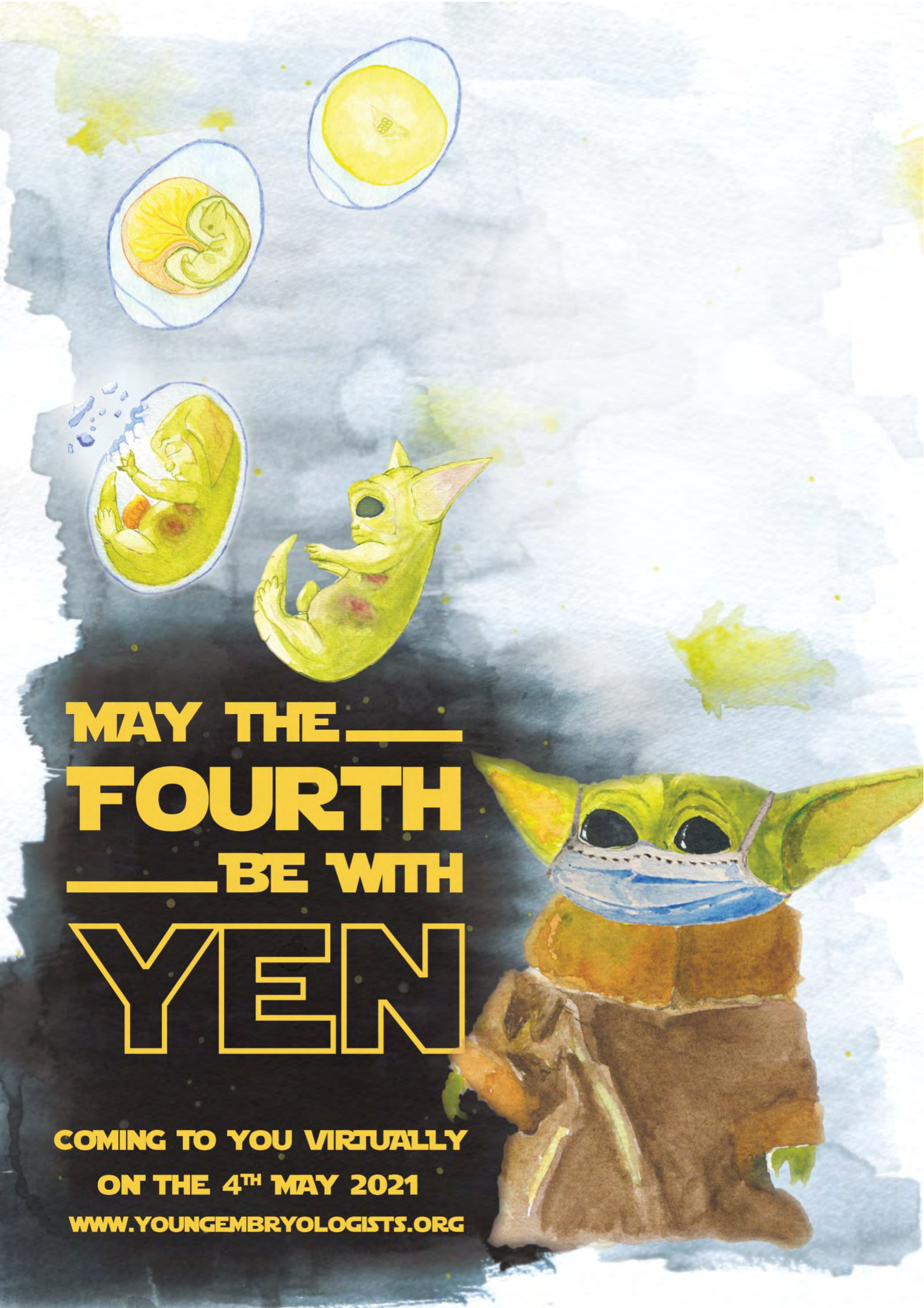
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About YEN

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 virtual, 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.

Acknowledgements

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

Committee: Sergio Menchero, Maddy Demuth, Nikolaos Angelis, Michelle Neumann, Ollie Inge, Eren Akademir, Jack Morgan, Eva Kane, Luca Zanieri, Ferran Garcia Llagostera, Daniele Kunz, Jeremie Subrini, Haskan Kaya, Richard Clayton, Foteini Papaleonidopoulou & Oliver Bower.

Judges: Karen Lee, Teresa Rayon & Naomi Morris

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Get in touch:

Twitter: @YEN_community (#YEN2021)
Website: http://www.youngembryologists.org/
Programme: http://www.youngembryologists.org/wp-content/uploads/2021/04/YEN2021Programme_final.pdf

We have also set up a Slack workspace where you will be able to post more questions for the speakers, interact with other participants outside of Remo and contact the YEN team if you require any assistance. This is the link to join:

Slack: https://join.slack.com/t/yen2021/shared_invite/zt-polur98j-9Y6tx3FECcnaveYQ4lYAIg

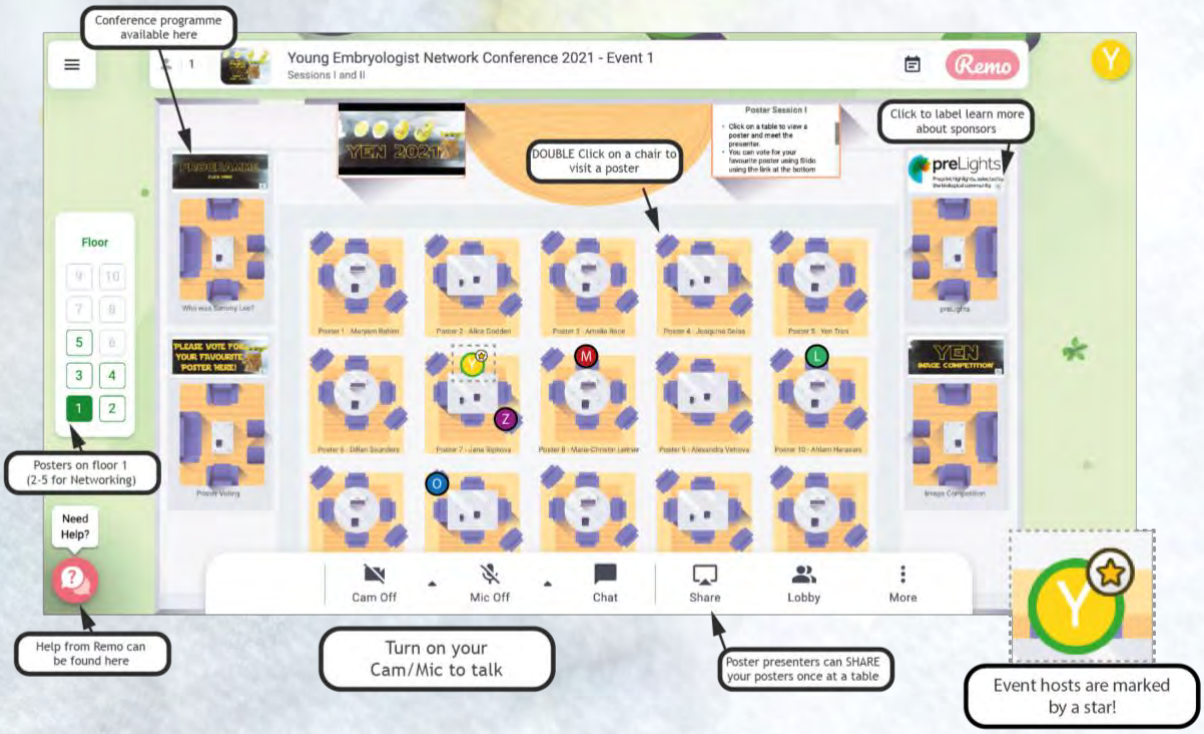
Join us virtually via Remo

This year, we are hosting our conference using Remo. Remo is an online platform which we hope will facilitate networking and provide interactive poster sessions. The conference will be split into two events:

Event 1: 9:00-13:25 (BST) https://live.remo.co/e/young-embryologist-network-confe-1
Event 2: 14:30-18:45 (BST) https://live.remo.co/e/young-embryologist-network-confe-5

Below, we're provided some information to prepare you for the day, as well as some troubleshooting tips if you encounter any technical difficulties. **We strongly recommend that you use a Google Chrome browser for the event** (we encourage you **not** to use Safari). The YEN team will be there to help you throughout the day and can be contacted through the Slack channels or via the Remo chat function.

