ASHBi Symposium 2021

Human Development, Genetics and Evolution

November 8–10, 2021

ASHBi International Symposium 2021

Symposium Time Slots (tentative plan)

16 September 2021

JST	Monday, 8 November (Day 1)	Tuesday, 9 November (Day 2)	Wednesday, 10 November (Day 3)	PST *1	EST *2	GMT *3
9:00		9:00–9:25 **Ryoichiro Kageyama** (RIKEN CBS/Kyoto U)		16:00(-1)	19:00(-1)	0:00
9:30		9:25–9:50 **Christopher Walsh** (Harvard Medical)				
10:00		9:50–10:05 *selected talk 1*		17:00(-1)	20:00(-1)	1:00
		10:05–10:35 Break (30-min)				
10:30		10:35–10:50 *selected talk 2*				
11:00		10:50–11:15 **Diana Laird** (UCSF)		18:00(-1)	21:00(-1)	2:00
		11:15–11:40 **Davor Solter** (Max-Planck Society)				
JST						
17:00	**Opening Remarks**			0:00	3:00	8:00
17:30	17:10–17:35 **Henrik Kaessman** (Heidelberg U)		17:00-17:25 **Takashi Hiiragi** (EMBL/ASHBi)			
	17:35–18:00 **James Turner** (Crick Institute)		17:25-17:50 **James Briscoe** (Crick Instirtue)			
18:00	18:00–18:25 **Claire Rougelle** (U Paris)		17:50–18:15 **J Gray Camp** (IMO Basel)	1:00	4:00	9:00
18:30	18:25-18:55 Break (30-min)	17:00–17:15 **Cantas Alev** (ASHBi)	18:15–18:45 Break (30-min)			
		17:15–17:40 **Barbara Treutlein** (ETH Zürich)				
		17:40–18:05 **Ryuichi Nishinakamura** (Kumamoto U)				
19:00	18:55–19:55 60-min	18:05–18:30 **Anne Grapin-Botton** (U Copenhagen)	18:45–19:45 60-min	2:00	5:00	10:00
19:30		18:30-19:00 Break (30-min)				
		19:00–20:00 60-min				
20:00	19:55–20:25 Break (30-min)	20:00–20:30 Break (30-min)	19:45–20:15 Break (30-min)	3:00	6:00	11:00
20:30	20:25–20:40 **Yasuhiro Murakawa** (ASHBi)	20:30–20:55 **Prisca Liberali** (F Miescher Inst)	20:15–20:40 **Katsuhiko Hayashi** (Kyushu U)			
	20:40–21:05 **Aida Andres** (U C London)	20:55–21:20 **Toshiro Sato** (Keio U)	20:40–21:05 **Azim Surani** (Wellcome Trust)			
21:00	21:05–21:30 **Janet Kelso** (Max Planck)	21:20–21:45 **Seishi Ogawa** (ASHBi)	21:05–21:30 **Anne Goriely** (U Oxford)	4:00	7:00	12:00
21:30			21:30–21:55 **Mitinori Saitou** (ASHBi)			
22:00			21:55–22:05 **Closing Remarks**	5:00	8:00	13:00

