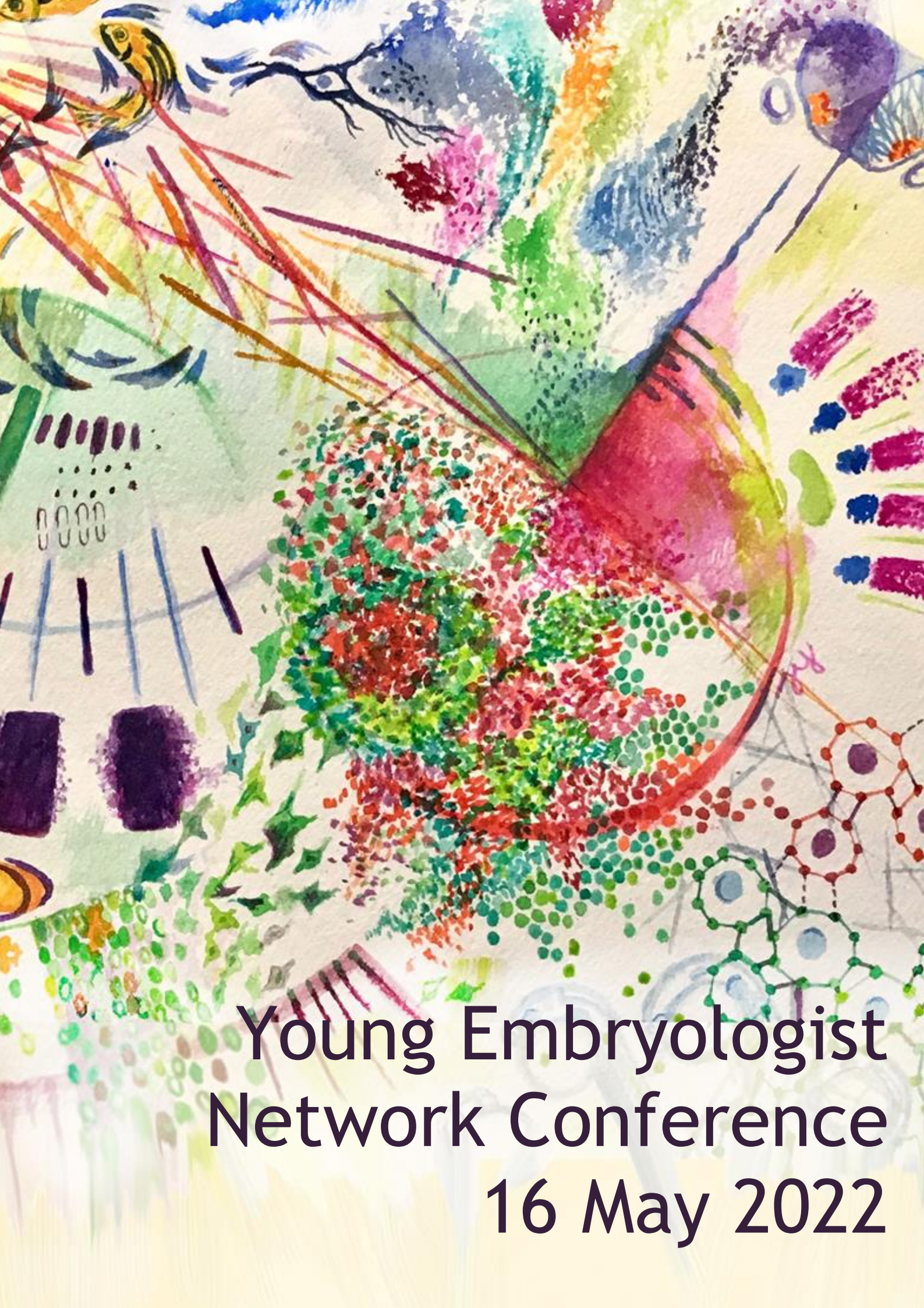

Young Embryologist Network Conference

16 May 2022

About YEN

The Young Embryologist Network (YEN), is an academic body aiming to bring together early career scientists within the wide field of developmental biology, in order to provide opportunities to present talks and posters, network and collaborate, and gain research or career advice.

YEN was set up in 2008 by graduate students in the prestigious Department of Cell and Developmental Biology at University College London. Every year, the YEN hosts an annual conference at a UK research institution with great success. The conference is entirely organised by graduate students and junior post-doctoral scientists, and has remained free to attend since 2008, due to the generosity of sponsors and grants.

The annual YEN conference is continually growing and expanding. This year, as our conference has gone hybrid, we are excited to welcome over 400 attendees from all over the world! We have invited speakers and selected talks and posters to reflect the diversity of questions in the field as well as capture the range of techniques used to address them. We hope that this unique opportunity of having a global audience fosters interactions between early career researchers in the field of developmental biology, expanding the Young Embryologist Network.

This year we are immensely proud to be hosting our first **"Scientific Perspectives: Working in Science with a Disability"** talks. Disability is a tremendous barrier not only to entry, but also to the progression of a scientific career, and researchers with disabilities remain immensely underrepresented at every career stage. Our community could be much better equipped in helping ensure inclusion and equal opportunities for disabled scientists, and so we have invited Dr. **Elisabeth Kugler (UCL), Prof. John Hutchinson (RVC) and Dr. Oscar Cazares (UCSF)** to share their perspectives and experiences of working in science with a disability. We also have the pleasure of welcoming **Dr. Cynthia Andoniadou (King's College London)**, who will be delivering the summary-address for these sessions. People with disabilities are often left out of inclusivity conversations and it is our hope that, by giving the conversation a platform at our conference, we can begin to enact positive change. It is our pleasure to share this platform with you at YEN 2022.

In-person attendance

Francis Crick Institute
1 Midland Rd, NW1 1AT
London

The conference will be held at the Manby Gallery located at the ground floor of the institute. The Francis Crick Institute is conveniently located in the heart of London and easily accessible by train, tube and bus. Nearby stations are King's Cross St Pancras, St Pancras International and Euston station

Virtual attendance

All registered attendees are welcome to join YEN 22 via the following Zoom link, which will go live at 09:00 BST on the day:

https://crick.zoom.us/j/66589598717?pwd=aFBUMmJxZWhlZjhsYUlxaFlvNUVLUT09
Passcode: 934217

The complete day's events will be viewable via the Zoom webinar link as per the attached programme, and posters will be viewable on our Google Drive.

Please use the following link to access the virtual posters:

https://drive.google.com/drive/folders/1fenRcUA520nS1zslG655_elhb1O2Kbuf?usp=sharing

Please vote for your top three posters on Slido using the code **#031821** or by using the following link: https://www.sli.do

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: Ollie Inge, Jack Morgan, Sergio Menchero, Maddy Demuth, Nikolaos Angelis, Michelle Neumann, Luca Zanieri, Ferran Garcia Llagostera, Jeremie Subrini, Foteini Papaleonidopoulou, Oliver Bower, Mint Htun, Claudia Belem, Maryam Clark, Ioakeim Ampartzidis & Olivia Dinwoodie.

Judges: Karen Lee, Zoe Mann & Rachel Moore

Poster Artist: Tim Justina Yeung

Sponsors: Hosting YEN each year would not be possible without the generosity of our sponsors and supporters. We kindly thank: REGEN, The Crick Partner Networking Fun, New England BioLabs, The Company of Biologists, BioLegend and the Society for Experimental Biology, and encourage all attendees to visit the associated stands during the refreshment breaks.

Get in touch:

Twitter: @YEN_community (#YEN2022)
Instagram: Youngembryologistnetwork
Website: http://www.youngembryologists.org/
Programme: http://www.youngembryologists.org/yen-2022/

Programme (BST)

Zoom: https://crick.zoom.us/j/66589598717?pwd=aFBUMmJxZWhIZjhsYUlxaFlvNUVLUT09
Passcode: 934217

8:00 - 09:00	Registration
9:00 - 9:15	Welcome address
9:15 - 9:45	**Andrew Gillis** (University of Cambridge, UK) *Polarity and patterning of the skate gill arch appendages*
9:45 - 10:45 **Selected Short Talks I**	**Rosie Marshall** (UCL, UK) *Characterisation of a novel population of neural crest arising from the mouse secondary neural tube* **Riley McMahon** (Children's Medical Research Institute, Australia) *LHX1 anchored gene regulatory network encompasses novel head organiser transcription factors* **M Joaquina Delas** (The Francis Crick Institute, UK) *Developmental cell fate choice employs two distinct cis regulatory strategies* **Lara Busby** (University of Cambridge, UK) *Telling the time - coordinating axial progenitor cell behaviour with the progression of embryogenesis*
10:45 - 11:10	Break - **Poster Session I** (even numbers) - Networking
11:10 - 12:10 **Selected Short Talks II**	**Ying Zhang** (The Francis Crick Institute, UK) *Characterisation of cellular and molecular changes during brain sparing* **Eirini Maniou** (UCL GOS Institute of Child Health, UK) *3D printing elastic 'springs' in the embryo: next generation biomechanics* **Bethan Clark** (Department of Zoology, University of Cambridge, UK) *Colouration evo-devo: the developmental basis of intra-specific variation in a cichlid pigmentation pattern* **Preetish Kadur Lakshminarasimha Murthy** (Duke University, USA) *Human distal lung maps and lineage hierarchies reveal a bipotent progenitor*
12:10 - 12:30 **Scientific Perspectives I**	**John Hutchinson** (Royal Veterinary College, UK) *Working in science with a disability*

12:30 – 13:00	**Kate McDole** (MRC LMB, UK) *Understanding early mammalian morphogenesis through advanced light-sheet microscopy*
13:00 – 14:00	Lunch break - Networking
14:00 – 15:00 **Selected Short Talks III**	**Marga Albu** (Max Planck Institute for Heart and Lung Research, Germany) *Building the muscular wall in the atrium involves cell elongation and reorganisation of tissue polarity* **Giacomo Gattoni** (University of Cambridge, UK) *Restricted proliferation during neurogenesis is essential to provide region-specific cell types in the amphioxus brain* **Grace Blakeley** (Oxford Brookes University, UK) *Spider segmentation and single-cell sequencing* **Yuchuan Miao** (Brigham and Women's Hospital and Harvard Medical School, USA) *Reconstructing human somitogenesis with pluripotent stem cells*
15:00 – 15:20 **Scientific Perspectives II**	**Elisabeth Kugler** (UCL, UK) *Working in science with a disability*
15:20 – 15:45	Break - **Poster Session II** (even numbers) - Networking
15:45 – 16:45 **The Sammy Lee Memorial Lecture**	**Elly Tanaka (IMP, Austria)** *Adaptations of developmental programs for appendage regeneration in axolotl*
16:45 – 17:05 **Scientific Perspectives III**	**Oscar Cazares** (UCSF, USA) *Working in science with a disability*
17:05 – 17:20 **Scientific Perspectives IV**	**Cynthia Andoniadou** (King's College London, UK) *Working in science with a disability: summary and future perspectives*
17:20 – 17:35	Talk and poster prizes, closing address
17:35 – 20:00	Drinks reception and Networking

Selected short talks

Rosie Marshall

Characterisation of a novel population of neural crest arising from the mouse secondary neural tube

Neural crest cells (NCCs) are a vertebrate migratory population of multipotent cells which contribute to a wide range of derivatives in the developing embryo, including peripheral and enteric nerves, craniofacial bone and cartilage and melanocytes. NCCs arise from three regions in mouse embryos – cranial, vagal and trunk - and migrate from the dorsal neural tube before closure in the cranial region and after closure in caudal regions. Whether NCCs arise from a potential fourth region, the secondary neural tube (SNT), is an open question. The SNT develops at the extreme caudal end of the embryo at the level of the tail, however very little is known about its development. In this study, we have discovered a novel population of NCCs in the dorsal SNT. This population contains fewer cells which migrate more slowly and a shorter distance in vitro than their primary neural tube counterparts. Furthermore, in vivo SNT NCCs only express a subset of classical NCC markers, expressing Wnt1, FoxD3 in a subset of cells, but not Sox9 and Sox10. How these cells migrate in vivo and the identity of their derivatives is now under investigation in our lab.