This will be your venue for the day!



Preparation for starting Remo (For All Participants)

Requirements: PC with camera, speaker & MIC or headset

Browser Chrome (highly recommended)
for more information, check the Remo_Technical Requirements
https://help.remo.co/en/support/solutions/articles/63000251000-what-computer-devices-and-browsers-are-supported-on-remo-conference-

High-speed Internet access
Using headphones or earbuds is recommended

Remo System Check (Important): You can check on this and allow your browser to access your mic and cam from the Security & Privacy section in your System Preferences.
https://remo.co/mic-cam-test/

Here are some useful troubleshooting links:

How to login for a remo event:
https://remo1.freshdesk.com/en/support/solutions/articles/63000240849

My camera/microphone isn't working: https://remo.co/my-camera-microphone-isnt-working/

Common issues with screen sharing:
https://help.remo.co/en/support/solutions/articles/63000251574

How to allow Chrome access to your camera/microphone:
https://help.remo.co/en/support/solutions/articles/63000253364

How to allow Microsoft Edge access to your computer/microphone:
https://help.remo.co/en/support/solutions/articles/63000259814

Programme (BST)

EVENT 1: https://live.remo.co/e/young-embryologist-network-confe-1

9:00 - 9:15	Registration
9:15 - 9:20	Welcome address
9:20 - 9:50	**Mattias Lutolf** (École Polytechnique Fédérale de Lausanne, Switzerland) *Self-organizing in vitro neural tube organoids mimic embryonic development*
9:50 - 10:35 **Selected Short Talks I**	**Nazmus Salehin** (Children's Medical Research Institute, University of Sydney, Australia) *Mixl1 activation remodels chromatin at lineage specific enhancers to poise epiblast stem cells towards endoderm specification* **Benjamin Swedlund** (Université Libre de Bruxelles, Belgium) *Unravelling the chromatin landscape and enhancer logic mediating spatiotemporal patterning of early mouse cardiovascular progenitors* **Meritxell Saez** (The Francis Crick Institute, UK) *Gene-free landscape models of development*
10:35 - 11:00 **Scientific Perspectives I**	**Ana Pombo** (MDC Berlin, Germany)
11:00 - 11:45	Break - **Poster Session I** - Networking
11:45 - 12:15	**Alexander Aulehla** (EMBL Heidelberg, Germany) *Glycolytic control of embryonic patterning and timing*
12:15 - 13:00 **Selected Short Talks II**	**Pranay Shah** (MRC Laboratory of Molecular Biology, UK) *Embryonic germ cell development requires DNA translesion synthesis factors* **Julia Pfanzelter** (Max Planck Institute of Molecular Cell Biology and Genetics, Germany) *The physics of life and death - a hydraulic instability drives the cell death decision in the nematode germline* **Alejandro Aguilera Castrejon** (Weizmann Institute of Science, Israel) *Ex utero development of mouse embryos from pre-gastrulation to advanced organogenesis*

13:00 - 13:25 **Scientific Perspectives II**	**Patrick P. L. Tam** (Children's Medical Research Institute, University of Sydney, Australia)
13:25 - 14:30	Lunch break

EVENT 2: https://live.remo.co/e/young-embryologist-network-confe-5

14:30 - 15:30 **Selected Short Talks III**	**Daisy Vinter** (University of Manchester, UK) *Dynamics of hunchback translation in real time and at single RNA resolution in the Drosophila embryo* **Justina Yeung** (The Francis Crick Institute, UK) *The role of a short-range FGF signal in neurogenesis patterning and glial fate specification* **Susannah McLaren** (University of Cambridge, UK) *Anterior expansion and posterior addition to the notochord mechanically coordinate embryo axis elongation* **Meng Zhu** (University of Cambridge, UK) *The timing and trigger of mouse embryo polarization and cell fate segregation*
15:30 - 16:30 **The Sammy Lee Memorial Lecture**	**Christiane Nüsslein-Volhard** (Max Planck Institute for Developmental Biology, Germany) *Animal Beauty: Function and Evolution of Biological Aesthetics*
16:30 - 17:15	Break - **Poster Session II** - Networking
17:15 - 17:40 **Scientific Perspectives III**	**Marianne Bronner** (Caltech, USA)
17:40 - 17:50	Talk and poster prizes, closing address
17:50 - 18:45	Networking

Selected short talks

Nazmus Salehin

Mixl1 activation remodels chromatin at lineage specific enhancers to poise epiblast stem cells towards endoderm specification

During mouse gastrulation, the transcription factor MIXL1 is vital for mesoderm and definitive endoderm germ layer specification. Mouse Mixl1 knock-out models fail to progress past gastrulation while cells without functional MIXL1 do not contribute to definitive endoderm lineages in mouse chimera studies. In vitro studies using mouse embryonic stem cells showed activation of Mixl1 resulted in an increased efficacy of mesoderm and endoderm formation. More recently, within mouse epiblast stem cells (EpiSC) subjected to undirected differentiation, endodermal specification appeared to be correlated to the timing and robustness of Mixl1 expression. In a novel EpiSC model of Mixl1-timed activation, we show that modulation of Mixl1 timing influences the proportion of definitive endoderm cells at the end of undirected differentiation – early induction of Mixl1 results in the greatest proportion of definitive endoderm.

To explore the functional role of Mixl1 during this narrow window, we performed bulk ChIP-seq, RNA-seq and ATAC-seq studies of differentiating cells immediately after early Mixl1 activation. MIXL1 acts as a transcriptional activator of genes implicated in mesendoderm formation and repressor of ectoderm lineage genes.

Furthermore, the induction of Mixl1 results in an increase in the accessibility of mesendoderm specific enhancers and regions harbouring double ATTA motifs, and a decrease in accessibility of ectoderm specific enhancers and regions harbouring motifs for POU and SOX transcription factors. Taken together, these results suggest that the timing of Mixl1 expression influences germ layer specification during gastrulation by direct transcriptional activation and downstream chromatin remodelling of lineage specific enhancers.

Benjamin Swedlund

Unravelling the chromatin landscape and enhancer logic mediating spatiotemporal patterning of early mouse cardiovascular progenitors

The mammalian heart arises from various populations of Mesp1-expressing cardiovascular progenitors (CPs) that are specified during the early stages of gastrulation. Mesp1 acts as a master regulator of CP specification and differentiation. However, how Mesp1 regulates the chromatin landscape of nascent mesodermal cells to define the temporal and spatial patterning of the distinct populations of CP remains unknown. Here, by combining ChIP-seq, RNA-seq and ATAC-seq during mouse pluripotent stem cell differentiation, we defined the temporal remodelling of the chromatin landscape mediated by Mesp1. We identified different enhancers that are temporally regulated to erase the pluripotent state and specify the pools of CPs that mediate heart development. We found that Mesp1 acts as a pioneer transcription factor (TF) and identified Zic TFs as essential cofactors that regulate Mesp1 pioneer activity at key mesodermal enhancers, thereby regulating the chromatin remodelling and gene expression associated with specification of the different populations of CPs in vivo. Our study identifies the dynamics of the chromatin landscape and enhancer remodelling associated with temporal patterning of early mesodermal cells into the distinct populations of CPs that mediate heart development.