*1 Pacific Standard Time

*2 Eastern Standard Time

*3 Greenwich Meant Time

S1

The evolution of spermatogenesis across mammals

Henrik Kaessmann

Center of Molecular Biology, Heidelberg University

The testis is a key male reproductive organ that produces gametes through the process of spermatogenesis. Testis morphologies and spermatogenesis phenotypes evolve rapidly in mammals, presumably due to the evolutionary pressure on males to be reproductively successful. The rapid evolution of the testis was shown to be reflected at the molecular level based on bulk-tissue work, but the molecular evolution of individual spermatogenic cell types across mammalian lineages remains largely uncharacterized. Here we report evolutionary analyses of single-nucleus transcriptome data for testes from ten species that cover the three major mammalian lineages (eutherians, marsupials, egg-laying monotremes) and birds (the evolutionary outgroup), and include seven key primates. Our analyses reveal that the rapid evolution of the testis is driven by accelerated evolutionary fixation rates of regulatory mutations, amino acid altering substitutions, and new genes in late spermatogenic stages – likely facilitated by reduced pleiotropic constraints, haploid selection, and a transcriptionally permissive chromatin environment. We identify temporal expression differences of individual genes across species, which probably contributed to species-specific phenotypes, but also conserved expression programs underlying ancestral spermatogenic processes. Sex-related analyses show that genes predominantly expressed in spermatogonia (i.e., germ cells fueling spermatogenesis) and Sertoli cells (i.e., somatic support cells) independently accumulated on X chromosomes across mammals during evolution, presumably due to male-beneficial selective forces. Further work uncovered that the process of meiotic sex chromosome inactivation (MSCI) is common to mammalian sex chromosome systems, contrary to previous suggestions. Thus, the general mechanism of meiotic silencing of unsynapsed chromatin, which underlies MSCI, represents an ancestral mammalian feature. Together, our study illuminates the cellular and molecular basis of the evolution of mammalian spermatogenesis and associated selective forces, and provides a resource for investigating the biology of the testis across mammals.

S2

The evolution of X-dosage compensation in mammals

James Turner

Francis Crick institute, London, UK

Females and males have the same autosome complement but differ with respect to their sex chromosomes: females are XX, while males are XY. The resulting disparity in X-gene dosage is solved by X-inactivation, the silencing of one X chromosome in females. X-inactivation is essential for development, is implicated in disease, and is a paradigm for understanding epigenetic regulation. In this seminar, I will describe how our work on an unusual model organism, the marsupial, is shedding light on the evolution of X-inactivation, embryonic lineage formation and epigenetic reprogramming in placental mammals.

S3

X chromosome inactivation in humans

Claire Rougeulle

Department of Epigenetics and Cell Fate; CNRS/Université de Paris, Paris, France.

X chromosome inactivation (XCI) in mammals is an essential developmental process and a paradigm of epigenetic regulations. X chromosome inactivation has been mainly studied in mouse, where the actors of the process, mainly long non-coding RNAs, and the kinetics of the molecular events leading to the transcriptional silencing of one of the two X chromosomes have been partly deciphered. However, it is now known that X-inactivation initiates through remarkable diverse strategies in different species. Understanding the molecular basis of such plasticity, and in particular how X-inactivation is regulated in human is critical, especially since atypical or abnormal X inactivation patterns are linked to human pathologies such as cancer or autoimmune diseases. We are using human embryonic stem cells as a model system for early human development, to characterize the early stages of X chromosome inactivation and to identify regulators of the process in human. We are in particular exploring the extent to which long non-coding RNA contribute to the variation in XCI strategies between species.

S4

Single-cell enhancer identification reveals genetic and evolutionary mechanisms of human diseases

Yasuhiro Murakawa

ASHBi, Kyoto University

Recent large-scale genome-wide association studies (GWAS) have yielded an increasing number of disease-associated genomic loci. However, the mechanistic interpretation is still far from complete. Recently, it has become apparent that disease-associated genetic variants are often found in cell-type-specific enhancers, suggesting the importance of sensitive identification of enhancers in disease-relevant tissues and cell-types. Here we developed our original computational and experimental approach to identify active enhancers using single-cell RNA sequencing. Using this approach, we comprehensively detected active enhancers from ~500,000 heterogeneous CD4+ T cells, and constructed a comprehensive atlas of transcribed enhancers. Integration of transcribed enhancers with GWAS data allowed us to predict putative disease-relevant specialized CD4+ T cell subsets as well as causal nucleotide variants within cell-type-specific enhancers. Furthermore, our conservation analysis highlighted the evolutionary diversity of autoimmune diseases. Thus, we provide a general framework to investigate molecular mechanisms underlying human diseases.

S5

Human evolution: how humans managed to adapt to life around the globe.

Aida Andrés

UCL Genetics Institute, Department of Genetics, Evolution and Environment, University College London, London, UK

Modern humans evolved in Africa, colonising the rest of the world in only 50-100,000 years. This rapid colonization of vastly different habitats was marked by pressures to adapt to each novel environment. The resulting local adaptations generated genetic differences among human populations. These population differences matter because natural selection can only act on variants with important effects in phenotype and fitness. Any population difference that emerged due to local adaptation is thus likely to have important consequences even today.

