ASHBi Symposium 2021

Human Development, Genetics and Evolution

November 8–10, 2021

ASHBi International Symposium 2021

Symposium Time Slots

08 November 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		Development/Neural development Chair: Tadashi Isa 9:00–9:25 **Ryoichiro Kageyama** (RIKEN CBS/Kyoto U) 9:25–9:50 **Christopher Walsh** (Harvard Medical) 9:50–10:05 **Bhagat Shruti** (ASHBi)		16:00(-1)	19:00(-1)	0:00
9:30						
10:00		10:05–10:35 Break (30-min)		17:00(-1)	20:00(-1)	1:00
10:30		Development/Neural development Chair: Mitinori Saitou 10:35–10:50 **Yasuhiro Takashima** (Kyoto Univeristy) 10:50–11:15 **Diana Laird** (UCSF)				
11:00		11:15–11:40 **Davor Solter** (Max-Planck Society)		18:00(-1)	21:00(-1)	2:00

JST	Monday, 8 November (Day 1)	Tuesday, 9 November (Day 2)	Wednesday, 10 November (Day 3)	PST	EST	GMT
17:00	**Opening Remarks** Evolutionary mechanisms Chair: Yasuhiro Murakawa 17:10–17:35 **Henrik Kaessman** (Heidelberg U)	Organoid biology/Cancer genome Chair: Motoko Yanagita 17:00–17:15 **Cantas Alev** (ASHBi)	Development/Neural development Chair: Cantas Alev 17:00-17:25 **Takashi Hiiragi** (EMBL/ASHBi)	0:00	3:00	8:00
17:30	17:35–18:00 **James Turner** (The Francis Crick Institute)	17:15–17:40 **J Gray Camp*** (IMO Basel)	17:25-17:50 **James Briscoe** (The Francis Crick Instirtue)			
18:00	18:00–18:25 **Claire Rougelle** (U Paris)	17:40–18:05 **Ryuichi Nishinakamura** (Kumamoto U)	17:50–18:15 **Barbara Treutlein*** (ETH Zürich)	1:00	4:00	9:00
18:30	18:25-18:55 Break (30-min)	18:05–18:30 **Anne Grapin-Botton** (U Copenhagen)	18:15–18:45 Break (30-min)			
19:00	Introduction of ASHBi Chair: Mitinori Saitou 18:55–19:10 **Yasuaki Hiraoka** (ASHBi) 19:10–19:25 **Misao Fujita** (ASHBi)	18:30-19:00 Break (30-min) Poster Session 19:00–19:30 Poster Session 1	Germ Cells: Genetic and epigeneitic regulation Chair: James Turner 18:45–19:10 **Katsuhiko Hayashi** (Kyushu U)	2:00	5:00	10:00
19:30	19:25–19:40 **Takuya Yamamoto** (ASHBi) 19:40–19:55 **Tomoyuki Tsukiyama** (ASHBi)	19:30–20:00 Poster Session 2	19:10–19:35 **Azim Surani** (Wellcome Trust) 19:35–20:00 **Anne Goriely** (U Oxford)			
20:00	19:55–20:25 Break (30-min)	20:00–20:30 Break (30-min)	20:00–20:25 **Mitinori Saitou** (ASHBi)	3:00	6:00	11:00
20:30	Evolutionary mechanisms Chair: Fumitaka Inoue 20:25–20:40 **Yasuhiro Murakawa** (ASHBi) 20:40–21:05 **Aida Andres** (U C London)	Organoid biology/Cancer genome Chair: Ryo Yamamoto 20:30–20:55 **Prisca Liberali** (F Miescher Inst)	20:25–20:35 **Closing Remarks**			
21:00	21:05–21:30 **Janet Kelso** (Max Planck)	20:55–21:20 **Toshiro Sato** (Keio U)	*updated	4:00	7:00	12:00
21:30		21:20–21:45 **Seishi Ogawa** (ASHBi)				
22:00				5:00	8:00	13:00

*1 Pacific Standard Time
*2 Eastern Standard Time
*3 Greenwich Meant Time

Monday, November 8 (Day 1)

Opening Remarks
17:00-17:10 Mitinori Saitou

Evolutionary mechanisms (Chair: **Yasuhiro Murakawa**)