Meritxell Saez

Gene-free landscape models for development

Fate decisions in developing tissues involve cells transitioning between a set of discrete cell states. Geometric models, often referred to as Waddington landscapes, are an appealing way to describe differentiation dynamics and developmental decisions. We consider the differentiation of neural and mesodermal cells from pluripotent mouse embryonic stem cells exposed to different combinations and

durations of signalling factors. We developed a principled statistical approach using flow cytometry data to quantify differentiating cell states. Then, using a framework based on Catastrophe Theory and approximate Bayesian computation, we constructed the corresponding dynamical landscape. The result was a quantitative model that accurately predicted the proportions of neural and mesodermal cells differentiating in response to specific signalling regimes. Taken together, the approach we describe is broadly applicable for the quantitative analysis of differentiation dynamics and for determining the logic of developmental cell fate decisions.

Pranay Shah

Embryonic germ cell development requires lesion bypass

Germ cells are responsible for transmitting genetic information between generations. Ensuring faithful genome replication during the development and maturation of gametes is critical to limit the number of mutations passed onto offspring. Our work has found that whilst the error-prone bypass of DNA lesions by translesion synthesis (TLS) factors is dispensable for somatic lineages, mice with compromised TLS are sterile due to a complete absence of germ cells. We have discovered that this sterility is due to an essential role of lesion bypass factors during embryonic primordial germ cell (PGC) development. TLS-deficient PGCs are specified but lose genome stability, fail to complete epigenetic reprogramming and enter apoptosis. This results in a >150-fold reduction in the number of PGCs prior to entry into meiosis. Contrary to this, maintenance of somatic lineages remains unperturbed across a range of tissues with survival unaffected in the absence of TLS-factors. Thus, these factors are required during embryogenesis for the production of germ cells but are dispensable for somatic cell development and maintenance.

Julia Pfanzelter

The physics of life and death – a hydraulic instability drives the cell death decision in the nematode germline

Oocytes are large cells that contain sufficient amounts of cellular material to develop into an embryo upon fertilization. As germ cells mature into oocytes, some of them grow, typically at the expense of others that undergo cell death. The transfer of cellular material from dying to growing cells is facilitated by a syncytial structure with a shared cytoplasm. How germ cells are selected to live or die out of a homogeneous population remains unclear. Here we present evidence that in the nematode Caenorhabditis elegans this cell fate decision is mechanical and related to tissue hydraulics. An analysis of germ cell volumes and cytoplasmic fluxes reveals a local transition in germline hydraulics involving an inversion of the pressure difference between germ cells and the shared cytoplasmic pool. This triggers a hydraulic instability which amplifies volume differences and causes some germ cells to grow and others to shrink, a phenomenon that is related to the well-known two-balloon instability. We also find that cell shrinkage occurs upstream of the conserved apoptosis machinery implying that there is a minimal size threshold below which cell death is triggered. Shrinking germ cells are extruded and die, as we demonstrate by artificially reducing germ cell volumes via thermoviscous pumping. Our work reveals a hydraulic symmetry-breaking transition central to the decision between life and death in the nematode germline.

Alejandro Aguilera Castrejon

Ex utero Development of Mouse Embryos from Pre-Gastrulation to Advanced Organogenesis

Establishment of the mammalian body plan occurs shortly after the embryo implants into the maternal uterus, and our understanding of post-implantation developmental processes remains limited. While methods for in vitro culture of pre- and peri-implantation mouse embryos are routinely utilized, approaches for robust culture of post-implantation embryos from egg cylinder stages until advanced organogenesis remain to be established. Here we develop highly stable ex utero post-implantation mouse embryo culture platforms, that enable appropriate development of embryos before gastrulation (E5.5)

until the hind limb formation stage (E11). Late gastrulating embryos (E7.5) are grown in 3D rotating bottles settings, while extended culture from pre-gastrulation stages (E5.5 or E6.5) requires a combination of novel static and rotating bottle culture platforms. Histological, molecular, and single cell RNA-seq analyses validate that the ex utero cultured embryos recapitulate in utero development precisely. This culture system is amenable to introducing a variety of embryonic perturbations and micro-manipulations that can be followed ex utero for up to six days. Establishment of a system to robustly grow normal mouse embryos ex utero from pre-gastrulation to advanced organogenesis represents a valuable tool to investigate embryogenesis, eliminating the uterine barrier to mechanistically interrogate post-implantation morphogenesis and tissue specification in mammals.

Daisy Vinter

Dynamics of hunchback translation in real time and at single RNA resolution in the Drosophila embryo

The Hunchback (Hb) transcription factor is critical for anterior-posterior patterning of the Drosophila embryo. Despite the maternal hb mRNA acting as a paradigm for translational regulation, due to its repression in the posterior of the embryo, little is known about the translatability of zygotically transcribed hb mRNAs. Here we adapt the SunTag system, developed for imaging translation at single mRNA resolution in tissue culture cells, to the Drosophila embryo to study the translation dynamics of zygotic hb mRNAs. By imaging single mRNAs in fixed and live embryos, we provide evidence for translational repression of zygotic SunTag-hb mRNAs. While the proportion of SunTag-hb mRNAs translated is initially uniform, translation declines from the anterior over time until it becomes restricted to a posterior band in the expression domain. We discuss how regulated hb mRNA translation may help establish the sharp Hb expression boundary, which is a model for precision and noise during developmental patterning. Overall, our data show how use of the SunTag method on fixed and live embryos is a powerful combination for elucidating spatiotemporal regulation of mRNA translation in Drosophila.

Justina Yeung

The role of a short-range FGF signal in neurogenesis patterning and glial fate specification

One key aspect of neural development is the spatial and temporal control of neurogenesis for the continuous production of diverse neuronal and glia cell types. In particular, mechanisms that inhibit neurogenesis are important for maintaining progenitor cells for the specification of later-born neuronal subtypes and gliogenesis. During zebrafish hindbrain development, the expression of proneural genes becomes spatially restricted in each hindbrain segment, producing a stripe pattern along the anterior-posterior axis of the tissue. Previous studies have shown that Fgf20a-signalling emanating from a subset of early-born neurons at the centre of each hindbrain segment is responsible for patterning through inhibition of proneural gene expression. However, the mechanism by which the morphogen regulates the patterning, and the significance of it in neuronal and glial fate specification remains obscure. We took advantage of the high-resolution and multiplexing capability of next generation in-situ hybridisation chain reaction (HCR) to visualise the patterning. We analysed the spatial relationship between the Fgf20-signalling source, the reporter genes for Fgfr-signalling, and proneural genes expression in wildtype and perturbed embryos. The 3D expression pattern suggests that Fgf20 is acting as a short-range signal to pattern the neuroepithelium. This is congruent with the biochemical properties of FGF20 previously described in the literature. Furthermore, we investigated the potential roles of Fgf20 signalling in progenitor maintenance and glial fate specification. We show that Fgf20 does not regulate proliferation among the hindbrain progenitors. Instead, the signal sets the timing of gliogenic precursor induction, establishing the proportion of oligodendrocyte cells through a temporal cue.

Susannah McLaren

Anterior expansion and posterior addition to the notochord mechanically coordinate embryo axis elongation

During development the embryo body progressively elongates from head-to-tail along the anterior-posterior axis. We investigated how the morphogenesis of one tissue can physically deform its neighbouring tissues to contribute to axis elongation. The rod-shaped notochord runs through the middle of the embryo and is flanked on either side by the somites in the segmented region of the axis and presomitic mesoderm in the posterior. Cells in the notochord undergo an expansion that is constrained by a stiff sheath of extracellular matrix, leading to an increase in notochord stiffness as the embryo develops - making it a candidate for driving the physical deformation of surrounding somitic tissue. Using multi-photon mediated cell ablation, we removed specific regions of the developing notochord and quantified the impact on axis elongation. We show that anterior notochord cell expansion generates a force that displaces notochord cells posteriorly and contributes to the elongation of segmented tissue during post-tailbud stages of development. Crucially, unexpanded cells derived from posterior progenitors provide resistance to anterior notochord cell expansion, allowing for force generation across the AP axis. Therefore, notochord cell expansion and addition of cells to the posterior notochord act as temporally coordinated morphogenetic events that shape the zebrafish embryo AP axis.