Ambient temperature was undoubtedly among the strongest selective pressures, as it varies widely around the world. Temperature perception is particularly important as the first essential step for thermoregulation and thus survival. In fact, protein changes in the TRP cation channel that mediates cold perception (*TRPM8*) has mediated adaptation to cold temperatures across mammals. We previously provided evidence that *TRPM8* also mediated adaptation to cold habitats in humans. rs10166942, a SNP upstream of *TRPM8*, shows remarkable population differentiation (5% frequency in Nigeria, 88% in Finland), its allele frequency correlate with latitude and temperature, and its patterns of linked variation are best explained by strong, local positive selection. Now, novel analytical methods and growing numbers of ancient genomes allow us to decipher the evolution of this variant in further detail. We infer that rs10166942's derived allele was under strong positive selection very shortly after the out-of-Africa migration, resulting in high frequency of the beneficial allele in early European groups. Nevertheless, preliminary analyses suggest that positive remained strong after subsequent population admixtures and until relatively recent times. This is interesting because the putatively selected allele has been associated with increased expression of the cold receptor, increased cold sensitivity and increased risk of migraine. It is thus likely that local adaptation has shaped both temperature perception and the risk of migraine in certain human populations.

S6

The contribution of ancient humans to understanding modern human genetic variation

Janet Kelso

Max Planck Institute for Evolutionary Anthropology

The genomes of archaic and ancient modern humans offer a unique way to learn about their histories and to gain insights into their unique physiologies. However, the sequencing and analysis of DNA from ancient humans is complicated by DNA degradation, chemical modifications and contamination. Recent technological advances have made it possible retrieve and sequence DNA from bones and other remains found at archaeological excavations, and we have been able to reconstruct the genomes of several Neandertals. We have also identified, based on their genome sequences, a previously unknown extinct Asian hominin group related to Neandertals, who we call “Denisovans”. The genomes of our extinct Neandertal and Denisovan relatives offer a unique opportunity to learn about the similarities and differences between us. We have used these archaic genome sequences to identify genetic changes that are unique to modern humans and not shared with Neandertals or Denisovans, and ongoing work aims to determine whether any of these genetic variants might underlie traits characteristic of modern humans. Further, we have also shown that the ancestors of some of us interbred with both Neandertals and Denisovans such that all present-day people outside of Africa carry approximately 2% Neandertal DNA, and that some populations, largely in Oceania, also carry DNA inherited from Denisovans. This introgressed DNA has been shown to have both positive and negative outcomes for present-day carriers: underlying apparently adaptive phenotypes such as high altitude adaptation, as well as influencing immunity and disease risk. In recent work we have identified Neandertal haplotypes that are likely of archaic origin and determined the likely functional consequences of these haplotypes using public genome, gene expression, and phenotype datasets.

S7

Dynamic transcriptional control of neural stem cells

Ryoichiro Kageyama[1,2]

[1]Kyoto University, Institute for Frontier Life and Medical Sciences, Kyoto, Japan
[2]RIKEN Center for Brain Science, Wako, Japan

Neural stem cells (NSCs) actively proliferate and generate neurons and glial cells (active state) in the embryonic brain while they are mostly dormant (quiescent state) in the adult brain. The expression dynamics of *Hes1* are different between active and quiescent NSCs. In active NSCs, Hes1 expression oscillates and periodically represses the expression of proneural genes such as *Ascl1/Mash1*, thereby driving their oscillations. By contrast, in quiescent NSCs, Hes1 oscillations become higher levels even at trough phases (thus continuous), thereby continuously suppressing the proneural gene expression. By using an optogenetic method, we found that high levels of Hes1 expression and the resultant suppression of *Ascl1* promote the quiescent state of NSCs, while oscillatory Hes1 expression and the resultant oscillatory Ascl1 expression regulate their active state, suggesting that the expression dynamics of these factors are important for active versus quiescent states. Indeed, when oscillatory Ascl1 expression is induced in quiescent NSCs in the aged mouse brain, these NSCs are efficiently activated, produce new neurons continuously and thereby ameliorate cognitive decline. We now plan to apply this method to monkeys to see whether their endogenous NSCs can be activated to generate new neurons continuously in the aged brain.

