative stem cells in the temporomandibular joint disc**

By Ticha Tuwatnawanit (King's College London)

The temporomandibular joint (TMJ) connects the mandibular condyle and the fossa of the temporal bone with a fibrocartilaginous disc in between. The TMJ is required for the intricate movements of mastication and communication. Despite its frequent use, the TMJ disc and surrounding structures have poor reparative capacity, with defects leading to highly prevalent Temporomandibular disorders (MDs). TMDs have limited effective treatment options with minimal understanding of the underlying TMJ disc biology. Here we assess whether the disc has a stem cell population. Gli1 is a transcription factor in the hedgehog (hh) signaling pathway, with Gli1 positive cells functioning as stem cells in a variety of tissues. We have tracked the lineage of Gli1 cells in the disc, and compared to label retaining cells (LRC), and proliferating cells, creating a spatial map of the disc. We show that the anterior disc houses a population of Gli1 positive cells and that the disc is very unproliferative, populated by large numbers of LRCs. The Gli1 cells decreased with age during postnatal development, with adults having few positive cells. Overall, the anterior disc appeared to possess a transient putative stem cell population, which will be explored further to understand its role in repair.

**Poster 31. Roles of ECM in Regulating Chick Gastrulation**
By Yuri Takahashi (Cambridge)

My project is focused on understanding potential ECM feedback mechanisms that guide proper tissue morphogenesis and pattern formation during vertebrate gastrulation. In particular, I am interested in how ECM physicochemical dynamics direct normal mesoderm migration, ingression, and cell-fate specification. Some of the models that I am using to pursue this question are whole chick embryos as well as chick explants. Our preliminary data suggest that the basement membrane may be crucial in guiding both proper mesoderm specification and induction of epithelial-to-mesenchymal transitions in gastrula stage chick embryos.

**Poster 32. Crosstalk between morphogen and metabolic gradients specifies tonotopic identity in developing hair cells.**
By Zoe Mann (King's College London)

In vertebrates with elongated auditory organs, mechanosensory hair cells (HCs) are organised morphologically such that complex sounds are broken down into their component frequencies along a proximal-to-distal long (tonotopic) axis. Proximal HCs respond to high frequency sounds, and distal HCs to low frequencies. Acquisition of tonotopic morphology at the correct positions along the cochlea requires that nascent HCs interpret their positional identity during development. The complex signalling that takes place within the auditory organ between a developing HC and its local niche along the cochlea is poorly understood. Here, using NAD(P)H fluorescence lifetime imaging (FLIM) to probe biochemistry in live HCs throughout their development. We identify a gradient in cytosolic glucose metabolism in HCs along the developing chick cochlea. We further show that re-shaping this gradient by modulating the fate of glucose catabolism, disrupts Bmp7 and Chordin like-1 signalling along the organ leading to a flattening of HC morphologies and loss of tonotopic identity. We identify a causal link between graded morphogen signalling and metabolism that specifies tonotopic identity in developing auditory HCs.

**Poster 33. Extracellular matrices modulate differentiation and zone-specific characteristics of human embryonic stem cell-derived hepatocyte-like cells.**
By Faiza Farhan (Imperial College London)

Human pluripotent stem cell (hPSC)-derived hepatocyte-like cells have the potential to offer a better predictive value in in-vitro liver-stage phenotypic drug screening and thus become a model for drug

development. There are several strategies to generate hepatic micro-tissues, however few have considered the existence of differences in extracellular matrix (ECM) from periportal to pericentral zone. Although the principal structural components of the liver ECM are COL and FN. COL-I and III are expressed in the portal area (Sánchez-Romero et. al., 2019). In line with these ECM gradients, liver functions are also non-uniformly distributed along the lobule radial axis, a phenomenon that has been termed "liver zonation" (Jungermann, K., & Kietzmann, T. 1996; Gebhardt, R., & Matz-Soja, M. 2014; Manco, R., & Itzkovitz, S., 2021). Processes that are energetically demanding, such as protein secretion and gluconeogenesis, are allocated to the portal layers (Manco, R., & Itzkovitz, S., 2021). While pericentral hepatocytes preferentially engage in xenobiotic metabolism, bile acid biosynthesis and glycolysis, which are less energetically demanding processes (Halpern, K. B. et al., 2017; Ben-Moshe, S., & Itzkovitz, S. 2019; Manco, R., & Itzkovitz, S., 2021). Therefore, we hypothesize that changes in the ECM composition in invitro conditions could modulate the hepatocyte differentiation resulting in zone-specific hepatocyte-like cells.

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**Characterization of Human Pluripotent Stem Cell Characteristics with Nano-Resolution Microscopy**
By Rasha Elmansuri (University of Turku)

Human pluripotent stem cells (hPSCs) are fast becoming a key instrument in regenerative medicine. One of the main hurdles remaining unsolved is understanding their characteristics and regulation of pluripotency. Investigating hPSCs is essential for further understanding stem cell biology in detail, thus enabling personalized medicine. Until now, research shows limited research on what characteristics make hPSCs unique.