Meng Zhu

The timing and trigger of mouse embryo polarization and cell fate segregation

During embryogenesis, developmental events are set to occur in a temporally ordered manner. In the mouse embryo, this event is programmed to occur at the 8-cell stage, and this timing follows an intrinsic developmental clock that is independent of embryo size or cell cycle progression. However, the molecular mechanisms to establish cell polarisation and the temporal regulation of this event have remained largely elusive.
Here, we identified stage-dependent transcription factors Tfap2c and Tead4 that synergises with RhoA to control the timing of the apical domain establishment. Importantly, the precocious activation of Tfap2c, Tead4 and RhoA sufficiently advances the timing of the apical domain formation, the timing of the following morphogenetic events, and the progression of cell differentiation. By combining quantitative imaging measurements and mathematical modeling, we show that apical domain formation is driven by the dynamic interplay between two key processes: 1) the cooperative recruitment of apical protein via the actin network and 2) the lateral mobility of apical protein on the membrane. Based on the experimental evidence and simulations of the biophysics of these interactions, we show that Tfap2c and Tead4 control the cooperative recruitment of apical protein whereas RhoA promotes membrane mobility. These results define a central step of the transition from totipotency to pluripotency during the early mammalian embryo development.

Selected posters

Please vote for your top three posters on Slido using the code **#232711** or by using the following link: https://app.sli.do/event/bjxzwc6u/embed/polls/0f4b3050-fd2b-48ec-837c-697edc22840f

Poster Session I: 11:00 – 11:45 (BST)

Poster 1 – Maryam Rahim (The Francis Crick Institute, UK)

Understanding molecular dynamics of enteric neuron subtype generation during gut organogenesis

The enteric nervous system (ENS) is the largest subdivision of the peripheral nervous system and plays an essential role in intestinal physiology and host defence. Despite recent progress, the molecular mechanisms governing the assembly of intestinal neural circuits remain obscure. Previous work at single cell resolution defined developmental trajectories and candidate regulators of neuronal and glial differentiation in the small intestine, but did not investigate the ENS in the large intestine; an organ with distinct anatomical and functional features to the small intestine. Additionally, the ENS of the large intestine is uniquely derived from both vagal and sacral contributions of Sox10-positive neural crest cells.

To elucidate the molecular mechanisms underpinning the development and generation of neuronal diversity in the large intestine, we combined single-cell transcriptomics (scRNA-seq) and mathematical modelling. We performed scRNA-seq on Sox10-labelled ENS populations at key developmental stages of the large intestine (E14.5, E16.5, E18.5 and P2). Louvain clustering identified progenitors, neurons and glial populations at varying developmental stages as defined by known markers (Elavl4, S100b). To unravel the molecular mechanisms underlying neuronal subtype specification, we performed pseudotime trajectory analysis on the neurogenic populations, revealing the emergence of two cardinal neuronal subtypes- an inhibitory Nos1+ and an excitatory Chat+. Our mathematical modelling approach allowed us to gain further insight into the generation of neuronal diversity at the level of variance of gene expression over pseudotime. Our study identifies candidate regulators of neuronal subtype specification in the ENS and further aims to unearth the regulatory networks underpinning the generation of enteric neuronal diversity.

Poster 2 - Alice Godden (University of East Anglia, UK)

Investigating the role of micro RNAs in the development of Xenopus neural crest

The neural crest is a multipotent stem-cell population. It is specified during early neurulation and that undergoes EMT and proceeds to migrate to various points in the developing embryo where they give rise to a number of tissues including parts of the peripheral nervous system and craniofacial skeleton. The detailed fine-tuning of neural crest specification is increasingly being elucidated but many questions remain. Dysregulation of neural crest results in many diseases grouped under the term neurocristopathies. These include in-born defects like Di George's syndrome which presents with craniofacial structural defects and reduced cardiac outflow septation and Waardenburg syndrome; presenting with Pax3 mutations, pigment defects and mild craniofacial dysmorphogenesis.

miRNAs are short non-coding RNAs 20-22 nucleotides long which affect gene expression through post-transcriptional repression. Our lab has shortlisted microRNAs-196a and -219 as potentially implicated in the development of Xenopus neural crest. The molecular pathways affected by these microRNAs have been investigated by using morpholinos and CRISPR-Cas9 revealing neural crest craniofacial phenotypes. Development of neural crest and other tissues is being evaluated using: whole-mount in situ hybridization of key neural crest and neural plate border markers such as Pax3, Xhe2, Sox10 and Snail2, q-RT-PCR, alcian blue testing, phenotype analysis and microRNA rescue experiments.

Upon model validation by different parameters such as glucose metabolism or body composition, fertility was assessed by the number of embryos obtained for each mating and early embryonic development by Quantitative Immunofluorescence analysis. In summary, we observe a delayed embryonic development that could explain the reduced fertility in aged, obese and diabetic females.

Poster 3 – Amelia Race (University of Cambridge, UK)

Mechanical regulation of zebrafish neural tube polarisation and lumenogenesis

During development, epithelial tubes are essential for building the complex structures of organs. Epithelial tubes can arise from cavitation in the centre of a solid tissue to form a lumen, which depends apico-basal cell polarity. But how do cells coordinate polarity across a tissue, and what are the mechanisms for precise localisation of polarity proteins within the cell? The zebrafish neural tube forms from two adjacent epithelia polarising 'de novo' from an initially unpolarised tissue, making it an excellent model for investigating apical polarity establishment. Work from our lab has suggested that cell adhesion may be important for the initiation of de novo polarisation, but it is unclear what role mechanical forces in the tissue have in regulating this process. In this study, mechanical forces are investigated over the course of polarisation and neural tube formation, first through characterising and perturbing subcellular myosin in the neuroepithelium. We show through fixed and live imaging that the dynamics of subcellular myosin vary over the course of lumenogenesis, suggesting that it may have a role beyond what was previously described. In the future we plan to investigate the mechanics of polarisation more directly through developing in vitro neural tube model.

Poster 4 – Joaquina Delas (The Francis Crick Institute, UK)

Two distinct modes of cis regulation control cell specification in response to SHH during mouse spinal cord development

Morphogen gradients act in a concentration-dependent manner in many developing tissues to organise the pattern of cellular differentiation. How a single signal is interpreted by responding cells to establish multiple cell-type specific gene expression programmes remains unclear. Ventral regions of vertebrate spinal cord serve as an example of this process. In this tissue, the morphogen Shh and its transcriptional effectors, the Gli family of proteins, control the expression of a set of transcription factors (TFs). These, in turn, regulate each another through repressive interactions to determine cell type identity. Using a cellular model of mouse spinal cord progenitor specification, we developed assays of cell type specific chromatin accessibility and gene regulation. These data revealed that the majority of cell types in the neural tube, whether or not they have been exposed to Shh signalling, share the same cis regulatory topology. This suggests that these progenitors use the same regulatory logic and that the accessible cis regulatory elements act as the information processing devices decoding cell type identity based on the TFs expressed in a cell. By contrast the ventral-most progenitor type, p3 cells, which are the only cell type to depend on Gli activation for their specification, display a dramatically different set of accessible regulatory elements. We propose that this indicates a distinct regulatory topology and consequently p3 cells interpret Shh differently. These data provide molecular evidence for the coexistence of two morphogenetic fields in the ventral spinal cord. This reveals an unexpected relationship between cis regulatory configuration and molecular identity and supports the existence of two different modes of Shh morphogen interpretation for the allocation of cell type identity in the spinal cord.