S8

Molecular genetics of human brain development and evolution.

Christopher A. Walsh, MD, PhD

Division of Genetics and Genomics and Howard Hughes Medical Institute, Boston Children's Hospital, Harvard Medical School, and Allen Discovery Center for Human Brain Evolution, Boston, MA 02115.

Although evolution must ultimately alter cognition and behavior at least in part through changes in the genome, it has been remarkably difficult to ascertain those genomic changes that enable the unique cognitive, cultural, and aesthetic capabilities of humans. Human Accelerate Regions (HARs) include ≈3000 genomic sequences that are highly conserved across nonhuman species--suggesting they have essential functions--but show accelerated divergence between humans and nonhumans, suggesting that their functions changed during human evolution. However, very little is known about whether any, some, or all of them have essential functions in human brain. Analysis of transcriptional activity of >3100 HARs, using Capture MPRA, reveals that 49% of HARs show enhancer activity in neural cell lines, while 42% show greater activity for the human sequence than the chimp, versus 19% showing greater activity for the chimp sequence. Analyzing DNA accessibility and chromatin marks in fetal human brain identified HARs with open chromatin in fetal neurons, neural precursor cells, or both, including a few hundred HARs with patterns suggestive of enhancer function in fetal human brain.
In order to identify HARs with essential neurological functions in humans, we sequenced HARs in >9600 individuals including thousands with social and cognitive disability. Confirming our previous work, we find higher rates of rare homozygous HAR mutation in in patients with social and cognitive disabilities than in matched controls, suggesting contribution of rare homozygous HAR mutations in ≈2% of autism and other disabilities, and identifying HARs that are recurrently mutated in disease. These data identify dozens of HARs, many with uncharacterized function, with key roles in human brain function.

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S9

TBA

S10

TBA

S11

A xenotransplantation model for studying the migration of human primordial germ cells

Diana J. Laird

Department of Obstetrics, Gynecology and Reproductive Science, Center for Reproductive Sciences, Eli and Edythe Broad Center for Regeneration Medicine and Stem Cell Research, University of California, San Francisco, USA

Fertility hinges upon events early in development before organogenesis, beginning with the specification of dedicated gametic precursors known as primordial germ cells (PGCs), which must traverse the growing embryo to reach the nascent gonads. PGC migration and some of the underlying signaling pathways are conserved across the Metazoan kingdom, although specific migratory routes can be dramatically different. Little is known about PGC migration in humans owing to the inaccessibility of embryos at the early developmental window in which it occurs, and the barriers to emulating the complex embryonic environment in organoids. A useful model for migratory-stage PGCs is furnished by their in vitro derivatives from pluripotent stem cells, known as PGC-like cells (PGCLCs), but their capacity to migrate remains unknown. We took advantage of the accessibility and tractability of the chick embryo to establish a system for studying human PGC migration. We developed methods for 3D quantitative imaging of PGCs in chick embryos as well as detection of xenografted PGCs. Despite anatomical differences in the migration routes, we found that mouse PGCs as well as human PGCLCs injected into the chick extraembryonic vasculature, which is the site of avian PGC specification, were capable of homing from bloodstream through the mesentery and colonizing the gonadal ridges. In addition to the remarkable conservation of critical navigation cues implied by these results, common signals for PGC proliferation and survival are suggested by an observed correlation in the rates of expansion between donor and host PGCs in this system. Using single cell transcriptomics, we found discrete transcriptional signatures in human PGCs as compared to mice across developmental time and identified new candidate signaling pathways between human PGCs and their niche. This xenotransplant system will serve as a platform for elucidating the mechanisms of human PGC migration as well as validating in vitro derived PGCLCs.

S12

Epigenetic Mechanisms Controlling Early Mammalian Development

Davor Solter

Max-Planck Society

Epigenetic state of each cell is crucial in determining its gene expression and consequently its phenotype. Changes in the epigenetic state of the genome are essential for normal development. While gradual changes in epigenetic states occur throughout development there are two crucial moments when massive reprogramming of the genome takes place: transition from somatic cells to primordial germ cells and in zygote, following fertilization, when the germ cell epigenome transits to somatic epigenome state. During those transition a general DNA demethylation takes places, however, there are several components of the genome e.g. imprinted genes that need to be protected. TRIM28 is one of the components of the protective mechanism and its absence during oogenesis leads to post-fertilization failure. Variations in failure phenotype suggest that, in absence of TRIM 28, epigenetic marks are randomly lost, and not replaced, from individual genes resulting in epigenetic chimerism. These results emphasize the importance of maintaining and protecting correct epigenetic state during mammalian development.