Riley McMahon

LHX1 anchored gene regulatory network encompasses novel head organiser transcription factors

Embryonic development is driven by molecular instructions encoded by transcription factors (TFs) that underpin the formation of the body plan and the specialisation of tissue precursor cells. Analysis of gastrulating mouse embryos has revealed that the LIM homeobox 1 (LHX1) TF is indispensable for head and face development. However, the precise function of LHX1 in the initiation of craniofacial morphogenesis at late gastrulation has not been fully elucidated. Here we present an LHX1 anchored gene regulatory network in embryos utilising multi-omics analytics including RNA-seq, ATAC-seq and DamID-seq. We identified the forkhead box gene, Foxd4 and the BTB domain gene, Kctd1, as direct downstream targets of LHX1. CRISPR-Cas9 edited mouse embryonic stem cell (mESC) lines were generated with bi-allelic frameshift mutations in the coding region of these two target genes. The function of these TFs was investigated using chimeric embryos harbouring the gene-edited mESCs and the stem cell-derived neuruloid model. We showed that FOXD4 is essential for neurulation in the rostral neural tube and for the specification of the cranial neural crest population and the loss of KCTD1 activity that impacted the canonical Wnt signalling pathway de-railed mesendoderm lineage development. Overall, our findings have highlighted the role of these LHX1 targets in the development of the head and face, which are major body parts of the early mammalian embryo.

M Joaquina Delas

Developmental cell fate choice employs two distinct cis regulatory strategies

In many developing tissues the spatial and temporal pattern of gene expression is organised by secreted signals functioning in a graded manner over multiple cell diameters. Cis Regulatory Elements (CREs) interpret signalling inputs to control gene expression. How this is accomplished remains poorly understood. The morphogen Sonic hedgehog (Shh) acts in a graded manner to direct neural progenitor specification in the neural tube. Here, we uncover two distinct ways in which CREs translate graded Shh signaling into differential gene expression. A common set of CREs are used to control gene activity in all but the most ventral neural progenitors. These CREs integrate cell type specific inputs to control gene expression. By contrast, in the most ventral progenitors, extensive chromatin remodelling is required for cell type specification. This is mediated by the pioneer factor Foxa2 engaging a distinct set of CREs, paralleling the role of Foxa2 in endoderm. Moreover, Foxa2 binds a subset of the same sites in neural and endoderm cells. Together the data identify distinct cis regulatory strategies for the interpretation of morphogen signaling and raise the possibility of an evolutionarily conserved regulatory strategy for Foxa2-mediated cell specification across tissues.

Lara Busby

Telling the time – coordinating axial progenitor cell behaviour with the progression of embryogenesis

Timing is a fundamental concept in developmental biology, but the mechanisms that control the timing of developmental events are poorly understood. Several studies have shown that when axial progenitor cells are grafted 'backwards in time' to younger embryos, they show a delayed contribution to the body axis, being present in more posterior structures relative to control grafts. This represents a difference in progenitor population behaviour at different times during development, but it is not clear what the mechanism underlying this delay is. Here, we focus on the contribution of medial somite progenitor (MSP) cells to the chick posterior body, beginning by carrying out a global transcriptomic analysis of heterochronic grafts to ask where these grafted cells are being delayed. We find that HH8 MSP cells in HH4 embryos are delayed at the transition from mesenchyme to invasive mesenchyme, a step which we term dispersion. We observe maintenance of the HH8 donor Hox gene expression profile after grafting, a feature which changes over time in axial progenitor cells in a process termed the Hox Clock and which has previously been suggested to time cell contribution to the body axis. We also use explanting to show that the Hox Clock also progresses when MSP populations are cultured in a neutral environment – supporting its regulation through a population intrinsic mechanism. Additionally, we show that cell behaviour can be uncoupled from Hox expression at small graft sizes, showing that the Hox Clock is not sufficient to explain the differential behaviour of MSP cells over time during development.

Ying Zhang

Characterization of cellular and molecular changes during brain sparing

During development, the growth of the brain is protected over that of the fetal body – a phenomenon known as 'brain sparing'. However, the cellular and molecular adaptions that occur during brain sparing are poorly understood. In this study, we established a maternal low protein (LP) dietary model for generating brain-spared mouse embryos. Using this model, we examined the proliferation of neural stem and progenitor cells, and also the activity of nutrient-dependent pathways during brain sparing from E13.5 to E18.5. We found that the overall mitotic index of the brain and the number of Pax6+ radial glia remain remarkably unchanged between standard (STD) and LP brains throughout the embryonic development, despite a strong growth deficit in organs such as the pancreas and liver. In contrast to radial glia, the number of Tbr2+ intermediate progenitor cells were reduced during E15.5 – E16.5, but then catches up during E17.5 - E18.5. Consistent with previous studies, a maternal LP diet significantly lowers the concentrations of both insulin and Igf1 in embryonic plasma and brain at E18.5. We found that this correlates with decreased Try972 tyrosine phosphorylation of the insulin receptor (Insr) in the LP brain. Interestingly, however, a kinase downstream of Insr, Akt1, retains similar activity (Ser473 phosphorylation) in LP and STD brains. Together, these results show that embryonic neural stem cells are spared during protein restriction, and they suggest that a tyrosine kinase other than Insr may sustain Akt1 signalling during protein restriction.

Eirini Maniou

3D printing elastic 'springs' in the embryo: next generation biomechanics

Successful completion of morphogenesis requires embryonic cells to generate forces and perform mechanical work. Incorrect application of these force fields can lead to congenital malformations including neural tube defects. Understanding this dynamic process requires quantification and profiling of three-dimensional mechanics in vivo, which has previously been limited by small temporal windows and indirect, semi-quantitative methods. We fabricated elastic spring-like force sensors into the closing neural tube of growing chicken embryos through intravital three-dimensional (i3D) bioprinting. This technique allows photo-crosslinking of biocompatible polymers in 3D elastic hydrogels with micron-level resolution. Combined with live-imaging and computational mechanics, i3D bioprinted force sensors allow real-time quantification of neurulation forces and work performed by embryonic tissues. We find that the two halves of the closing neural tube at the embryonic midline reach over a hundred nano-Newton compression during neural fold apposition. Pharmacological inhibition of Rho-associated kinase reveals active anti-closure forces, which progressively widen the neural tube. This suggests that an imbalance between pro- and anti-

closure forces in favour of the former needs to be maintained to prevent neural tube defects. Overall, we present a highly versatile technology, readily detecting differences in the direction of force generation and allowing dynamic quantification of morphogenetic mechanical forces.

Bethan Clark

Colouration evo-devo: the developmental basis of intra-specific variation in a cichlid pigmentation pattern

In evo devo there is often a focus on traits in model organisms for which the adaptive functions and natural variability are not well understood, limiting the understanding of the role of developmental mechanisms in speciation and adaptive radiations. Cichlid egg-spots are an ideal system to investigate the development of adaptively diversifying traits. Egg-spots are an ecologically-relevant colouration trait in East African cichlid fishes: circular orange markings on male anal fins with roles in visual signalling in reproductive behaviour. In a crater lake population of Astatotilapia calliptera, there is variation in egg-spot number and colour between shallow and deep habitats, likely maintaining signal visibility in different light conditions. A GWAS for spot number variation identified associated SNPs in the UTR of oca2, a gene with a role in melanin pigment synthesis. A GWAS for colour variation identified SNPs in the non-coding region of cdc42, a RhoGTPase involved in cytoskeleton regulation and pigment cell migration. To understand the developmental basis of egg-spot divergence, I am imaging pigment cell pattern development in juvenile fins and knocking out candidate genes with CRISPR-Cas9. Imaging wild-type fin development reveals several stages involving melanophores, iridophores, xanthophores, and erythrophores, with a key role for iridophores initiating xanthophore aggregations for egg-spots. Cdc42 knock-out embryos are not viable but have a melanophore phenotype. Oca2 knock-out and UTR deletion mutants both form egg-spots, though oca2 knock-out fish are amelanistic. Comparing mutant egg-spot development to wild-type will elucidate the developmental role of oca2 and the UTR in generating egg-spot number variation.

Preetish Kadur Lakshminarasimha Murthy

Human distal lung maps and lineage hierarchies reveal a bipotent progenitor

Mapping the spatial distribution and molecular identity of constituent cells is essential for understanding tissue dynamics in health and disease. We lack a comprehensive map of human distal airways, including the terminal and respiratory bronchioles (TRBs) implicated in respiratory diseases. Here, using spatial transcriptomics and single cell profiling of microdissected distal airways, we identify previously uncharacterized, molecularly distinct TRB cell types. These include airway associated LGR5+ fibroblasts and TRB-specific alveolar type-0 (AT0) and TRB-secretory cells (TRB-SCs). Connectome maps and organoid-based co-cultures reveal that LGR5+ fibroblasts form a signalling hub in airway niche. AT0 and TRB-SCs are conserved in primates and emerge dynamically during human lung development. Using non-human primate lung injury model, and human organoids and tissue specimens, we show that alveolar type-2 cells (AT2) in regenerating lungs transiently acquire an AT0 state from which they can differentiate into either alveolar type-1 cells or TRB-SCs. This differentiation program is distinct from that identified in the mouse lung. Our study revealed mechanisms driving bi-potential AT0 cell-state differentiation into normal or pathological states. In sum, our study revises human lung cell maps and lineage trajectories, and implicates a novel epithelial transitional state in primate lung regeneration and disease.