Poster 5 – Yen Tran (Victor Chang Cardiac Research Institute, Australia)

Single-nuclei RNAseq analysis of embryonic cardiac ventricles reveals cell heterogeneity and molecular networks involved in trabeculation

The heart is the first organ to function during embryogenesis. During cardiogenesis, the ventricular wall develops a luminal sponge-like network called trabeculae, adjacent to the outer compact layer. Trabeculae facilitate nutrient and oxygen exchange before coronary artery formation and later contribute to a number of different structures of the heart. Defects in trabeculation lead to non-compaction cardiomyopathy and, in extreme cases, embryonic lethality. The mechanisms guiding trabeculation are poorly understood. We have explored the cellular heterogeneity and molecular networks underlying this process using single-nuclei RNA-sequencing of micro-dissected mouse embryonic ventricles. We chose two critical developmental timepoints: embryonic day 8.0 to 8.5 (E8.0-8.5), when the endocardium undergoes 'sprouting' and 'touch-down', critical for trabecular patterning; and E9.0 to 9.5 (E9.0-9.5), when individual myocardial trabeculae become encapsulated within an endocardial layer, creating an individual trabecular unit. 16,168 nuclei passed quality control.

Clustering revealed novel cell populations and previously unknown features within myocardial and endocardial populations. First, we were able to resolve the anatomical location of different myocardial subsets. At E8.0 - 8.5, the outer compact layer and inner trabecular layers could be distinguished by the enrichment of cell-polarity and conventional trabecular markers, respectively. Additionally, we uncovered an intermediate myocardial cell subtype which co- expressed Nppa – trabecular – and Hey2 – compact markers. Cell trajectory analysis with Monocle suggests that these cells may be in transition between cell-polarity feature and trabecular myocardial fates. We also identified specific endocardial populations which showed signatures of sprouting vascular endothelial cells, including the enrichment of 'tip-cell' markers, highlighting that trabeculation critically involves a sprouting-like process. Overall, our dataset provides a deeper mechanistic understanding of myocardial and endocardial cell states and signalling processes and helps build the first molecular network of trabeculation.

Poster 6 – Dillan Saunders (University of Cambridge, UK)
Investigating Pattern Regulation in the Zebrafish Tailbud

The differentiation of axial progenitors is an essential part of forming the posterior body axis of the embryo. The zebrafish tailbud contains progenitor populations with regionalised fates, giving rise to either neural tube or somitic mesoderm (Attardi et al., 2018). However, manipulation of Wnt signal transduction has shown that there is flexibility in these cells fates (Martin and Kimelman, 2012). It remains an open question where in the tailbud this property is regionalised.

Here, we utilise 2-photon thermal ablation to investigate which regions of the tailbud can withstand the loss of cells. We show that within the neural and somitic mesodermal progenitors ablation location does not affect tail development. We go on to begin to quantify the cell behaviours that facilitate this pattern regulation.

Poster 7 – Jana Sipkova (University of Cambridge, UK)
Mechanical regulation of Eph/ephrin signalling in the developing Xenopus brain

Eph receptors and their membrane-bound ligands, ephrins, provide key signals in many developmental processes including neuronal guidance. However, despite immense progress in our understanding of Eph/ephrin signalling, discrepancies between in vitro and in vivo work remain. As neuronal growth is regulated by chemical and mechanical signals, and the mechanical regulation of Eph/ephrin signalling is currently poorly understood, we here investigated the role of mechanical cues in this signalling pathway. Xenopus retinal ganglion cells cultured on glass responded to ephrinB1 as previously described: growth cones from the EphB receptor-bearing ventral retina collapsed significantly more than those from the ephrinB ligand-bearing dorsal retina. However, when cultured on a soft substrate mechanically resembling brain tissue, we observe the opposite effect of ephrinB1 application. Furthermore, in vivo atomic force microscopy data suggest that the developing Xenopus brain is mechanically heterogenous, with a change in mechanical properties at the diencephalon-tectum boundary, where retinal ganglion cells unbundle to target specific locations. Since EphB/ephrinB signalling in Xenopus retinal ganglion cells is affected by substrate stiffness in vitro, and a complex mechanical landscape exists in the optic tectum at the time of innervation, our data suggest that mechanical cues could be important in tuning retinotectal mapping through chemical signalling.

Poster 8 – Marie-Christin Leitner (Institute of Molecular Biotechnology, Austria)
Deciphering the specification of posterior lateral plate mesoderm

Mesoderm gives rise to a diverse range of tissues and the first stages of mesoderm specification are decisive to achieve this variety. Subtypes of mesoderm include cardiac, posterior lateral plate (a progenitor of limb mesoderm) and somitic mesoderm but the underlying mechanisms of early cell fate bifurcations that separate them remain unresolved.

To dissect how posterior lateral plate mesoderm specification differs from its cardiac and somitic counterparts, we established developmentally-guided human pluripotent stem cells differentiation into hindlimb-specific TBX4+ and forelimb-specific TBX5+ posterior lateral plate progenitors. These stem cell-derived precursors were functional as they efficiently specified into endothelial cells and chondrocytes.
We found that while the dosage of WNT and retinoic acid signalling patterned mesoderm into either cardiac, forelimb or hindlimb precursors, BMP had a key role in promoting limb over somitic mesoderm.
Among other factors, we identified and are in the process of genetically validating MSX2 and SOX2 as possible direct targets of BMP signalling and found strong indications that these transcription factors are on top of the posterior lateral plate mesoderm specification hierarchy.
Interestingly, despite profound differences, limb and cardiac mesoderm share a network of key transcription factors. As a future outlook, we will use our cardiac and limb differentiation platforms to molecularly decipher how mutations in these shared developmental regulators cause cardiac and limb malformations in human syndromes.

Poster 9 – Alexandra Vetrova (Institute of Developmental Biology, Russia)

From apolar gastrula to polarised larva: embryonic development of a marine hydroid, Dynamena pumila

The primary body plan of Cnidaria is formed with the involvement of a wide range of developmental pathways, which reach maximum diversity at the gastrula stage. In cnidarians, canonical Wnt (cWnt) signaling patterns the primary body axis and controls oral identity during body plan formation. In the case of polar gastrulation, the area of cWnt signaling activity coincides with a single region of gastrulation morphogenesis. In cnidarians, both gastrulation morphogenesis and the role of cWnt signaling in the primary body axis formation were thoroughly studied only in species with a polar gastrulation (Clytia, Nematostella). However, within the class Hydrozoa (Cnidaria), apolar modes of gastrulation are widespread. Hense, the question arises whether cWnt signaling providing the establishment of a body axis controls morphogenetic processes of apolar gastrulation.

We focused on the embryonic development of Dynamena pumila, a hydrozoan species with apolar gastrulation. We revealed that D. pumila utilizes several equifinal developmental pathways. We have shown that gastrulation morphogenesis occurs as a peculiar combination of primary and secondary delamination. The absence of morphological polarity is combined with molecular prepatterning of the embryo at the gastrula stage as in situ analyses of several cWnt signaling components revealed. The morphological body axis forms late in the embryonic development of D. pumila, but we experimentally demonstrated that it is highly robust to modulations in cWnt signaling activity. Thus, gastrulation morphogenetic processes are uncoupled from molecular axial polarity based on cWnt signaling in D. pumila.
The work is supported by RFBR, 20-04-00978a.