S13

Reconstituting human somitogenesis *in vitro*

Yoshihiro Yamanaka[1], Kumiko Yoshioka-Kobayashi[1], Sirajam Munira[1], Sofiane Hamidi[1], Yi Zhang[1], Shunsuke Kihara[2], Yuzuru Kurokawa[1], Taro Tsujimura[1], Takuya Yamamoto[1,2], Cantas Alev[1]

[1] Institute for the Advanced Study of Human Biology (ASHBi), Kyoto University, Kyoto, Japan
[2] Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

Somitogenesis is a fundamental process during which the segmented vertebrate body plan is laid out via the controlled emergence of epithelial somites from paraxial/presomitic mesoderm (PSM). It has been extensively studied using model organisms such as mouse, zebrafish or chick, but remains largely elusive and poorly understood when it comes to human and other primates. Using embryonic development-inspired induction of human PSM from pluripotent stem cells (PSC), we recently succeeded to recapitulate features of human paraxial mesoderm development in vitro. We hereby visualized and quantified oscillatory activity of putative segmentation clock associated genes and identified core molecular and functional features of human normal as well as abnormal spine development and disease. Building upon these findings we then asked whether we could recapitulate the actual process of human segmentation in vitro. Utilizing pluripotent stem cells as starting material and following the guiding principles of the embryo we were able to establish a novel 3D model of human axial development, enabling us to observe and assess the sequential formation of human epithelial somites in vitro. Our newly established bottom-up in vitro model system provides a promising approach to study various aspects of axial development & disease in human and other mammalian species.

S14

Tracing and perturbing lineages during human brain organoid development

Barbara Treutlein

ETH Zurich, Department of Biosystems Science and Engineering, Basel Switzerland

Induced pluripotent stem cell (iPSC) derived organoids provide models to study human organ development. Organoids are complex, containing numerous cell states and integrative, multi-modal single-cell technologies are needed to understand the mechanisms underlying organoid development.
In my talk, I will present two efforts from our lab where we develop novel integrative single-cell methods to understand human brain organoid development. First, I will present iTracer, a lineage recorder that combines reporter barcodes with inducible CRISPR/Cas9 scarring, and is compatible with single-cell and spatial transcriptomics. We apply iTracer to explore clonality and lineage dynamics during cerebral organoid development, and identify a time window of fate restriction as well as variation in neurogenic dynamics between progenitor-neuron families. We also establish long-term 4-D lightsheet microscopy for spatial lineage recording in cerebral organoids and confirm regional clonality in the developing neuroepithelium. We incorporate gene perturbation (iTracer-perturb), and assess the effect of mosaic *TSC2* mutations on cerebral organoid development.
Second, I will present a data set of paired single-cell transcriptome and accessible chromatin profiling data over a dense time course of human brain organoid development, which we utilize to infer a gene regulatory network of human brain organoid development. To this aim, we have developed Pando, a flexible computational framework that incorporates multi-omic data and transcription binding site predictions to infer a global GRN describing organoid development. We use pooled genetic perturbation with single-cell transcriptome readout to assess transcription factor requirement for cell fate and state regulation *in organoid* and show interesting alterations of abundance of cell fates.
Together, these techniques can be adapted in any iPSC-derived culture system to dissect lineage relationships and regulomes during normal or perturbed development.