Marga Albu

Building the muscular wall in the atrium involves cell elongation and reorganization of tissue polarity

During vertebrate cardiac development, cardiomyocytes undergo cellular rearrangements important for the formation of complex myocardial structures. Previous studies have mostly focused on the formation of the trabecular network in the ventricle; however, morphogenetic processes that drive atrial myocardial complexity, which is crucial to propagate the action potential for cardiac contraction, have largely been overlooked. Our study uses zebrafish larvae to elucidate cardiomyocyte behaviours during atrial development, as they allow for high-resolution live imaging and are easily amenable to genetic

modifications. Using live 3D confocal imaging of zebrafish hearts, combined with mosaic labelling and temporal tracking of individual atrial cardiomyocytes, we found that atrial myocardial morphogenesis is driven by complex cell behaviours. Specifically, we observed that atrial cardiomyocytes in zebrafish larvae form membrane protrusions and adopt an elongated shape in a non-stochastic orientation that establishes atrial tissue-level polarity. These shape changes lead to restricted multilayering between neighbouring cardiomyocytes, and the formation of new cell contacts, resulting in populations of elongated cardiomyocytes that span the atrium in an orientation parallel to the direction of blood flow. These cell behaviours lead to the appearance of muscle ridges on the inner surface of the atrium. Notably, these atrial cardiomyocyte behaviours appear to be independent from factors important in ventricular morphogenesis such as Erbb2 and Notch signalling. Altogether, these data suggest that atrial morphogenesis is driven by oriented cell elongation as well as by distinct molecular and environmental/physical factors, all of which are under investigation.

Giacomo Gattoni

Restricted proliferation during neurogenesis is essential to provide region-specific cell types in the amphioxus brain

The central nervous system of the cephalochordate amphioxus consists of a dorsal neural tube with an anterior brain. While previous studies have revealed a complex molecular regionalization of the amphioxus nervous system, little is known about the morphogenetic processes regulating the emergence of cell types during neurogenesis. Proliferation is a key driver of morphological complexity in vertebrate nervous systems, but in amphioxus it has never been studied in detail. Here, we describe the dynamics and contributions of cell division during neurogenesis in amphioxus embryos. By labelling proliferating cells and inhibiting cell division, we show that proliferation is spatially restricted to the anterior and posterior ends of the neural tube during neurulation. Between these proliferative domains, trunk nervous system differentiation is independent from cell division, which decreases during neurulation and resumes at the early larval stage. We further demonstrate that anterior proliferating cells are integrated in the amphioxus brain and are required to establish the full cell type repertoire of the frontal eye complex and the hypothalamic region. Taken together, our results highlight the importance of proliferation as a tightly controlled mechanism for shaping the amphioxus nervous system and correctly regionalizing the developing brain by providing new cells fated to particular types.

Grace Blakeley

Spider segmentation and single-cell sequencing

Arthropods are amongst the most morphologically diverse phyla, accounting for approximately 75% of animals. Their diversity can be partially attributed to segmentation which generates segments along the anterior-posterior (A-P) axis that act as distinct autonomous modules for evolution to act upon, reducing effects on the organism as a whole. In arthropods, segmentation research has focused upon the insect Drosophila melanogaster which uses maternally initiated morphogen gradients to almost simultaneously prescribes segments through a hierarchical gene cascade. To better understand the mechanisms and evolution of segmentation, we study the spider Parasteatoda tepidariorum, a chelicerate which basally branch from mandibulates including insects. Parasteatoda forms its opithosomal (abdominal) segments sequentially from a posterior segment addition zone (SAZ) in a way that is analogous to the segmentation clock in vertebrates. To better understand Parasteatoda segmentation, we performed single-cell RNA sequencing (scRNA-seq) at three embryonic stages from when the first segment forms and the Hox genes are expressed along the A-P axis. Our results firstly identified all Hox genes as top markers in the cluster map, correctly reflecting their position along the A-P axis. Secondly, we identified the SAZ as a distinct cell cluster that we verified through in situ hybridisation of top marker genes and I am now performing RNAi on several genes to assess their function in segmentation. Finally, we identified another cluster that abuts the SAZ defining a region in which nascent segments may start to differentiate. Overall, our scRNA-seq has provided new insights into Parasteatoda development and the formation of segments.

Yuchuan Miao

Reconstructing human somitogenesis with pluripotent stem cells

The metameric organization of vertebrates is first implemented when somites, which contain the precursors of skeletal muscles and vertebrae, are rhythmically generated from the presomitic mesoderm. This process of somitogenesis is vital for body plan development, yet little is known about human somitogenesis given limited access to early embryos. Stem cell-based embryo models provide a promising alternative to in vivo studies. Mouse pluripotent stem cells (PSCs) have been used to achieve a striking recapitulation of all somitogenesis stages in 3D, yet no such protocols have so far been reported for human PSCs. Here we introduce two novel 3D culture systems of human PSCs, called Somitoids and Segmentoids, which can recapitulate the formation of epithelial somite-like structures with antero-posterior (AP) identity revealed by live cell imaging and single-cell RNA sequencing. In contrast to gastruloids which harbor cell lineages derived from the three germ layers, our two models contain almost exclusively paraxial mesoderm. Somitoids recapitulate the temporal sequence of somitogenesis, with all cells undergoing differentiation and morphogenesis in a synchronous manner. This system can provide unlimited amounts of cells precisely synchronized in their differentiation and will allow exploring these patterning processes at an unprecedented level of detail. On the other hand, Segmentoids reconstruct the spatio-temporal features of somitogenesis, including gene expression dynamics, tissue elongation, sequential somite morphogenesis, and AP polarity patterning. They therefore provide an excellent proxy to study human somitogenesis. Together, these two complimentary models provide a valuable platform to decode general principles of somitogenesis and advance knowledge of human development.

Selected posters

In-person: If you have been selected to present a poster in-person on the day, please ensure it is in place on the poster board at the associated number by the end of the registration period. All presenters have been assigned a poster number, and these will be found below.

Virtual: Virtual posters will be hosted on the YEN website for one week following the event, with the uploaders email address attached such that questions can be asked directly. We strongly encourage all poster presenters, including those in-person, to upload a virtual copy of their poster, such that our virtual attendees also have the opportunity to view them.

Please use the following link to both upload and access the virtual posters:
https://drive.google.com/drive/folders/1fenRcUA520nS1zslG655_elhb1O2Kbuf?usp=sharing

Please vote for your top three posters on Slido using the code **#031821** or by using the following link: https://www.sli.do

Poster Session I (even numbers): 10:45 – 11:10 (BST)

Poster 2 – Timo Kohler (University of Cambridge, UK)

Plakoglobin is a mechanoresponsive regulator of naïve pluripotency

Biomechanical cues are instrumental in guiding embryonic development and cell differentiation. Understanding how these physical stimuli translate into transcriptional programs could provide insight into mechanisms underlying mammalian pre implantation development. Here, we explore this by exerting microenvironmental control over mouse embryonic stem cells (ESCs). Microfluidic encapsulation of ESCs in agarose microgels stabilized the naïve pluripotency network and specifically induced expression of Plakoglobin (Jup), a vertebrate homologue of beta-catenin. Indeed, overexpression of Plakoglobin was sufficient to fully re-establish the naïve pluripotency gene regulatory network under metastable pluripotency conditions, as confirmed by single cell transcriptome profiling. Finally, we found that in the epiblast, Plakoglobin was exclusively expressed at the blastocyst stage in human and mouse embryos – further strengthening the link between Plakoglobin and naïve pluripotency in vivo. Our work reveals Plakoglobin as a mechanosensitive regulator of naïve pluripotency and provides a paradigm to interrogate the effects of volumetric confinement on cell fate transitions.

Poster 4 – Cato Hastings (University College London, UK)

Comparing models of cell decision-making to establish the site of primitive streak formation

Primitive streak formation is a key moment in development: the site of streak formation defines the anterior–posterior axis of the embryo. Proteins are expressed in opposing gradients before streak formation, but it is not clear how cells interpret these gradients to determine the site of primitive streak formation. We compare two models of how cells might assess their position in a large developing epithelium. The first model proposes that cells assess protein concentrations locally and autonomously. The second model assumes that cells communicate with their neighbours to establish their position. We use Bayesian parameter inference to find parameter values allowing both models to replicate the results of experiments performed in chick embryos. In addition, we use both models to make predictions and show that only the model involving cell-cell communication is consistent with experimental results.

Poster 6 – Sami Leino (The Francis Crick Institute, King's College London, UK)

Plzf mediates a switch between FGF signalling regimes in the developing hindbrain

Developing tissues are sequentially patterned by extracellular signals that are turned on and off at specific times. In the zebrafish hindbrain, fibroblast growth factor (FGF) signalling has different roles at different developmental stages: in the early hindbrain, Fgf3 and Fgf8 expressed in rhombomere 4 are required for correct segmentation, whereas later, Fgf20 expressed in specific neurons regulates the pattern of neuronal

differentiation by acting as an anti-neurogenic signal. How the switch between these two signalling regimes is coordinated is not known. We present evidence that the promyelocytic leukaemia zinc finger (Plzf) transcription factor is required for this transition to happen in an orderly fashion. In mutants lacking functional plzf paralogues (plzfa/b) the expression of fgf3, but not fgf8, persists in the hindbrain and anterior spinal cord until a late stage and consequently overlaps with fgf20 expression. Accordingly, the plzfa/b mutant hindbrain shows an abnormal pattern and high levels of FGF signalling. In addition, the inner ear is transiently anteriorised in plzfa/b mutants, consistent with ectopic Fgf3 signalling from the hindbrain. Immunofluorescence analysis indicates that the Plzf protein is initially present at low levels in the posterior hindbrain and anterior spinal cord, and the onset of higher levels of Plzf expression correlates with the downregulation of Fgf3. These results suggest that Plzf downregulates Fgf3 to ensure the correct temporal and spatial pattern of FGF signalling in the hindbrain.

Poster 8 – Daria Korotrova (EPFL, Switzerland)

The role of zDHHC4 in cilia epithelium development in zebrafish

The role of cilia epithelium in otic vesicle development has been established long time ago and the majority of proteins responsible for otolith formation have already been well-described. Here we report one of palmitoylating enzymes, zDHHC4, that is for the first time was shown to be involved in otolith formation in zebrafish. Our lab investigates one of post-translational protein modifications called S-palmitoylation. This reversible process of lipid tail attachment to the protein allows for control of the modified protein's localization, trafficking and turnover in the cell. There are 30 palmitoylating enzymes (zDHHCs) in zebrafish that perform palmitoylation. In order to establish their function in vivo we studied their expression pattern by in situ hybridization. As a resulted we for the first time created a library of zDHHCs expression during zebrafish embryonic development. Expression of few zDHHCs turned out to be restricted to the otic vesicle. We consequently downregulated them with antisense MO oligonucleotides and for one of them, zDHHC4, observed strong phenotypical effects - absence or fusion of otoliths, "circler" swimming behavior and curved body axis in larvae. According to the literature, such phenotype can be caused by disruption in cilia epithelium functioning. In order to test this we performed ac-tubulin staining of 3dpf larvae and observed by means of confocal and light-sheet microscope that hair cells in morphants were malformed as compared to wild type ebryos. Interestingly, previous studies in our lab showed that zDHHC4 is the only palmitoyltransferase whose silencing resulted in statistically significant inhibiting effect on cilia epithelium development in human cell culture.