Poster 10 – Ahlam Harasani (University of Edinburgh, UK)

Consequences of EZH2 reduction during mouse preimplantation embryo development

During mouse preimplantation development, totipotent blastomeres give rise to the first three cell lineages of the embryo: trophectoderm (TE), epiblast (EPI) and primitive endoderm (PrE). The first decision separates the cells forming the TE and the inner cell mass (ICM). The ICM differentiates into EPI and PrE. This cell lineage specification is thought to be mediated by transcription factors and epigenetic processes that control the expression of genes both temporally and spatially without any changes in the genetic code.
Polycomb group repressive complex 1 and 2 (PRC1 and PRC2) are important regulators of chromatin structure, cell identity, and gene expression during development. PRC2 contains Ezh2, Eed, and Suz12 proteins. Ezh2 is an essential epigenetic modifier that regulates chromatin structure and gene expression silencing through H3K27me3 and recruitment of DNA methyltransferases for gene silencing.
In this project, we studied the impact of Ezh2 inhibition onmouse embryo development and pluripotency using GSK-126 or EPZ-6438, small molecule chemical inhibitors of Ezh2. Zygotes were cultured until E4.5 stage in the presence of either inhibitor or DMSO vehicle control. The effects of Ezh2inhibition were assessed by immunocytochemistry and qPCR. Results were analysed to determine whether Ezh2 mediated gene repression may control cell fate and affect cell proliferation in the preimplantation mouse embryo.

Poster 11 – Riley McMahon (Children's Medical Research Institute, Australia)

LHX1 GENE REGULATORY NETWORK REVEALS A KEY ROLE FOR *FOXD4* IN EMBRYONIC HEAD FORMATION

Embryonic development is driven by a series of molecular instructions encoded by the transcription factors (TFs) that drive the formation of the body plan and the specialisation of tissue precursor cells. Analysis of gastrulating mouse embryos has demonstrated that key TFs such as LIM homeobox 1 (Lhx1) are indispensable for head and face development. The aim of this project is to identify and functionally characterise the genetic targets of LHX1 to refine the gene regulatory network for embryonic head formation. We have conducted RNA-seq, ATAC-seq and used DamID-seq on gastrulating mouse embryos to identify genomic regions that are directly regulated by LHX1. We identified the forkhead box gene FoxD4 as a downstream target of LHX1 in E7.75 embryos. We found that FoxD4 is essential for driving the differentiation and migration of neural tissue in mouse embryos using FoxD4$^{-/-}$ stem cell derived chimera embryos. FoxD4$^{-/-}$ chimeras showed defects in the anterior neural tube as well as craniofacial malformations similar to those seen in Zic2$^{-/-}$ mutant embryos. Also, using FoxD4$^{-/-}$ embryonic stem cell derived organoids resembling the anterior epiblast, we validated downstream genetic targets of FoxD4. By determining novel gene regulators in the LHX1 network, we gained a valuable insight into the cellular and molecular mechanisms building the body plan in the early mammalian embryo.

Poster 12 – Christopher Bolt (École Polytechnique fédérale de Lausanne, Switzerland)

Mesomelic dysplasias associated with the HoxD locus are caused by regulatory reallocations

Some human families display severe shortening and bending of the radius and ulna, a condition referred to as mesomelic dysplasia. Many of these families contain chromosomal rearrangements at 2q31, where the human HOXD locus maps. In mice, the dominant X-ray-induced Ulnaless inversion of the HoxD gene cluster produces a similar phenotype suggesting that the same mechanism is responsible for this pathology in humans and mice. Amongst the proposed explanations, the various alterations to the genomic structure of HOXD could expose Hoxd13 to proximal limb enhancers, leading to its deleterious gain-of-expression in the embryonic forelimb. To assess this hypothesis, we used an engineered 1Mb large inversion including the HoxD gene cluster, in order to position Hoxd13 within a chromatin domain rich in proximal limb enhancers. We show that these enhancers contact and activate Hoxd13 in proximal cells, concomitant to the formation of a mesomelic dysplasia phenotype. A secondary mutation in the coding frame of the HOXD13 protein in-cis with the inversion completely rescued the limb alterations, demonstrating that ectopic HOXD13 is indeed the unique cause of this bone anomaly. Single cell expression analysis and evaluation of HOXD13 binding sites in cells from this ectopic expression domain suggests that the phenotype arises primarily by acting through genes normally controlled by HOXD13 in distal limb cells. Altogether, these results provide a conceptual and mechanistic framework to understand and unify the molecular origins of human mesomelic dysplasia associated with 2q31.

Poster 13 – Afnan Alzamrooni (University of Pourtsmouth, UK)

Cardiac competence of the head mesoderm fades concomitant with a shift towards the head skeletal muscle programme

The vertebrate head mesoderm provides the heart, the great vessels, smooth and most head skeletal muscle, and parts of the skull base. The ability to generate cardiac and smooth muscle is thought to be the evolutionary ground-state of the tissue, and initially the head mesoderm has cardiac competence throughout, even in the paraxial region that normally does not engage in cardiogenesis. How long this competence lasts, and what happens as cardiac competence fades, is not clear.

Using a wide palette of marker genes in the chicken embryo, we show that the paraxial head mesoderm has the ability to respond to Bmp, a known cardiac inducer, for a long time. However, Bmp signals are interpreted differently at different time points. Bmp triggers cardiogenesis up to early head fold stages; the ability to upregulate smooth muscle markers is retained slightly longer. Notably, as cardiac competence fades, Bmp activates the head skeletal muscle programme instead.

Poster Session II: 16:30 – 17:15 (BST)

Poster 14 – Joaquin Lilao-Garzan (University of Las Palmas de Gran Canaria, Spain)
Maternal age and prediabetes impact on fertility and early embryonic development

Modern societies lifestyle results in the in birth rate decrease, and a considerable increase in metabolic diseases such as obesity and diabetes mellitus (DM). This is becoming one of the biggest challenges in economically developed societies. One factor behind the decrease in birth rates is the increased maternal age. European first-time mother's age has increased up to 29 years old in 2017 (eurostat).
DM is considered a global pandemic, affecting 422 million people in 2014 and increasing, causing the death of 1.5 million people each year (WHO). Type 2 diabetes (T2DM) is a combination of insulin deficiency and insulin resistance resulting from a sedentary lifestyle and fat enriched diets. It is usually preceded by mild hyperglycaemia or prediabetes. The worldwide estimated prevalence of prediabetes is 7.3% of the adult population in 2017.
Maternal age, obesity and DM have direct effect on the offspring's health, increasing the risk of obesity, DM and cardiovascular diseases in adulthood. Hence, these factors impact on the future generation´s health.
We developed a prediabetic mouse model to study how these different factors affect early embryonic development by switching mice normal chow diet for a fat enriched diet at adolescence or at pre-menopause age.

Poster 15 – Teresa Olbrich (National Institutes of Health, USA)
CTCF is a Barrier for 2C-like Reprogramming

Totipotent cells have the ability to generate embryonic and extra-embryonic tissues. Interestingly, a rare population of cells with totipotent-like potential, known as 2 cell (2C)-like cells, has been identified within ESC cultures. They arise from ESC and display similar features to those found in the 2C embryo. However, the molecular determinants of 2C-like conversion have not been completely elucidated. Here, we show that the CCCTC-binding factor (CTCF) is a barrier for 2C-like reprogramming. Indeed, forced conversion to a 2C-like state by the transcription factor DUX was associated with DNA damage at a subset of CTCF binding sites. Depletion of CTCF in ESC efficiently promoted spontaneous and asynchronous conversion to a 2C-like state and was reversible upon restoration of CTCF levels. This phenotypic reprogramming was specific to pluripotent cells as neural progenitor cells did not show 2C-like conversion upon CTCF-depletion. Furthermore, we showed that transcriptional activation of the ZSCAN4 cluster was necessary for successful 2C-like reprogramming. In summary, we revealed an unexpected relationship between CTCF and 2C-like reprogramming.