S15

Kidney organoids for modeling human development and disease

Ryuichi Nishinakamura

Institute of Molecular Embryology and Genetics, Kumamoto University, Japan

Recapitulating the three-dimensional organ structure in vitro is a major challenge for developmental biology and regenerative medicine. The kidney develops by the reciprocal interactions between the nephron progenitor and ureteric bud. We established an induction protocol toward the former from pluripotent stem cells (PSCs) (Taguchi et al. Cell Stem Cell, 2014). Induced nephron progenitors robustly formed glomerular podocytes equipped with slit diaphragm (SD), a filtration barrier. Mutations in the NEPHRIN are known to cause congenital nephrotic syndrome, resulting from impaired SD formation in glomerular podocytes. By establishing iPS cells from a patient with a NEPHRIN missense mutation, we reproduced the initial phase of this congenital disease (Tanigawa et al. Stem Cell Reports 2018). However, these nephron organoids lacked the collecting ducts that connect the nephrons and constitute the lower part of the urinary tract. As the collecting ducts are derived from the second precursor of the kidney: the ureteric bud, we also established protocols to induce the ureteric bud from mouse and human PSCs. These protocols are applicable to model autosomal dominant polycystic kidney disease (Kuraoka et al. J Am Soc Nephrol 2020). Furthermore, mouse organoids reassembled from the differentially induced ureteric bud and nephron progenitors, together with embryo-derived stromal progenitors, developed the inherent architectures of the embryonic kidney (Taguchi et al. Cell Stem Cell 2017). Induction of stromal progenitors from PSCs and generation of an organotypic structure in humans is under way.

S16

Modelling human pancreas development with organoids

Anne Grapin-Botton

Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauerstr. 108, 01307 Dresden, Germany

To attempt to understand human pancreas development, as a complement to investigations in mice and with mouse pancreas organoids, we established 3D (three-dimensional) culture conditions that enable the efficient expansion, differentiation and morphogenesis of pancreatic progenitors isolated from human fetuses or produced from human pluripotent stem cells (hPSCs). Using single-cell sequencing, we compared the transcriptional profile of cells grown in vitro in 2D, and 3D from hPSC-derived, to those isolated from fetal pancreas. Our experiments show a good molecular stability of pancreas progenitors over time, retention of the endocrine differentiation capacity and a molecular signature closer to the in vivo counterparts when grown in 3D than in 2D. We used this system to perform a screen for molecules controlling human pancreas progenitor expansion and to study differentiation dynamics towards the endocrine lineage. The systems developed enable to address the mechanisms of pancreas development, a process normally hidden from us in the womb, notably the function of genes controlling architectural events and morphogenesis operating in 3D that would be difficult to address in 2D culture, for example ductal lumen formation. The human organoids also open a way to investigate whether some genes identified in genome-wide association studies (GWAS) control the development of beta cells thereby predisposing to diabetes later in life. We initiated this process by studying the effect of GLIS3 in human development and started to clarify the mechanisms by which it promotes diabetes and pancreatic cysts.

S17

Design principles of multicellular systems

Prisca Liberali

Friedrich Miescher Institute for Biomedical Research (FMI) Basel, Switzerland

Multicellular organisms are composed of cells and tissues with identical genomes but different properties and functions. They all develop from one cell to form multicellular structures of astounding complexity. During development, in a series of spatio-temporal coordinated steps, cells differentiate into different cell types and establish tissue-scale architectures and functions. Throughout life, continuous tissue renewal and regeneration is required for tissue homeostasis, which also requires fine-tuned spatio-temporal coordination of cells. I will discuss how cellular interactions generate the specific contexts and spatio-temporal coordination underlying development and regeneration and how we specifically investigate what are the molecular and physical mechanisms that allow a cell, in a tissue, to sense its complex environment, to take individual coordinated decisions. Moreover, I will discuss the molecular mechanisms of intestinal organoid self-organization and the role of cell-to-cell variability in populations of differentiating cells during symmetry breaking.

S18

TBA

S19

TBA

S20

Self-organisation in mammalian development

Takashi Hiiragi

ASHBi Kyoto University, Hubrecht Institute

A defining feature of living systems is the capacity to break symmetry and generate well-defined forms and patterns through self-organisation. Our group aims to understand the principle of multi-cellular self-organisation using early mouse embryos as a model system. Mammalian eggs lack polarity and symmetry is broken during early embryogenesis. This results in the formation of a blastocyst consisting of three cell types, each distinct in its position and gene expression. Our studies revealed that morphogenesis and gene expression are highly dynamic and stochastically variable during this process. Determining how the blastocyst establishes a reproducible shape and pattern despite the preceding variability remains fundamental open questions in early mammalian development. We have recently developed an experimental framework that integrates biology, physics and mathematics. We aim to understand how molecular, cellular and physical signals are dynamically coupled across the scales for self-organisation.