To delve into the characteristics of hPSCs, the cells were cultured and fixed in specific terms to be imaged under different nano-resolution microscopy techniques. First, hPSCs were visualized by Transmission Electron Microscopy (TEM) to characterize their biological components. Then hPSCs were imaged with Scanning Electron Microscopy (SEM) to determine their cellular surface features. As a result of our total proteome and cell surface proteomic data with Stale Isotope Labelling by Amino Acids in Cell Culture (SILAC), we verified that hPSCs have specific proteins on the cellular surface. Imaging these proteins with nano-resolution microscopy techniques and measuring them provides tremendous significance in understanding the biology of hPSCs.

**The molecular basis of epigenetic inheritance in humans**
By Sarah Stucchi (Human Technopole)

Epigenetic inheritance refers to the transmission of information across generations that is independent of the DNA sequence carried in gametes. To what extent environments shape such inheritance and with which implications for human health remain open questions. While animal experiments uncovered clear

instances of such phenomena, overall impact and mechanisms of epigenetic inheritance in humans remain elusive, mainly due to difficulties in precisely measuring environmental exposures across generations and to the lack of access to developing germ cells from prenatally exposed individuals. Considering the paradigmatic case of the heritable impact of prenatal exposure to endocrine disrupting chemicals (EDCs) on neurodevelopment, we are overcoming these challenges by employing human induced pluripotent stem cells (hiPSC)-derived 3D in vitro models, namely human primordial germ cell-like cells (hPGCLCs) and cortical brain organoids (CBOs). We are exposing hPGCLCs to mixtures of EDCs, defined on the basis of epidemiological data, and assessing their impact on gene expression and epigenetic layers, using -omics approaches. Interestingly, previous evidence showed that some loci associated with neurological disorders are resistant to global erasure of DNA methylation occurring during early germ cell development, revealing potential for transgenerational epigenetic inheritance that may have phenotypic consequences. Therefore, to unravel the causality between epigenetic changes in germ cells and adverse neurodevelopmental outcomes, we will edit hiPSC lines to introduce the key chemical-induced (epi)genetic changes that we will identify in hPGCLCs upon EDCs exposure. We will then differentiate these edited lines into CBOs and investigate the molecular effects of the manipulation. Overall, this work will contribute to shed light on the extent to which environmentally induced, epigenetically encoded and inherited changes affect development and health in humans, providing scientific evidence to guide the reduction of adverse effects on present and future generations caused by widespread environmental exposures.

**The Sammy Lee Memorial Lecture**

**Sammy Lee**, Visiting Professor in Cell and Developmental Biology at UCL, passed away suddenly on 21 July 2012, aged 54. Sammy was a great friend to many in the community; a gregarious person who could and would happily talk to everyone he met. He was a lateral thinker whose enthusiasm was infectious.

Sammy's scientific journey began in the 1970s. He chose to study Physiology at Chelsea College, KCL based on the fact he was a Chelsea Football supporter. After graduating, he went into UCL to ask Professors Ricardo Miledi and Sir Bernard Katz for a place to study for a PhD and he was offered an MRC scholarship. His post-doctoral research on gap junctions in research on gap junctions in early mammalian embryos, with Professors Anne Warner and Dame Anne McLaren, produced new information on factors affecting communication between cells and their developmental potential.

In 1985, Sammy changed direction to work in the newly emerging field of IVF with Professor Ian Craft, quickly becoming head of the laboratory at the Wellington Hospital which was at the time one of the largest IVF units in the world. He dedicated many years to his work with fertility treatment in several IVF units including London's Portland Hospital and Bourn Hallam. Sammy developed numerous new successful techniques including pioneering the first UK gamete Intra-fallopian Transfer (GIFT) program and in later years, whilst head of the lab in the Chelsea and Westminster hospital he developed a successful technique, allowing infected patients to give birth to HIV-free babies.

When he returned to academic work, Sammy's focus at UCL was very much on the students who he was always willing to help. He enjoyed teaching the next generation of scientists both undergraduate and postgraduate. He also wanted to continue his research in stem cell and regenerative medicine research which included sponsoring a PhD studentship through his charity REGEN. It was his wish to present a medal to a young scientist to encourage them in their career. With that in mind, it is the honour of Sammy's family to present a medal annually at the YEN meeting in his name.

The medal is presented annually to an outstanding piece of research at the YEN meeting. The bronze medal was designed by the late Felicity Powell and is an artwork with depth and meaning both for Sammy's family and her own. The front of the medal shows Sammy's smile emphasising the humanity and his ability to communicate. On the reverse is an oocyte with the needle-like insertion of the name of Sammy's charity REGEN into its nucleus. The inscription around the edge of the medal 'The Sammy Lee Award for Research in Embryology' maintains the simplicity of the design. 2022 will be the 9th Sammy Lee award to be presented.


The Lee Family and REGEN have been proud supporters of the YEN conference since 2013. We are so pleased to see the event grow from strength to strength each year and honoured that YEN has chosen Sammy with an annual lecture.