Poster 16 – Irene Zorzan (University of Padova, Italy)
The transcriptional regulator ZNF398 mediates pluripotency and epithelial charater downstream of tgf-beta in human pluripotent stem cells

Human pluripotent stem cells (hPSCs) have the capacity to give rise to all differentiated cells of the adult. TGF-beta is used routinely for expansion of conventional hPSCs as flat epithelial colonies expressing the transcription factors POU5F1/OCT4, NANOG, SOX2. Here we report a global analysis of the transcriptional programme controlled by TGF-beta followed by an unbiased gain-of-function screening in multiple hPSC lines to identify factors mediating TGF-beta activity. We identify a quartet of transcriptional regulators promoting hPSC self-renewal including ZNF398, a human specific mediator of pluripotency and epithelial character in hPSCs. Mechanistically, ZNF398 binds active promoters and enhancers together with SMAD3 and histone acetyltransferase EP300, enabling transcription of TGF-beta targets. In the context of somatic cell reprogramming, inhibition of ZNF398 abolishes activation of pluripotency and epihelial genes and colony formation. Our findings have clear implications for the generation of bona fide hPSCs for regenerative medicine.

Poster 17 – Maria Vega Sendino (NIH, USA)

The ETS Transcription Factor ERF controls the exit from the naïve pluripotent state

The naïve epiblast undergoes a transition to a pluripotent primed state during embryo implantation. Despite the relevance of the FGF pathway during this period, little is known about the downstream effectors regulating this signaling. Here, we examined the molecular mechanisms coordinating the naïve to primed transition by using inducible ESC to genetically eliminate all RAS proteins. We show that differentiated RAS^{KO} ESC remain trapped in an intermediate state of pluripotency with naïve-associated features. Elimination of the transcription factor ERF overcomes the developmental blockage of RAS-deficient cells by naïve enhancer decommissioning. Mechanistically, ERF regulates NANOG expression and ensures naïve pluripotency by strengthening naïve transcription factor binding at ESC enhancers. Moreover, ERF negatively regulates the expression of the de novo methyltransferase DNMT3B, which participates in the extinction of the naïve transcriptional program. Collectively, we demonstrated an essential role for ERF controlling the exit from naïve pluripotency during the progression to primed pluripotency.

Poster 18 – Violeta Trejo (The Roslin Institute, UK)

Genetic analysis of SMOC1 and SMOC2 in chicken eye and limb development

Secreted modular calcium-binding proteins (SMOCs), SMOC1 and SMOC2 have proven to hold important roles for development of eyes, craniofacial structures and limbs To date, no study has been undertaken in the chicken, despite its utility as a highly-tractable model organism. RT-PCR and whole mount in situ hybridisation confirmed the presence of SMOC genes in chicken embryos, and when comparing their expression patterns with mouse Smocs, similar expression patterns were observed. These data suggest that SMOCs perform similar developmental roles across divergent vertebrates. Further analysis with qRT-PCR confirmed high expression in the developing eye and limb. The optic fissure is a region of the ventral eye that must fuse for normal eye development to occur; prior data suggested SMOC1 expression is enriched in the ventrally developing eye. RNA-seq data from whole eyes and optic fissure regions of humans, mice, chick and zebrafish were compared to assess SMOC gene expression before, during, and after fissure closure. Evolutionarily conserved gene expression underscores SMOC1's importance in eye development. SMOC2 also showed up-regulation in eye tissues, but this was enriched during later stages of development and in non-fissure regions of the eye. Finally, the SMOC1 locus in chicken was extensively studied from the perspective of isoform expression. Taken together, these findings confirm the utility of the chicken embryo for revealing developmental genetic information at the single gene and whole transcriptome levels. This work also provides a framework to follow these genetic studies up at the protein function level, including understanding the roles of SMOC proteins and individual isoforms, as well as their molecular interactions and importance for normal development and disease.

Poster 19 – Hugo Fernandes-Silva (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Portugal)

Retinoic acid signalling regulates the metabolic component of embryonic lung branching morphogenesis

Retinoic Acid (RA) signaling is crucial for pulmonary development since it coordinates lung patterning and early-branching morphogenesis. Recently, the metabolic profile of pulmonary branching was described, showing a glycolytic lactate-based metabolic preference. Here, we studied the impact of RA signaling modulation on the metabolic component of early lung branching. We used an ex vivo lung explant culture system, and embryonic chicken lungs (Gallus gallus) were cultured under the following experimental conditions: DMSO, 1µM RA (stimulation), or 10µM of BMS493 (inhibition). The explant culture media was collected, and the consumption/production of extracellular metabolites evaluated by ^{1}H-NMR spectroscopy. Lung explants were obtained to determine the respiratory capacity by Seahorse analyzer and to conduct a mitochondrial DNA copy number assay. The expression of key metabolic components was assessed by qPCR and in situ hybridization. Results revealed that RA stimulation promotes lung branching, while RA inhibition decreases branching and changes its morphology. RA inhibition increases glucose consumption and alanine production when compared to RA stimulation. Conversely, RA stimulation

promotes an increase in pyruvate production. At the mitochondrial level, RA inhibition leads to increased basal respiration and elevated ATP production. Moreover, we observed a similar mitochondrial abundance and a stable expression of mitochondrial transcription factor A (tfam). Lactate dehydrogenase (ldha and ldhb) expression is affected by RA modulation. In conclusion, we describe an intimate interaction between RA pathway and lung metabolism. RA signaling modulation differently impacts the metabolic component of lung development, standing out as a crucial player in the metabolic regulation of branching morphogenesis.

Poster 20 – Tiago Ribeiro (Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Portugal)

Electronic vs. Conventional Cigarette: a comparative study of aerosol and smoke effect on the embryonic chick lung *in vitro*

Smoking is a major public health problem responsible for countless deaths/year in Europe. Recently, electronic cigarettes (e-cig) emerged as an alternative to conventional cigarettes (c-cig). Previous studies revealed that c-cig smoke exposure during fetus development promotes congenital disorders, impairs lung development and triggers inflammation. However, the impact of e-cig aerosol during pulmonary development is not clear. To determine how e-cig aerosol affects lung development, ex vivo chick (Gallus gallus) embryonic lungs were cultured in vitro for 48 hours in e-cig aerosol exposed-medium or unexposed medium. For comparison purposes, chick lung explants were also cultured in c-cig smoke exposed-medium. Lung explants were morphologically analyzed to assess the impact on lung growth. Additionally, inflammation was evaluated by checking TNF-α levels. Results showed that, when compared to control, aerosol-exposed explants displayed a significant reduction in lung total area and perimeter. However, smoke c-cig exposed explants exhibited a significant decrease, in all morphometric parameters analyzed, comparing both with control and aerosol exposed-explants. Additionally, aerosol and smoke exposed-medium induced similar amounts of TNF-α released, which were nearly 7 times higher than control. Our results suggest that e-cig aerosol impairs lung growth and promotes lung inflammation. However, its impact on early lung growth seems less detrimental than c-cig smoke. Considering that e-cig is recommended as a smoking cessation tool for pregnant women, more studies are required for proper validation of these effects, which could lead to new tobacco control recommendations to pregnant women.