S21

The Dynamics of Spinal Cord Development

James Briscoe

The Francis Crick Institute, London, UK

The generation of the correct cell types in the correct position, at the correct time is the first step in the assembly of functional tissues. One well-studied example of this is the development of the vertebrate spinal cord. In this tissue, distinct classes of neurons are generated in a precise spatial and temporal order from progenitor cells arrayed along the dorsal-ventral axis of the neural tube. Underpinning this organization is a complex network of extrinsic and intrinsic factors. Particularly well understood is the mechanism that determines the generation of different neuronal subtypes in ventral regions of the spinal cord. In this region of the nervous system, the secreted protein Sonic Hedgehog (Shh) acts in graded fashion to organize the pattern of neurogenesis. This is a dynamic process in which exposure to Shh generates progenitors with successively more ventral identities. At the same time tissue growth alters the arrangement of cells and the proportions of cell types and contributes to the elaboration of pattern. A gene regulatory network composed of transcription factors regulated by Shh signaling play an essential role in this process. Together, the mechanism determines pattern, pace, precision and proportions in the forming neural tube. Thus, accurate development of the neural tube and the specification of neuronal subtype identity relies on the interplay of cellular and molecular processes.

S22

Charting human development using a multi-endodermal organ atlas and organoid models

J. Gray Camp[1,2]

[1]Roche Institute for Translational Bioengineering (ITB), Roche Innovation Center Basel, Switzerland
[2]University of Basel, Basel, Switzerland

Organs are composed of diverse cell types that traverse transient states during organogenesis. To interrogate this diversity during human development, we generate a single-cell transcriptome atlas from multiple developing endodermal organs of the respiratory and gastrointestinal tract. We illuminate cell states, transcription factors, and organ-specific epithelial stem cell and mesenchyme interactions across lineages. We implement the atlas as a high-dimensional search space to benchmark human pluripotent stem cell (hPSC)-derived intestinal organoids (HIOs) under multiple culture conditions. We show that HIOs recapitulate reference cell states and use HIOs to reconstruct the molecular dynamics of intestinal epithelium and mesenchyme emergence. We show that the mesenchyme-derived niche cue NRG1 enhances intestinal stem cell maturation in vitro and that the homeobox transcription factor CDX2 is required for regionalization of intestinal epithelium and mesenchyme in humans. This work combines cell atlases and organoid technologies to understand how human organ development is orchestrated.

S23

Oogenesis *in vitro*: what we can and cannot reconstitute.

Katsuhiko Hayashi[1,2]

[1]Department of Stem Cell Biology and Medicine, Graduate School of Medical Sciences, Kyushu University
[2]Department of Germline Genetics, Graduate School of Medicine, Osaka University

Oocytes are highly specialized cells to confer totipotency on fertilized eggs. To create oocytes, the female germ line, originating from primordial germ cells, undergoes a unique sequence of differentiation processes that includes biologically important events such as epigenetic reprogramming, meiosis and oocyte growth associated with follicular development. Reconstitution of these processes using pluripotent stem cells provides a unique platform for a comprehensive understanding of mechanisms underlying oogenesis, as well as an alternative means of egg production.
Pioneering in vitro gametogenesis in mammals, reconstitution of oogenesis in mice has progressed rapidly over the past decade, during which we developed a culture system to produce cells closely resembling primordial germ cells, oocytes, and follicular somatic cells from mouse embryonic stem cells and induced pluripotent stem cells. By combining these culture methods, it is now possible to produce a robust number of mouse oocytes from pluripotent stem cells without sacrificing mice and their embryos. Is the role of the mouse as a pioneer in this field over? This seems highly unlikely, as we found several important processes that cannot be reconstituted using pluripotent stem cells. In this symposium, I will present our recent work on reconstitution of oogenesis in vitro and discuss further directions of this technology for a deeper understanding mammalian oogenesis.