Poster 10 – Ana Garcia Urbano (King's College London, UK)

Modelling inner ear development in human iPSC derived organoids

The sense of hearing allows awareness of the surroundings and orientation in the environment, and it is arguably the most important sense for human communication. In children, congenital hearing loss affects language development and cognitive skills, and in many cases, it is due to genetic mutations that disrupt ear development. In that context, understanding the molecular and cellular processes that drive the formation of a functional inner ear is critical to elucidate the consequences of genetic mutations. While extensive data are available on the molecular mechanisms involved in inner ear development, most of this knowledge comes from animal models and only limited information exists for human-specific aspects. This is largely due to the lack of human tissue available for research.
To elucidate whether these mechanisms are conserved in humans we rely on in vitro systems such as human iPSC-derived organoids, a system that recapitulates ear development in a stepwise manner. I aim to establish and optimize the pre-existing inner ear organoid protocol in our lab, and to use it as a model to explore human ear development. The differentiation of iPSCs towards an otic fate is driven by the addition of specific modelling signals at precise timepoints, a process that can be finely tuned in the lab to better replicate in vivo ear development. Thus, this model provides a suitable platform to study human-specific mutations and gene function, and in the long term, it could be used to develop better diagnosis and potential therapies.

Poster 12 – Daniela Costa (ICVS, University of Minho, Portugal)
Establishment and validation of a hyperglycemia-induced model in ovo

Mammalian animal models are crucial to understanding the pathophysiology of diabetes-induced defects throughout gestation but face ethical, practical, or technical limitations. Conversely, the chicken embryo model is suitable for studying embryo malformations because it is accessible to simulate specific gestational disorders and is similar to the mammalian embryo. Here we used the in ovo model to induce a hyperglycemic environment in the early stages of embryonic development. The induction of hyperglycemia was performed in fertilized chicken eggs with the administration of 0.2 and 0.4 mmol of glucose on day 1 of incubation through a window above the air sac. As a control, the same volume of NaCl 0.72% was administered. An experimental group (sham) was included to exclude the impact on egg manipulation. Subsequently, the window was sealed with tape, and eggs were incubated for 5 days at 37ºC and 49% humidity. After 5 days, embryos were photographed and macroscopically analyzed to determine the presence of morphological malformations. Egg glucose levels were measured in all groups. Results showed that it is possible to induce different and highly reproducible scenarios of hyperglycemia in ovo. Sham and NaCl treatment did not affect embryonic development; however, glucose administration created a hyperglycemic environment in the egg, with glucose values ranging between 256-352mg/dl, whereas the normal value is 142mg/dl. In the hyperglycemic conditions, embryonic mortality rate and malformations increased in a dose-dependent manner compared to controls. This model allows a systematic, inexpensive, and easily reproducible way to create different hyperglycemic scenarios during embryonic development.

Poster 14 – Francisco M Martin-Zamora (Queen Mary University of London, UK)
Histone modifications in spiral cleavage and annelid development

Spiralia is a major animal clade encompassing almost half of the animal phyla, which is defined by a stereotypic early development program termed spiral cleavage. Spiralian species display one of two modes of spiral cleavage, in which cell fates are specified either conditionally –via cell-cell interactions– or autonomously –through the asymmetric segregation of maternal factors. How these different dynamics of early cell fate commitment evolved remains, however, largely unknown. Here we describe and functionally characterise the role and dynamics of histone modifications (hPTM) and their associated machinery in spiral cleavage, which we hypothesise to be drivers of the repeated convergent evolution of autonomous specification. To do this, we are studying three annelid species, two of them with contrasting cell fate commitment modes and one with a miniature genome, that we use as proxies to all Spiralia. We show that annelids display a conserved histone complement and have retained the core hPTM machinery, yet mass spectrometry shows significant inter-species differences in key hPTM abundance. Importantly, targeting histone modifiers with epigenetic inhibitors impairs cleavage, gastrulation, and normal organ differentiation, suggesting a role of hPTM in cell fate decisions. Furthermore, we report here the first implementation in a spiralian species of the hPTM-profiling technique CUT&Tag. We anticipate CUT&Tag to be pivotal in describing hPTM and functional genomic element dynamics throughout spiral cleavage and annelid development. Altogether, our results will contribute to a richer understanding of hPTM in Spiralia and Metazoa and the mechanisms that generate new dynamics of early cell fate specification.

Poster 16 – Joachim De Jonghe (The Francis Crick Institute, UK)
Droplet-based Single-cell Total RNA-seq Reveals Differential Non-Coding Expression and Splicing Patterns during Mouse Development

In recent years, single-cell transcriptome sequencing has revolutionized biology, allowing for the unbiased characterization of cellular subpopulations. However, most methods amplify the termini of polyadenylated transcripts capturing only a small fraction of the total cellular transcriptome. This precludes the detection of many long non-coding, short non-coding and non-polyadenylated protein-coding transcripts. Additionally, most workflows do not sequence the full transcript hindering the analysis of alternative splicing. We therefore developed VASA-seq to detect the total transcriptome in single cells. VASA-seq is compatible with both plate-based formats and droplet microfluidics. We applied VASA-seq to over 30,000 single cells in the developing mouse embryo during gastrulation and early organogenesis. The dynamics of the total single-cell transcriptome result in the discovery of novel cell type markers many based on non-

coding RNA, an in vivo cell cycle analysis and an improved RNA velocity characterization. Moreover, it provides the first comprehensive analysis of alternative splicing during mammalian development.

Poster 18 – Eva Hamrud (King's College London, UK)
Deciphering Cell Fate Decisions at the Neural Plate Border

The neural plate border gives rise to 4 different fates: neural plate, neural crest, placode progenitors and epidermis. Each of these fates in turn give rise to distinct components of the central nervous system and head. The different fates begin to be specified around gastrulation and are classically thought to segregate spatially with time. Recent single-cell analysis shows that this early lineage segregation is more complicated than anticipated, and progenitor cells at the neural plate border are highly heterogenous and often co-express competing fate determinants. This project aims to build the gene regulatory networks that drive fate segregation in individual cells by integrating single cell RNA-seq and ATAC-seq datasets from the developing chick ectoderm.

Poster 20 – Matteo Perino (EMBL, Germany)
WINEseq: low-input, label-free identification of nascent transcription initiation and pausing

The RNA content of early zygotes consists of abundant transcripts deposited in the egg during oogenesis, and represents a large fraction of the total potential transcript of an organism. This large pool of total RNA makes it very challenging to identify and quantify the first molecules transcribed from the newly awaken zygotic genome. Many genes are expressed both maternally and zygotically, and involve a switch in their Transcriptional Start Site (TSS) usage. In addition to efficient transcriptional initiation, RNA polymerase II (PolII) pausing and release can also play a critical role in the final transcriptional output. To address these points, many techniques have been developed over time: Cap-trapping techniques accurately map TSS locations but do not specifically target nascent transcripts. RUN-ON based approaches are very reliable for nascent transcript quantification, but require high input and metabolic labelling, a combination not always practical or even feasible for in-vivo experiments in developing embryos. PolII-DNA-RNA ternary complex enrichment techniques provide accurate positional information about transcriptional dynamics but are similarly challenging when sample amount is limiting. Here we present Whole-transcriptome Identification of Nascent Expression by sequencing (WINEseq) a new approach that couples label-free identification of TSS and PolII pausing site usage with low input, thus being highly suited for the identification of early zygotic transcription down to the single embryo level. This method provides a new very sensitive approach that identifies nascent promoter transcripts without metabolic labelling, and therefore can be applied to a wide-range of in vivo contexts that were not feasible before.

Poster 22 – Lisa Dobson (King's College London, UK)
GSK3 and Lamellipodin cooperate to regulate actin dynamics in mouse neural crest cells

Neural crest cells are multipotent cells that give rise to diverse tissues such as the craniofacial skeleton and peripheral nervous system. During embryogenesis, neural crest cells delaminate from the neural plate border and migrate to populate distant organs. Cultured mouse neural crest cells require broad, fan-shaped protrusions (lamellipodia) for efficient locomotion; however, cytoskeletal regulation of in vivo migration is poorly understood. Actin regulators such as Lamellipodin (Lpd) promote lamellipodia formation by balancing actin branching (Scar/WAVE) versus elongation (Ena/VASP) at the leading edge. Here, using a neural crest-specific conditional knock-out mouse model, we show that Lpd is required for lamellipodium formation and migration of mouse neural crest cells. Interestingly, GSK3 is also required for lamellipodium formation in the neural crest, suggesting that GSK3 and Lpd may act cooperatively. Consequently, we identified Lpd as a novel GSK3 substrate, at serine residues in the C-terminus. We found that GSK3 phosphorylation negatively regulates Lpd recruitment of Ena/VASP proteins to the leading edge, promoting stable protrusions. Our results suggest that a novel GSK3-Lpd-Ena/VASP pathway controls actin dynamics in the mouse neural crest. An improved understanding of cytoskeletal regulation in neural crest migration will provide insights into normal development and pathologies such as neurocristopathies and neuroblastoma.

Poster 24 – Sara Anuar (UCL Great Ormond Street Institute of Child Health, UK)
Molecular and Cellular Mechanisms Underlying Primary Microcephalies

The rare heterogeneous disease autosomal recessive primary microcephaly (MCPH) has multiple genetic causes, characterized by a smaller cerebral cortex with a significant reduction of the occipital frontal head circumference of less than 3 standard deviations compared to age and sex match controls. Commonly found cause of MCPH is attributed to mutations in the abnormal spindle-like microcephaly-associated (ASPM) gene encoding centrosomal protein. ASPM sequence and function are highly conserved across species. In rodents, a decline in neural progenitors population due to premature neurons production has been reported in the aspm mutant. However, majority of previous observation were performed at tissue level and it remains ignored whether the MCPH phenotype is present in other brain regions. Our project aims to understand the underlying causes of ASPM derived MCPH at individual cell level and zebrafish embryo is used as an animal model to study the disease. During zebrafish development, ASPM expression is restricted to neural progenitor populations. We observed that all brain regions (telencephalon, midbrain and hindbrain) were significantly smaller in the zebrafish ASPM mutant when compared to wild type. Histological analysis and quantification of neurons and neural progenitors in the zebrafish telencephalon and caudal hindbrain, revealed that neural progenitors and mitotic divisions proportion were significantly reduced in the mutant suggesting that zebrafish ASPM mutant mimics major aspects of mammalian ASPM derived MCPH and that ASPM regulates growth of other brain regions. In future, we will use live-imaging in monitoring single progenitors behaviours in their intact environment to investigate the neural progenitor phenotype.