Poster 21 – Ioakeim Ampartzidis (University College London, UCL)

Mitotic apical constriction is conserved in human neuroepithelium-like sheets *in vitro*

Neuroepithelial (NE) cells fulfil sequential functions during mammalian development; they biomechanically fold their tissue into a closed neural tube and differentiate into mature neurons. Failure of force-generating NE behaviours, which include apical constriction and interkinetic nuclear migration (IKNM), produces neural tube defects such as spina bifida. We recently reported NE cells of mice and lower vertebrates predictably undergo apical constriction during mitosis(1), but whether this occurs in human cells is unknown. To address this, we extensively characterised early stages of NE induction from human iPSCs through a well-established dual-SMAD inhibition protocol. Over 12 days of induction these cells progressively up-regulate NE markers such as CDH2 while down-regulating Lamin A/C, both of which are pre-requisites for efficient IKNM in vivo. Other hallmarks of IKNM evident in NE-like sheets include progressive pseudostratification, apicobasal elongation and apically-restricted mitoses. Their apical surface becomes enriched in actomyosin, suggesting they undergo apical constriction. We compared apical areas between NE-like cells in G1/S, G2 or M phase, identified using cell cycle stage-specific distribution of Ki-67. The apical area of G2-phase cells is larger than that of cells in G1/S or M phase, suggesting NE-like cells apically dilate in G2 prior to constricting in M phase. Thus, mitotic apical constriction is a conserved neuroepithelial behaviour recapitulated in human NE-like sheets in vitro. Next, we will mechanistically compare force-generating behaviours of NE-like sheets between iPSCs from healthy donors versus cells from individuals who have spina bifida.

1. Butler and Short et al, J Cell Sci, 2019

Poster 22 – Marta Grzonka (University Hospital of Cologne, Germany)
Defining the roles of SAS-6 during mouse development

Centrosomes are microtubule-organizing structures fulfilling different functions throughout the cell cycle in animal cells. They organize mitotic spindles and serve as microtubule organizing centers and templates for cilia during interphase. Centrosomes are comprised of two perpendicularly arranged centrioles as well as of pericentriolar material. Centrioles duplicate once per cell cycle to ensure that each daughter cell inherits a centriole pair. At the onset of centriole duplication in human cell lines and C. elegans, SAS-6 forms a cartwheel structure, which is the precursor for the forming procentrioles. However, the function of SAS-6 in mice remains to be elucidated. Here we investigate the roles of mouse Sass6 in centrosome formation, cell division and early development. Our data showed that similar to mutants of other genes essential for centriole duplication, Sass6-null embryos lack centrioles and arrest at midgestation with widespread p53-dependent apoptosis. To study the loss of Sass6 in vitro, we generated Sass6 knockout mESCs by CRISPR/Cas9. Surprisingly, the loss of Sass6 did not lead to the loss of centrosomes, instead the cells exhibited thread-like γ-tubulin accumulations. Colocalization of the centriolar marker centrin with γ-tubulin and the localization of CEP164 to one end of the threads suggested that these were elongated centrioles. Upon partial differentiation of Sass6-/- mESCs, the elongated centrioles failed to template cilia. Thus, we conclude that the mechanism of centriole biogenesis does not strictly require SAS-6 and depends on the cellular context even within the same organism.

Poster 23 – Yin Ho Vong (University of Warwick, UK)
The RNA-binding protein Igf2bp3 regulates the yolk syncytial layer and primordial germ cells in zebrafish

The ability to reproduce is essential in all branches of life. In metazoans, this process is initiated by formation of the germline, a group of cells that are destined to form the future gonads, the tissue that will produce the gametes. The molecular mechanisms underlying germline formation differs between species. In zebrafish, development of the germline is dependent on the specification, migration and proliferation of progenitors called the primordial germ cells (PGCs). PGC specification is dependent on a maternally provided cytoplasmic complex of ribonucleoproteins (RNPs), the germplasm. Here, we show that the conserved RNA-binding protein (RBP), Igf2bp3, has an essential role during early embryonic development and germline development. Loss of Igf2bp3 leads to an expanded yolk syncytial layer (YSL) in early embryos, reduced germline RNA expression, and mis-regulated germline development. We show that loss of maternal Igf2bp3 function results in translational de-regulation of a Nodal reporter during the mid-blastula transition. Furthermore, maternal igf2bp3 mutants exhibit reduced expression of germplasm transcripts, defects in chemokine guidance, abnormal PGC behaviour and germ cell death. Consistently, adult igf2bp3 mutants show a strong male bias. Therefore, Igf2bp3 is essential for normal embryonic and germline development, and acts as a key regulator of sexual development.

Poster 24 – Sami HA. Sultan (King's College London, UK)
Notch signalling differentially regulates muscle stem cells in homeostasis and during regeneration

Muscle regeneration is mediated by the activity of resident muscle stem cells (muSCs) that express Pax7. In mice Notch signalling regulates both quiescence and asymmetrical division of muSCs. Inhibition of Notch during regeneration leads to the loss of the muSC pool as a result of premature differentiation, perturbing muscle repair and resulting in fibrosis and smaller myofibers. Our understanding of how Notch regulates muscle regeneration is almost entirely derived from studies in mice. We have therefore investigated the role of Notch in regulating muSC homeostasis and regeneration in the zebrafish. Current studies reveal several caveats for using zebrafish to investigate the response of resident muSCs towards injury. These are: it is not known 1) whether injury size correlates with the magnitude of the muSC response, 2) whether muSC responses to injury are altered following perturbation of key regulatory pathways, 3) how comparable these muSC responses are at different stages of larval development. We have therefore tested the importance of injury size and developmental stage on how muSCs respond to injury, characterizing the

effects of Notch inhibition on the muSC response. In an absence of injury, Notch is important for maintaining muSC quiescence. In contrast, Notch signalling promotes proliferation and prevents differentiation in the context of injury. Larval stage does not affect this Notch-dependent response of muSCs to injury. These results reveal a conserved role for Notch signalling in maintaining muSC quiescence and for enabling a proliferative response during regeneration in teleost fish and mammals.

Poster 25 – Raquel Rouco García (University of Geneva Medical School, Switzerland)
Cell-specific alterations in *Pitx1* regulatory landscape activation caused by the loss of a single enhancer

Most developmental genes rely on multiple transcriptional enhancers for their accurate expression during embryogenesis. Because enhancers may have partially redundant activities, the loss of one of them often leads to a partial loss of gene expression and concurrent moderate phenotypic outcome, if any. While such a phenomenon has been observed in many instances, the nature of the underlying mechanisms remains elusive. We used the Pitx1 testbed locus to characterize in detail the regulatory and cellular identity alterations following the deletion in vivo of one of its enhancers (Pen), which normally accounts for 30 percent of Pitx1 expression in hindlimb buds. By combining single cell transcriptomics and a novel in embryo cell tracing approach, we observed that this global decrease in Pitx1 expression results from both an increase in the number of non- or low-expressing cells, and a decrease in the number of high-expressing cells. We found that the over-representation of Pitx1 non/low-expressing cells originates from a failure of the Pitx1 locus to coordinate enhancer activities and 3D chromatin changes. The resulting increase in Pitx1 non/low-expressing cells eventually affects the proximal limb more severely than the distal limb, leading to a clubfoot phenotype likely produced through a localized heterochrony and concurrent loss of irregular connective tissue. This data suggests that, in some cases, redundant enhancers may be used to locally enforce a robust activation of their host regulatory landscapes.

Poster 26 – Lara Busby (University of Cambridge, UK)
Time and Cell Fate Decisions in the Development of the Avian Anteroposterior Axis

During development, the vertebrate posterior body plan is laid down in a sequential manner, with anterior structures being generated before more posterior ones. The pool of cells that contribute to the conserved structures of the anteroposterior axis, including the notochord, somites and neural tube, are termed axial progenitor cells. During posterior body development, axial progenitor cells coordinate their cell fate decisions and contributions to the body axis with the overall progression of developmental time. This is necessary for normal morphogenesis.

In this project, we are examining the mechanisms underlying how axial progenitor cells “tell the time” during development, in particular focusing on making the distinction between cell-intrinsic and cell-extrinsic timing mechanisms in controlling the expression of Hox genes in somite progenitors.

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. 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.

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 art work 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. 2021 will be the 7th Sammy Lee award to be presented.