S24

Genomic imprinting, human development and the ‘immortal’ germline

Azim Surani

Gurdon Institute, University of Cambridge

The germline generates the totipotent state at fertilization, where the parental genomes make an equal genetic contribution. However, in therians (mammals and marsupials) but excluding monotremes, the parental genomes exhibit an epigenetic asymmetry due to genomic imprinting, which requires epigenetic resetting starting soon after the specification of primordial germ cells (PGC), with the erasure of imprints and their re-initiation in gametes. “Imprinted genes”, which show mono-allelic expression during development and in adults depending on their parental origin, are thought to play an essential role in mammals, balancing embryonic growth and development, mammalian physiology, behaviour and metabolism.

Investigations of early germline development are challenging in human embryos that are largely inaccessible. Nonetheless, significant advances have been possible with the generation of *in vitro* models using pluripotent stem cells and non-rodent animals as surrogates for human development.

Research on human germline can contribute widely to advances in human development and diseases through, for example, advances in germline epigenome reprogramming that is critical for the ‘immortal’ germline and obligatory requiring comprehensive epigenetic erasure because of imprinting in mammals.

S25

The selfish testes of ageing men and *de novo* mutations

Anne Goriely

MRC Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, OX39DS, UK.
E-mail: Anne.goriely@imm.ox.ac.uk

It is now well-established that our genomes mutate at a slow but constant rate of 30-100 new point mutations per generation. Although most *de novo* mutations (>80%) originate from the father and increase in frequency at the rate 1-2 mutation/paternal year, very little is known about the cellular mechanisms that allow the adult testis to reconcile the contradictory demands for abundant sperm production - that takes place over many decades - and a low mutation rate across generations.
We have previously described a process where some pathogenic mutations hijack the homeostatic mechanisms of sperm production to their own advantage. This mechanism called 'selfish selection' was originally proposed to explain the paternal age-effect and high birth prevalence observed for some Mendelian disorders, such as Apert syndrome (*FGFR2*) or achondroplasia (*FGFR3*). It relies on principles similar to oncogenesis to explain why these pathogenic mutations occur spontaneously at levels up to 1000-fold higher than the background rate. Importantly, this process emphasizes the intimate link that exists between testicular homeostasis and germline mutation rate.
I will summarise our current understanding of *de novo* mutations in humans, the impact of paternal age and their importance for human disease and for genome heterogeneity/interpretation. I will then describe the data that have led to the discovery of the selfish selection process and the novel strategies we are developing to study *de novo* mutations directly within human testes. Finally, I will speculate on the broader implications of selfish selection and the importance of the regulation of spermatogenesis for human disease, genome diversity and evolution.

S26

Mechanism and In Vitro Reconstitution of Mammalian Germ-Cell Development

Mitinori Saitou[1,2,3]

[1]Institute for the Advanced Study of Human Biology, Kyoto University,
[2]Department of Anatomy and Cell Biology, Graduate School of Medicine, Kyoto University,
[3]Center for iPS Cell Research and Application, Kyoto University.

The germ-cell lineage ensures the creation of new individuals, perpetuating/diversifying the genetic and epigenetic information across the generations. We have been investigating the mechanism for germ-cell development, and have shown that mouse embryonic stem cells (mESCs)/induced pluripotent stem cells (miPSCs) are induced into primordial germ cell-like cells (mPGCLCs) with a robust capacity both for spermatogenesis and oogenesis and for contributing to offspring. These works have served as a basis for exploring the mechanism of key events during germ-cell development such as epigenetic reprogramming/programming, sex determination, and meiotic entry.

By investigating the development of cynomolgus monkeys, we have defined a developmental coordinate of the spectrum of pluripotency among mice, monkeys, and humans, and have identified the origin of the germ-cell lineage in cynomolgus monkeys in the amnion. Accordingly, we have induced human iPSCs (hiPSCs) with a primed pluripotency into human PGCLCs (hPGCLCs) and then into oogonia and early oocytes with appropriate epigenetic reprogramming. More recently, we have shown that hPGCLCs can be propagated to ~10^6-fold over a period of 4 months under a defined condition. These studies have created a foundation for human in vitro gametogenesis.

Here, I would like to discuss our latest findings regarding the mechanism and in vitro reconstitution of mammalian germ-cell development.

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