Poster 26 – Syed Munim Husain (University of Oxford, UK)
Differences in the transcriptional regulation of psychiatric risk gene CACNA1C across tissues and between species

Alternative splicing is a regulatory mechanism that modifies mRNAs in a cell-type and cell-state dependent manner, allowing fine-tuning of protein function based on cellular or physiological needs. The CACNA1C gene is associated with bipolar disorder and schizophrenia, and is known to undergo extensive alternative splicing with splice variants preferentially expressed in specific tissues and at different developmental stages. We compared full-length CACNA1C transcript isoforms across mouse tissues using a within-subjects design. We dissected four brain regions (frontal cortex, hippocampus, striatum, and cerebellum), and three peripheral tissues (heart, aorta, and colon) from 10 adult C57BL6 mice, and sequenced CACNA1C using targeted long-read nanopore sequencing. Top isoforms were compared cross tissues using a customised multidimensional reduction strategy based on their exon composition. Although there was overlap between brain and smooth muscle isoforms, heart isoforms segregated fully – demonstrating that heart CACNA1C transcripts are qualitatively distinct from those in other tissues. The mouse CACNA1C isoform profile was compared with that obtained in human tissues. We complemented the long-read sequencing study with a reanalysis of publicly available short-read RNA-seq data, quantifying differential expression at the exon level across mouse tissues. There were significant differences between heart and brain for 10 exons (fold change>1.5, $p<0.05$). Of these, one is a novel exon discovered through our long-read sequencing pipeline. Our multi-platform sequencing analyses reveal a previously underestimated complex splicing profile across tissues. Moreover, comparisons of human and mouse data show inter-species transcriptomic differences that may have implications for the use of animal models in functional genetics research.

Poster 28 – Katarina Grobicki (University of Cambridge, UK)
The evolution of ribosomal proteins and their contribution to translational regulation during germline development

Ribosomal proteins, together with ribosomal RNAs, form the backbone of the cellular machinery responsible for catalysing protein synthesis. Ribosomal protein genes have been duplicated during evolution, leading to co-existing pairs of paralogous genes within species, and the implications of this remain to be determined. In Drosophila, at least one ribosomal protein gene in these pairs is expressed ubiquitously and is essential for translation; mutations in these genes are homozygous lethal and heterozygotes usually display the minute phenotype. These are referred to as canonical ribosomal protein

genes. Less is known about their paralogues arising from duplications (referred to as non-canonical), however a number show tissue-specificity and many have enriched expression in the germline. We performed systematic mutagenesis and showed that non-canonical ribosomal protein genes are generally not required for viability or fertility, while identifying one which is essential for female fertility, RpS5b, and another which is essential for male fertility, RpS28a. Investigation of the RpS5b mutant phenotype indicates that it plays a key role in the inter-tissue signalling between the germline and the soma that is required for successful oogenesis. We are currently dissecting the molecular mechanisms by which non-canonical ribosomal proteins regulate germline biology to ensure proper gametogenesis.

Poster 30 – Archita Mishra (Indian Institute of Technology Kanpur, India) *Virtual

Understanding how NeuroD1 regulates neurogenesis in the chick pallium

Neurogenesis is a multi-step process that sequentially involves neural stem cell specification, proliferation, cell-cycle exit, differentiation, migration and maturation. In Xenopus, a comprehensive screen for transcripts with ¬the ability to induce precocious neurogenesis led to the identification of NeuroD1, a basic-helix-loop-helix (bHLH) transcription factor. A knock-out mouse was created to further investigate the role of NeuroD1 that showed no significant effect on the process of neurogenesis in the forebrain. It is likely that the absence of NeuroD1 was compensated by other members of NeuroD-family such as NeuroD2 and, NeuroD6 that share spatio-temporally overlapping expression domains in the developing mouse forebrain. Due to functional redundancy among multiple members of the NeuroD family, there are very limited reports that describe the function of NeuroD1 in the forebrain using a loss-of-function strategy. Interestingly, birds express only one member of NeuroD family i.e., NeuroD1 in the forebrain /pallium. Hence, we have used the avian model to investigate the role of NeuroD1 in the overall process of neurogenesis in the pallium using both loss-of function and gain-of-function approaches. We found that NeuroD1 regulates the levels of expression of Pax6, factor regulating stemness and the process of differentiation of neural stem cells. Furthermore, the gain-of-function of NeuroD1 promoted precocious differentiation and migration of pallial neural progenitor cells. Additionally, manipulation of NeuroD1 also perturbed some of the components of the FGF signaling pathway that have been implicated in regulation of proliferation of neural stem cells in the mouse forebrain. Therefore, NeuroD1 appears to be crucial for regulating multiple steps of neurogenesis in an evolutionary conserved manner. Acknowledgement-This project is funded by SERB Govt. of India.

Poster 32 – Irene Amblard (LMS MRC, UK)

Investigating the regulatory logic controlling epiblast regionalisation and the production of caudal cell types of the body plan

A long-held question at the heart of developmental biology is understanding how extrinsic signals can be interpreted by cells to produce a wide variety of cell types. In the post-implantation epiblast, anterior versus posterior progenitors are established 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, in response to WNT and FGF signals. However, as epiblast cells undergo differentiation, they lose the competence to express CDX2 in response to the same cues. Using embryonic stem cells to model the transition from epiblast to spinal cord or paraxial mesodermal progenitors, we are investigating the molecular basis that underpins this loss of competence. Our data suggest that CDX2 expression is controlled by tissue-specific regulatory mechanisms which can be recapitulated in vitro. Using genome engineering approaches to generate targeted enhancer deletions with CRISPR/Cas9 we are systematically testing regulatory element function. This strategy will shed light on the regulatory logic that underpins the competence to produce trunk derivatives in the body plan, and provides an opportunity to control this competency window through the manipulation of regulatory elements.

Poster 34 – Natalia Lopez Anguita (Max Planck Institute for Molecular Genetics, Germany) *Virtual

Hypoxia induces a transcriptional early primitive streak signature in pluripotent cells enhancing spontaneous elongation and lineage representation in gastruloids

The cellular microenvironment together with intrinsic regulators shapes stem cell identity and differentiation capacity. Mammalian early embryos are exposed to hypoxia in vivo and appear to benefit from hypoxic culture in vitro. Yet, components of the hypoxia response and how their interplay impacts cell transcriptional networks and lineage choices remain poorly understood. Here we investigated the molecular effects of acute and prolonged hypoxia on distinct embryonic and extraembryonic stem cells types as well as the functional impact on differentiation potential. We find a temporal and cell type-specific transcriptional response including an early primitive streak signature in hypoxic embryonic stem (ES) cells mediated by HIF1a. Using a 3D gastruloid differentiation model, we show that hypoxia-induced T expression enables symmetry breaking and axial elongation in the absence of exogenous WNT activation. When combined with exogenous WNT activation, hypoxia enhances lineage representation in gastruloids, as demonstrated by highly enriched signatures of gut endoderm, notochord, neuromesodermal progenitors and somites. Our findings directly link the microenvironment to stem cell function and provide a rationale supportive of applying physiological conditions in models of embryo development.

Poster 36 – Suad Alghamdi (University of Liverpool, UK) *Virtual

Lineage tracing of Wt1 role in the development of peritoneal and intestinal vasculature of mice embryos and gastruloids

During embryonic development, the epicardium, the mesothelium of the heart, contributes VSMCs to the coronary vessels, however, the developmental origin of vascular smooth muscle cells (VSMCs) in the embryo is not clearly defined. Wilm et al. 2005, have previously reported that cells expressing the Wilms tumour protein (Wt1) give rise to VSMCs in the intestine, mesentery, and coronary vessels. Because Wt1 is expressed in the mesothelium of the body cavities, we concluded that Wt1-expressing mesothelial cells contributed to the VSMC compartment in the respective organs. However, our result has revealed that the VSMC lineage may be more complicated. This study used temporally controlled lineage-tracing experiments for different embryonic stages between E18.5 and E8.5 of embryonic development. These experiments led to the finding that Wt1 is switched on and remains expressed in the visceral mesothelium from around E9.5 onwards, while earlier transient expression of Wt1 in the nascent mesoderm specifies the future vascular and visceral smooth muscle of the intestine and mesentery. Further analysis of the Wt1-expressing mesoderm population was done by using the gastruloid as a valuable tool to study early embryonic development. In this study, the gastruloid was marked with different intermediate, lateral plate and paraxial mesodermal markers to figure the contribution of these markers in the cells that expressed Wt1. Interestingly, our findings conclude that the origin of the Wt1 expressed cells that give rise to VSMSC in the intestine and the mesentery, appears to be mesodermal derived progenitor cells following a specific journey during the embryonic development.

Poster 38 – Mallika Chatterjee (Amity University, India) *Virtual

Heparan sulfate modifications determine navigation properties of thalamocortical axons in the developing mouse forebrain

Development of precise topographical connections between the thalamus and cortex is imperative for accurate sensory and motor functioning of the vertebrate body. Thalamocortical axons (TCAs) navigate complex territories before reaching their final cortical destinations. This complex route is designed by the intricate, context dependent function of various guidance molecule-receptor complexes like Slit-Robo, Erbb-neuregulin, Nrp2-semaphorin etc. Of late, heparan sulfate proteoglycans (HSPGs) has been shown to be key functional interactors of signalling and axon- guidance molecules. Various post-translationally modified HSPGs have been shown to play important roles in determining corpus callosum and optic chiasm development. However, their function in determining the trajectory of forebrain projection fibers has not been yet looked into. Gbx2, a homeodomain containing transcription factor is expressed in the developing mouse thalamus. Our microarray data shows that Gbx2 regulates thalamic expression of all three isoforms

of Hs6st -a key enzyme of the heparan sulfate synthesis pathway known to be involved in the 6 Ortho (6O) sulfation of heparan sulfate. Gbx2 loss causes significant down-regulation of expression of all three isoforms resulting in an aberrant sulfation pattern within the mutant TCAs. Analyses of Hs6st1/2 mutants revealed significant trajectory defects with some of these mutant axons being directed ventrally towards the hypothalamus – a partial phenocopy of Gbx2 mutants. This behaviour also recapitulates Slit/Robo mutant TCA defects. Using explant cultures we show that Slit/Robo interaction is indeed compromised in Hs6st1/2 mutants. The binding kinetics of Slit/Robo/HSPG in presence and absence of 6O sulfation are presently being characterized through in silico molecular dynamics simulation.

Poster 40 – Pallavi Dethe (Indian Institute of Technology Kanpur, India) *Virtual
Role of Wnt signaling in forebrain development

During embryonic development, patterning of several tissue types is orchestrated by interplay of one or more signalling centers. Developing chick brain is one such example where, anterior neural ridge acts as a source FGF ligands, dorsal forebrain acts as source of BMP, Wnt ligands and Retinoic Acid (RA). The forebrain roof plate midline undergoes invagination to divide the forebrain into two hemispheres, failure of which leads to holoprosencephaly. The roof plate midline has a characteristic W shape, wherein the middlemost part is thinner than the rest of the neuroepithelium. The roof plate midline also exhibits a very low rate of cell proliferation. Previous studies from our laboratory have demonstrated that BMP and RA signalling are active in the forebrain roof plate midline in mutually exclusive domains, RA in the middle, BMP in the flanks. Wnt signalling is present in the dorsal and lateral forebrain. BMP and RA signalling are known to regulate the invagination process. However, the role of Wnt signalling in the midline invagination process has never been investigated. We have performed a thorough screening for 40 key players of canonical Wnt signalling pathway and observed that many key players are differentially expressed in dorsal forebrain. These molecules tightly regulate the domain of active canonical Wnt signaling in the chick forebrain. Perturbing Wnt signaling by gain-of-function or loss of function affects patterning of the region undergoing midline invagination. However, Wnt signaling alone does not govern all aspects of the invagination process. Therefore, it is crucial to investigate interaction between different signaling pathways and their overall effect on the process of midline invagination.

Poster 42 – James Hammond (University of Oxford, UK) *Virtual
Exploring Evolvability of Segment Number in Teleost Fishes

Teleost fish show remarkable diversity in the number of vertebrae in their axial column. The number of vertebrae corresponds with the number of embryonic segments, somites, formed in the developing embryo during somitogenesis. The number of segments formed in the embryo is an emergent property of the dynamics of a complex molecular oscillator known as the segmentation clock, and the morphogenetic dynamics of axial elongation. This project aims to elucidate some of the features of this process which contribute to its evolutionary plasticity, using a mixture of experimental and computational techniques. Here, by using a phenomenological model describing cell movements in the zebrafish pre-somitic mesoderm (PSM), and a kuramoto-type model describing the zebrafish segmentation clock dynamics as a network of coupled phase oscillators, I show that the teleost segmentation clock is robust to cell ingressions into the PSM that occur during morphogenesis, but that mitosis can act to alter the emergent frequency of the clock. Additionally, I outline the proposed next steps for the project, involving the Lake Malawi Cichlids Astatotilapia calliptera and Rhamphochromis sp. ‘chilingali’ as a model system to study somitogenesis evolvability in teleost fish.

Poster Session II (odd numbers): 15:20 - 15:45 (BST)

Poster 1 - James O'Sullivan (King's College London, UK)

Mitochondrial function and remodelling along the tonotopic axis of the developing chick cochlea

In the vertebrate inner ear, the positions of sensory hair cells (HCs) along the basal-to-apical long axis of the cochlea determine the frequencies to which they are tuned (tonotopy). Despite growing understanding of how HC fate is specified versus the surrounding astrocyte-like supporting cells (SCs), the specific factors driving phenotypic refinement within these lineages along the tonotopic axis remain unclear. Since mitochondrial metabolism is emerging as a key driver of excitable cell maturation, we aimed to characterize mitochondrial morphology and activity in HCs and SCs along the tonotopic axis of the developing chick cochlea. Live and super-resolution imaging of cochlear whole mounts between E7 and E14 revealed spatial and temporal metabolic patterns and unique metabolic signatures between cell types. Immunohistochemistry revealed significant differences in mitochondrial distribution between high and low frequency cells by E14, and an extensive remodelling of the mitochondrial network during development. Using tetramethyl rhodamine methyl ester (TMRM) to monitor the mitochondrial membrane potential, we observe changes in mitochondrial activity throughout development and significant differences in mitochondrial metabolism between cell types. Our data suggest that mitochondrial activity and remodelling are linked to the functional refinement of HCs and SCs along the tonotopic axis of the developing cochlea.

Poster 3 - Shannon Taylor (University of Oxford, UK)

Dynamical regimes creating robustness in zebrafish axial patterning

Zebrafish posterior axis elongation is an ideal system with which to study the integration of cell movement with gene regulatory networks (GRNs). Within the pre-somitic mesoderm (PSM), cells undergo extensive, heterogenous rearrangements in space, yet still manage to form a sequential pattern of tbxta, tbx16, and tbx6 expression across the PSM (Fulton et al. 2022). To investigate how this pattern is formed despite cellular heterogeneity, Speiss et al. 2022; Fulton et al. 2022 developed a 'live modelling' approach where GRNs are simulated onto in vivo cell tracks, explicitly incorporating cell movement into a GRN simulation. We have further analysed this GRN model and found that it has two 'attractors', or stable gene expression states. One corresponds to the expression state of a cell in the anterior PSM, expressing high tbx6 and low tbxta/tbx16. The other is a posterior-like state expressing low tbxta, tbx16, and tbx6. Most cells in the PSM exist within the basin of attraction of the high tbx6 attractor, despite many having a tailbud-like gene expression state. This explains the surprising observation that cells removed from the tailbud and cultured in vitro upregulate tbx6, despite experiencing uniform signalling profiles (Fulton et al. 2022). We also simulated perturbing cells' signalling, finding that gene expression at the tissue level was largely robust to these perturbations. This suggests that patterning at the tissue level is robust to perturbations in signalling, potentially explaining how cells cope with the variability of extrinsic signals associated with heterogeneity in cell movements.

Poster 5 - Callum Bucklow (Department of Zoology, University of Oxford, UK)

Exploring Regionalisation of the Vertebral Column in East African Cichlids

Vertebrae are critical components of the axial skeleton, whose number and identity are established by the periodic addition of somites and subsequent anterior-posterior patterning by Hox genes. However, little is known about vertebral column regionalisation and the establishment of vertebral identities in teleosts. East African cichlids, particularly those native to the African Great Lake systems of Lake Tanganyika, Malawi, and Victoria, have undergone explosive adaptive radiations leading to remarkable morphological diversity. Preliminary evidence of vertebral count and regionalisation data from over 350 cichlid species across East Africa suggests interesting inter- and intra-lake trends in these traits. For example, Lake Tanganyika cichlids have evolved more disparate vertebral counts and axial proportions than the younger Lake Malawi or Victoria radiations. Moreover, in contrast to the closely related haplochromine cichlids, the Tropheini tribe from Lake Tanganyika have evolved elongated precaudal regions, which may indicate evolutionary modification of anterior-posterior Hox-patterning and the uncoupling of axial patterning and

somitogenesis during embryogenesis. We have now constructed a µCT-scan library of a diverse range of Lake Malawi cichlid species, representing an extremely closely related radiation of East African cichlids. Future work will apply 3D geometric morphometrics to examine vertebral shape variation and column regionalisation across the lake phylogeny and investigate how modified Hox patterning may modulate vertebral identity in a subset of Lake Malawi cichlid embryos.

Poster 7 – Sheila Xie (LMS, MRC, UK)

Recruitment of Dux to nucleoli is required for embryonic 2-cell state exit

During early embryo development, transcriptional activation of endogenous retrotransposons (MERVL) inducing ZGA is critical for embryo progression. The extensively upregulation of MERVL and MERVL-driven genes is restricted at the 2-cell stage, indicating totipotency. However, the mechanism and requirement for MERVL and 2-cell gene upregulation are poorly understood. Furthermore, this MERVL-driven transcriptional program must be rapidly turned off to allow 2-cell exit and developmental progression. Here, we report that MERVL activator Dux repositioning to nucleolar heterochromatin and proper rRNA synthesis are necessary for embryo exit from the 2-cell state. We reveal that blocking rRNA synthesis or preventing nucleolar phase separation enhances conversion to a 2C-like state in mESCs by releasing Dux from the nucleolar heterochromatin. In embryo, inhibition of rRNA synthesis prevents Dux switching off and leads to 2-4-cell rest. Mechanistically we found that chromatin decompensation using an acidic DEL peptide of crisp/dCas9, without affecting transcription, is sufficient to induce Dux repositioning, and recruiting a synthetic activator can further enhance Dux relocation. These indicate that Dux positioning is driven by its activation and nuclear reorganisation. Our findings demonstrate an important link among rRNA synthesis, nucleolar organisation, gene positioning and its repression during early development.

Poster 9 – Dillan Saunders (University of Cambridge, UK)

Neural and mesodermal tissues proportions are robust to progenitor ablation in the zebrafish tailbud

Posterior body axis elongation requires multiple tissues to form in a coordinated manner such that a correctly proportioned body plan is established. Within the tailbud, a neuro-mesodermal competent (NMC) progenitor can differentiate into either a neural or paraxial mesodermal cell. We hypothesise that NMC cells provide a flexible progenitor pool to compensate for any potential imbalance in progenitor numbers, thereby providing a regulative mechanism to ensure the correct proportioning of the spinal cord and paraxial mesoderm. To test this, we utilised 2-photon ablation to remove cells from 35% of the zebrafish neuro-mesodermal region. Ablation causes localised cell death with tissue healing complete within 2hrs. Following ablation, neural and mesodermal gene expression patterns are disrupted and then correctly re-established as surrounding cells move into the wound site. This regulation eventually leads to the correct formation of posterior neural tube and somites. Crucially, regulation does not involve elevated division levels within the tailbud. Overall, this provides a model to study tissue proportion regulation at a single-cell level which we intend to utilise to understand how NMC cells compensate for progenitor loss.

Poster 11 – Laura Cowell (University of York, UK)

Transcriptional regulation by FGF in the switch from pluripotency to cell lineage commitment

During development, key signalling pathways activate transcription factor regulators to direct cells down specific lineages. The exact mechanisms required for stem cells to undergo the change from pluripotency to lineage commitment are not fully understood. Overexpression of the bHLH transcription factor myod (the ‘master regulator’ of the myogenic lineage) in Xenopus or mouse embryonic stem cells is not sufficient for muscle differentiation. This indicates additional factors are needed for effective myogenesis. Our hypothesis is that FGF signalling is a competence factor required for the transition from pluripotency to lineage commitment. We have used an organoid culture protocol to investigate myogenesis in explants of pluripotent cells from Xenopus embryos. We show the efficient induction of skeletal muscle in organoids expressing Myod and treated with Fgf4. These data support the hypothesis that FGF signalling acts as a

gatekeeper during cell lineage specification to allow differentiation of pluripotent cells into skeletal muscle. We have used NGS to determine the transcriptional output of the myogenic programme initiated by this protocol to identify key genetic players for further investigation.

Poster 13 – Jakke Neiro (University of Oxford, UK)

Identification of enhancer-like elements defines regulatory networks active in planarian adult stem cells

Planarians have become an established model system to study regeneration and stem cells, but the regulatory elements in the genome remain almost entirely undescribed. Here, by integrating epigenetic and expression data we use multiple sources of evidence to identify enhancer elements active in the adult stem cell populations that drive regeneration. We have used ChIP-seq data to identify regions with histone modifications consistent with enhancer identity and activity, and ATAC-seq data to identify accessible chromatin. Overlapping these signals allowed for the identification of a set of high confidence candidate enhancers predicted to be active in planarian adult stem cells. These enhancers are enriched for conserved transcription factor (TF) binding sites for TFs and TF families expressed in planarian adult stem cells. Foot-printing analyses provided further evidence that these potential TF binding sites are occupied in adult stem cells. We integrated these analyses to build testable hypotheses for the regulatory function of transcription factors in stem cells, both with respect to how pluripotency might be regulated, and to how lineage differentiation programs are controlled. Our work identifies active enhancers regulating adult stem cells and regenerative mechanisms.

Poster 15 – Amber Harper (Oxford Brookes University, UK)

Studying the outcomes of whole-genome duplication in arachnids

Spiders belong to the Chelicerata, an arthropod group that branch basally to the Mandibulata (insects, crustaceans, and myriapods), and so are good models to study whether a trait is ancestral or derived with respect to the arthropod common ancestor. Within the Chelicerata there have been independent whole genome duplications (WGD) in the lineages leading to horseshoe crabs and arachnopulmonates (spiders, scorpions, and their kin). We aim to better understand the consequences of the ancestral arachnopulmonate WGD event for the development and evolution of spiders. We surveyed Hox, Wnt and frizzled genes in de novo embryonic transcriptomes from a wolf and jumping spider, belonging to the derived retrolateral tibial apophysis (RTA) clade, and two amblypygids in addition to twelve existent genomic and transcriptomic resources for chelicerates. We identified retained ohnologs across spiders, scorpions, and amblypygids in these three key developmental gene families, consistent with previous surveys of homeobox and other developmental genes. The improved taxonomic sampling in our study provided further evidence for the ancestral arachnopulmonate WGD and allowed us to identify likely patterns of ohnolog retention and loss. Furthermore, we identified the first reported duplicates of Wnt1/wg in any animal and the first Wnt10 in any arachnid. I have now started to analyse ohnologs in the common house spider Parasteatoda tepidariorum, to determine their roles in development and if they have been subject to sub or neofunctionalization. My future work in Parasteatoda to compare open chromatin regions of ohnologs, including the two Hox clusters, will help to understand regulatory divergence in retained ohnologs after WGD.

Poster 17 – Kero Guynes (Queen Mary University of London, UK)

The evolution and role of DNA Methylation in Annelida

DNA methylation (5mC) is a conserved epigenetic mark found across eukaryotes and 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 shown the importance of 5mC throughout development and especially in silencing TEs. In invertebrates, however, the functional implications of this mechanism remain unclear, particularly in terms of its role in gene regulation and TE repression. To address this knowledge gap, we dissected the DNA methylation machinery, including the levels and dynamics of 5mC in Annelida, one of the largest and more diverse groups within Lophotrochozoa. We focused on three annelid species with varying genome sizes, gene repertoires and TE landscapes, and

performed whole-genome bisulphite sequencing (WGBS) at embryonic and adult stages. Dimorphilus gyrociliatus, a meiobenthic annelid with a compact genome of just ~70Mb and ~5% TEs, lacks 5mC methylation, whereas Owenia fusiformis and Capitella teleta, with larger genomes (500Mb and 330Mb, respectively) and TE landscapes (~60% and ~30%, respectively) exhibit higher levels of methylation (20.6% and 7.6%, respectively). In these two species, DNA methylation predominantly concentrates in gene bodies and the global levels decrease as development progresses in O. fusiformis. However, we also observe specific and dynamic targeting of certain TE classes with 5mC methylation in these two annelids. Together, our work reveals an array of 5mC landscapes in Annelida, which positively correlates with TE abundance, further suggesting a potential role of 5mC in transcriptional regulation during embryogenesis in this animal group.

Poster 19 – Scott Wilcockson (The Francis Crick Institute, UK)

An improved Erk-specific reporter reveals oscillatory Erk dynamics linked to the cell cycle during early development

In the early zebrafish blastoderm, long-range FGF signalling at the embryonic margin patterns the mesendoderm. 'Snap-shot' views of development in fixed tissues reveal that although FGF signalling forms a gradient at the margin, using phosphorylated Erk (pErk) as a read-out, the levels of activity are highly variable between neighbouring cells. Interestingly, differential Erk signalling dynamics over time have been shown to influence cell fate decision making and cellular behaviour. We therefore sought to investigate the temporal dynamics of FGF/Erk signalling during early development to ask whether this is the source of heterogeneity in signalling levels. To do this, we have improved the specificity of an Erk-Kinase Translocation Reporter (KTR) to enable real-time visualisation of Erk activity in developing tissues in vivo. We find that Erk signalling is extremely dynamic and suggest that mitotic erasure of Erk activity introduces oscillations in Erk signalling, a phenomenon we also observe during early Drosophila anteroposterior patterning. We also observe that the rate of Erk signal restoration post-mitosis is a source of heterogeneity in the developing zebrafish mesendoderm. Finally, we show that signalling downstream of pErk does not reflect the shape of the pErk gradient, however, the mechanisms that shape the pErk gradient regulate the sensitivity of cells to changes in signalling levels over time. Going forward, our modified KTR will be an important resource for the in vivo study of Erk signalling dynamics during development and our future work will ask how Erk signalling dynamics and heterogeneity influence tissue patterning.

Poster 21 – Miha Modic (The Francis Crick Institute, UK)

Stepwise activation of selective mRNA decay coordinates the pluripotency progression and epiblast lumenogenesis

The peri-implantation period involves a rapid progression through rosette formation and lumenogenesis, coupled with rosette to formative pluripotency transition. Guided by machine learning, metabolic RNA sequencing, proteomics and RBP interactome analyses we identify a stepwise activation of mRNA decay that ensures that the naïve pluripotency-associated mRNAs are cleared in the narrow time window at the exit of the rosette stage. Loss of WNT signalling is initially required for OTX2-dependent transcriptional induction of LIN28A, and at the rosette stage is followed by MEK-dependent phosphorylation that relocates LIN28A and activates its capacity to directly induce selective mRNA decay of mRNAs while creating a delayed negative feedback system that restricts the MEK response to the rosette. Strikingly, ablation or nuclear retention of LIN28A arrests the cell fate at the rosette expression programme whereas morphological determination proceeds to lumenogenesis, leading to an unforeseen embryonic multiplication with impaired gastrulation. These findings show that cross-regulation between signalling, transcription factors and post-transcriptional mechanisms is essential for the appropriate coordination of cell fate and morphogenetic transitions in early development.

Poster 23 – Gemma Sutton (University of Exeter, UK)

Spotting changes in zebrafish stripes: Wnt/ß-catenin regulation of pigment development

Vertebrate pigment cells are derived from the neural crest, a multipotent embryonic cell population with extraordinary migratory capacity. As well as pigment cells, the neural crest forms a variety of cell derivatives including neurons and glia of the peripheral nervous system, cardiomyocytes and ectomesenchymal cells in craniofacial tissue. Zebrafish have three neural crest-derived pigment derivatives; black melanocytes, iridescent iridophores and yellow xanthophores. The Wnt/ß-catenin signalling pathway has an ongoing role in neural crest development. In the process of neural crest fate restriction, Wnt/ß-catenin promotes the specification of the melanocyte cell lineage at the expense of neuronal derivatives. To identify a developmental time window in which Wnt/ß-catenin signalling is required for neural crest development in zebrafish, we treat embryos with chemical inhibitors that inhibit or over-activate the Wnt/ß-catenin pathway at discrete developmental stages. Changes in pigment derivatives are subsequently quantified at the larval stage. These manipulations of Wnt/ß-catenin signalling have revealed an ongoing role of the pathway in regulating numbers of melanocytes and their migration pathways in the embryo. Notably, we have also found evidence of a role of Wnt/ß-catenin signalling in iridophore development; with over-activation of the signalling pathway resulting in a marked reduction of zebrafish trunk iridophores. Overall, our findings are indicative of a crucial role of Wnt/ß-catenin signalling in the promotion of the melanocytic lineage and repression of the iridophore pigment derivative during neural crest specification in zebrafish.

Poster 25 – Lea Darnet (King's College London, UK)

Development of dendritic arborisations of amacrine cells underlying orientation selectivity in the vertebrate visual system

The retina is not only a camera but pre-processes visual information detected by photoreceptors before transmitting the results to the brain. This computation is a process of extracting visual features in different channels such as colour, contrast or orientation. It had been suggested that orientation selectivity (OS) originates from specific ACs that exhibit asymmetric dendritic field shape. Indeed, our laboratory identified in zebrafish a specific subset of ACs (type II and III), thus suggesting that the elongated dendritic field shape is critical for generating this visual feature. However, despite the importance of dendritic structure for its function, little is known about how the orientation-selective ACs develop their specific dendritic arborisations and orient them correctly within the retina. Here, using in vivo confocal time-lapse imaging, two-photon microscopy and optogenetics, we study the cellular interactions underlying the structural and functional development of orientation-selective ACs. We first examined the development processes of dendritic arborisations. We found that elongated dendritic arborisation in type II cells is established through extension of selective dendrites along one axis. We found that light deprivation generates a decrease of orientation selectivity, of their dendritic field size but did not affect the complexity of these ACs. Finally, to study how cellular interactions shape the type II/III dendritic morphology, we removed the postsynaptic targets of these cells by using the lakritz mutant line that lacks all RGCs. This results in a strong phenotype, including aberrant cell positioning and defects in dendrite formation, arborisations and field size. Taken together, these results suggest that the development of orientation selectivity of these cells is dependent on sensory stimulation, as well as the presence of their postsynaptic targets. The work presented is funded by grants from the BBSRC and the MRC.

Poster 27 – Punkita Lohiya (King's College London, UK)

Identifying key factors in the formation of mammalian heart valves

Heart valve defects are the most common birth defects in new-borns worldwide. Endocardial (EC) cells are a subpopulation of endothelial (ET) cells that have a unique ability to undergo endothelial-to-mesenchymal (EndoMT) transition, a process critical for heart valve formation. To date, only one molecular maker, NFATc1, is known that uniquely labels EC cells during valve development. However, it is not the master regulator of EC cell specification since mice deleted for Nfatc1 forms underdeveloped heart valves. The ability of EC cells to undergo EndoMT makes them distinct from ET cells, despite both sharing a common precursor origin during cardio-genesis. We hypothesize that EC cells become distinct from ET cells via the expression of a sub-set of genes that are regulated differently at genetic and epigenetic levels.

The integrated analysis of bulk RNA-sequencing and whole-genome bisulphite sequencing data alongside single-cell RNA-sequencing datasets from public domain has identified candidate genes involved in EC-fate determination. These candidates have been validated at the gene expression level. Additionally, inducible shRNA knockdowns of candidate genes have been studied in an Nfatc1- mCherry mouse ES cell line using an in vitro hanging drop culture differentiation system. This has provided a functional assay for the temporal knockdown effects of these genes on the EC differentiation and specification. One of the top candidates is Zfpm1 and the effect of its knockdown on the functional ability of EC cells to undergo EndoMT is assessed using trans-well invasion and migration assays. Altogether, this will not only facilitate our understanding of the role of Zfpm1 in heart development but will also provide a basis for stem cell-based therapies for valve-related defects.

Poster 29 – Amber Ridgway (Oxford Brookes University, UK)
Using evolution to uncover development in Drosophila male genitalia

External male genitalia have frequently been described as one of the most rapidly evolving insect body parts. Within Drosophila, D. simulans and D. mauritiana exhibit striking morphological differences in the size, shape, and bristles of the male periphallic genitalia, despite only diverging 200,000 years ago. This raises the question of what genetic differences have led to this phenotypic divergence, however, the underlying developmental programme required to form these structures is still not well understood. We generated RNA-Seq data from developing genitalia of D. simulans and D. mauritiana males and focused on a subset of highly expressed and differentially expressed transcription factors (TFs) within and between species to pinpoint those that could be both developmentally, and evolutionary, important. RNAi knockdown of candidate genes revealed thirteen TFs that had a significant phenotypic effect. From those differentially expressed TFs between D. simulans and D. mauritiana, RNAi of Sox21b revealed a repressive role on posterior lobe growth. To see whether Sox21b has evolved between the two species, we designed reciprocal hemizygotes that differed only in the working allele of Sox21b. This study showed that Sox21b has evolved between the two species. C15, a homeobox TF, results in an increase of clasper bristles and disrupts bristle patterning when gene expression is reduced, pinpointing this gene as essential to clasper development. To understand where in the clasper C15 functions, we conducted immunofluorescence at key developmental stages, which identified distinct C15-expressing cells. This has led us closer to uncovering the gene regulatory network directing clasper development.

Poster 31 – Dan Holder (UCL Great Ormond Street Institute of Child Health, UK)
Investigating neurogenesis in the developing human brainstem

Animal models do not recapitulate major features of human neural development, and interrogation of human neurogenesis, which is driven by apical and basal radial glia cells, has largely focused on the cortex. Thus, it remains unclear whether mechanisms of cortical neurogenesis are conserved in the human hindbrain, which comprises the cerebellum and brainstem, structures essential for survival. Recent work has indicated the presence of radial glia-like cells in the cerebellum, and this study aims to characterise progenitor populations in the developing human brainstem. Here we demonstrate that the progenitor zone of the human brainstem ventricular zone exhibits spatial compartmentalisation, with radial glia-like cells being identifiable both at the ventricular surface and in both inner and outer subventricular zones by immunohistochemistry. These cells exhibit variable morphology but extend basal processes and can be identified across the embryonic and early foetal periods, with the relative proportion of basally dividing radial glia-like cells increasing over time. Furthermore, we use immunohistochemistry to identify CS17 as the peak of proliferation in the developing brainstem and find dynamic differences in the proliferation rate along the medio-lateral axis across developmental time. Together, these data suggest that the human brainstem shares key similarities to cerebellar and cortical neurogenesis, with the presence of variable radial glia-like cells. This initial characterisation can provide the basis for further functional assessment of human brainstem progenitors.

Poster 33 – Raasib Mahmood (UCL Great Ormond Street Institute of Child Health, UK)

Why does the brain fail to close more commonly in females than males

A very intriguing question in neural tube defect research is why females present with anencephaly (open brain) more commonly than males. This female abundance is observed in humans and genetic mouse models, suggesting a fundamental sex difference in early brain development. The X chromosome inactivation (XCI) hypothesis has been suggested to explain this female excess. XCI is a methylation-dependent process that 'balances up' X-linked gene expression in female cells by inactivating one X chromosome. The inactive X is suggested to create an 'epigenetic sink' putting cellular methylation reactions at risk in females at the time of cranial neurulation – increasing female susceptibility to anencephaly. The XCI hypothesis was tested to assess the anencephaly sex bias in mice. Methyl group deficiency in CD1 embryos was induced by 24 h whole embryo culture with cycloleucine, a drug that inhibits S-adenosyl-methionine (SAM) production, the methyl group donor for DNA, RNA and proteins. Cycloleucine treatment resulted in mouse exencephaly which presented with a female excess. To test whether SAM depletion underpinned this defect, cycloleucine-treated embryos were treated with MGBG, a SAM decarboxylase inhibitor, to enhance SAM availability for methylation use. MGBG rescued cycloleucine-induced exencephaly in female but not male embryos. Our findings support the XCI hypothesis in explaining the female bias in anencephaly.

Poster 35 – Anna Koerte (Max Planck Institute of Cell Biology and Genetics, Germany) *Virtual

Spatial and temporal interplay between mitochondrial metabolism and energy expenditure during early embryogenesis

In animals, fertilization is followed by a period of rapid cell divisions that expand the number of cells during cleavage stage. These cells become smaller as they divide, producing a multicellular embryo with the same volume as the fertilized egg. Despite the absence of volumetric growth, energy expenditure increases substantially with each division to fuel active cellular processes underlying cleavage development. This poses a dynamic challenge for metabolism to satisfy the increasing requirements for energy. However, how embryonic energy metabolism meets these increasing energy demands remains elusive. Energy supply in early embryos relies on mitochondrial oxidative phosphorylation. Mitochondria are maternally deposited into the oocyte and mitochondrial replication is absent during early development, suggesting that a gradual activation of mitochondrial function occurs during cleavage divisions. Live imaging of mitochondrial localization and membrane potential as a readout for mitochondrial activity in zebrafish embryos shows that mitochondria are fragmented and evenly distributed throughout the embryonic cells. Strikingly, only mitochondria close to the plasma membrane harbor a measurable membrane potential. As development proceeds and the cellular surface to volume ratio increases, we observe that the fraction of mitochondria with a measurable membrane potential increases concomitant with the increase in energy expenditure. These findings suggest that an interplay between energy expenditure at the plasma membrane and spatial regulation of mitochondrial function exists in early embryos. In the future, we aim to gain mechanistic understanding of this proposed spatial and temporal coupling between mitochondrial activity and energy expenditure at the plasma membrane.

Poster 37 – Meenu Sachdeva (Indian Institute of Technology Kanpur, India) *Virtual

Understanding the role of cell adhesion in the process of forebrain roof plate invagination resulting in separation of the cerebral hemispheres

The invagination of the forebrain roof plate leads to the division of the single telencephalic vesicle into two cerebral hemispheres, the failure of which often leads to a congenital disorder known as holoprosencephaly (HPE). While loss of function of several genes have been linked to HPE, there is very little mechanistic insight available. The invagination of the roof plate is conserved between birds and mammals, which prompted us to study this process in the chick (Gallus gallus) embryo. To understand the forces driving roof plate invagination we carried out in silico modeling studies. Preliminary results indicated that stiffness of the roof plate neuroepithelium and its interaction with the overlying mesenchyme are likely to play important roles. Since the stiffness of tissues is often determined by the

expression of cell adhesion molecules, we screened for their expression in the developing chick forebrain, and found some members of the Cadherin family to be differentially expressed in the roof plate. Subsequent functional manipulation of these Cadherins lead to dramatic defects in the forebrain roof plate such as evagination instead of invagination. At present we are in the process of quantifying the stiffness of the roof plate under normal conditions as well as under conditions where these cell adhesion molecules have been functionally manipulated. The results of these studies will provide further evidence in support of our hypothesis that differential stiffness of the forebrain roof plate is an important factor driving its invagination.

Poster 39 – Inês Pereira (ICVS, University of Minho, Portugal) *Virtual

Studying the impact of hyperglycemia in chick lung branching morphogenesis

Hyperglycemia in pregnancy is a major teratogenic factor, mainly associated with abnormalities in the cardiovascular and central nervous system, resulting in impaired embryonic development. However, the impact of hyperglycemia in early lung development is poorly understood. Accordingly, this study aims to unveil the effect of high glucose levels in early lung branching morphogenesis, using in vitro chick lung explants as a model. The chicken embryo model is an alternative to studying branching morphogenesis, as the molecular events underlying early lung organogenesis are very similar to mammalian. Fertilized chicken eggs were incubated for 4.5-5.5 days at 37ºC with a relative humidity of 49%. Embryonic lungs were collected and processed for in vitro lung explant culture. Chick lung explants were supplemented with 5.5mM (control), 25mM, 50mM or 75mM of D-Glucose and incubated at 37°C, 5% CO2 for 48h. Lung branching was monitored daily by photographing the explants. On day 0 and day 2, the total number of peripheral airway buds, the epithelial and mesenchymal area and perimeter of the lung were determined. Lung explants supplemented with 50mM and 75mM of D-glucose showed a statistically significant decrease in the number of secondary buds formed compared to controls. The total area of the lung and the epithelial area and perimeter were also decreased in 50mM and 75mM doses compared to the controls. However, no differences were observed between the 25mM dose and the control. This work shows that high glucose levels impair chick lung branching morphogenesis.

Poster 41 – Sumit Garai (Indian Institute of Science Education and Research and Max Planck Institute of Cell Biology and Genetics, India) *Virtual

Reassesing the single-cell transcriptomic landscape of stem-cell based embryo models

Mammalian post-implantation development occurs in-utero, precluding easy monitoring and manipulation. To circumvent this impediment, pluripotent stem cells (PSCs) can be coaxed to form embryonic organoids (or stembryos). Gastruloids are stembryos that, when grown in a favourable medium and pulsed with a WNT agonist, break symmetry, elongate, and eventually self-organise their body axes, resulting in structures reminiscent of the post-cephalic ~E8.5 embryo but lacking its stereotypical architecture. Recent work has shown various degrees of in-vivo-like architecture (gut tube, heart tube, somites, neural tube) can be unlocked by modulations of the extracellular environment. Here, we reassess the scRNA-seq data of different stembryos. Our initial results highlight similarities and differences between the different protocols. Interestingly, even gastruloids generated with highly similar protocols in different laboratories display vastly different transcriptomic signatures, which could be rooted in the use of PSCs of distinct genetic backgrounds. Most importantly, our analysis revealed that classifiers trained on two different in vivo references in many cases output different predictions for a given cell. We show that this is caused by inconsistencies in the annotation of the 'ground truth' in vivo cell states: cells with a similar transcriptional profile are differentially annotated in the two major in vivo compendia published to date. This lack of consensus represents a fundamental problem in the analysis of stembryo scRNA-seq data, as the annotation of “ground truth” cell states results in the divergent classification of the cell states detected in the models of embryo development.

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 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.