17:10-17:35 **S-1**
The evolution of spermatogenesis across mammals
Henrik Kaessmann (Heidelberg University)

17:35-18:00 **S-2**
The evolution of X-dosage compensation in mammals
James Turner (The Francis Crick Institute)

18:00-18:25 **S-3**
X chromosome inactivation in humans
Claire Rougeulle (University of Paris)

18:25-18:55 Break

Introduction of ASHBi (Chair: **Mitinori Saitou**)

18:55-19:10 **A-1**
Fusion researches between biology and mathematics in ASHBi
Yasuaki Hiraoka (ASHBi, Kyoto University)

19:10-19:25 **A-2**
Biothics and Science at ASHBi
Misao Fujita (ASHBi, Kyoto University)

19:25-19:40 **A-3**
Introduction of Single-Cell Genome Information Analysis Core (SignAC) at ASHBi
Takuya Yamamoto (ASHBi, Kyoto University)

19:40-19:55 **A-4**
Generation of genetically modified monkeys in PRiME.
Tomoyuki Tsukiyama (PRiME, Shiga University of Medical Science)

19:55-20:25 Break

Evolutionary mechanisms (Chair: **Fumitaka Inoue**)

20:25-20:40 **S-4***
Single-cell enhancer identification reveals genetic and evolutionary mechanisms of human diseases
Yasuhiro Murakawa (ASHBi, Kyoto University)

20:40-21:05 **S-5**
Human evolution: how humans managed to adapt to life around the globe.
Aida Andres (University College London)

21:05-21:30 **S-6**
The contribution of ancient humans to understanding modern human genetic variation
Janet Kelso (Max Planck Institute for Evolutionary Anthropology)

*Selected talks

Tuesday, November 9 (Day 2 - Morning)

Development/Neural development (Chair: **Tadashi Isa)**

9:00-9:25 **S-7**
Dynamic transcriptional control of neural stem cells
Ryoichiro Kageyama (Kyoto University / RIKEN Center for Brain Science)

9:25-9:50 **S-8**
Molecular genetics of human brain development and evolution.
Christopher Walsh (Harvard Medical School

9:50-10:05 **S-9***
Elucidating the genetic and evolutionary differences between promoters and enhancers at high resolution
Bhagat Shruti (ASHBi, Kyoto University)

10:05-10:35 Break

Development/Neural development (Chair: **Mitinori Saitou)**

10:35-10:50 **S-10***
Analyzing human peri-implantation development using naïve pluripotent stem cells
Yasuhiro Takashima (Kyoto University)

10:50-11:15 **S-11**
A xenotransplantation model for studying the migration of human primordial germ cells
Diana J. Laird (University of California, San Francisco)

11:15-11:40 **S-12**
Epigenetic Mechanisms Controlling Early Mammalian Development
Davor Solter (Max-Planck Society)

*Selected talks

Tuesday, November 9 (Day 2 -Evening)

Organoid biology/Cancer genome (Chair: **Motoko Yanagita)**

17:00-17:15 **S-13***
Reconstituting human somitogenesis in vitro
Cantas Alev (ASHBi, Kyoto University)

17:15-17:40 **S-14**
Charting human development using a multi-endodermal organ atlas and organoid models
J Gray Camp (Roche Innovation Center Basel / University of Basel)

17:40-18:05 **S-15**
Kidney organoids for modeling human development and disease
Ryuichi Nishinakamura (Kumamoto University)

18:05-18:30 **S-16**
Modelling human pancreas development with organoids
Anne Grapin-Botton (MPI-CBG)

18:30-19:00 Break

Poster session

19:00-19:30 Poster Session 1

Room #	Poster #	Name	Affiliation
Room 1	P-1	Rio Tsutsumi	ASHBi, Kyoto University
Room 2	P-3	Mitsuru Sasaki-Honda	CiRA, Kyoto University
Room 3	P-5	Hanna Lu	The Chinese University of Hong Kong
Room 4	~~P-7~~	~~Shino Shimada~~	~~National Human Genome Research Institute~~
Room 5	P-9	Anjani Kumar Tiwari	Babasaheb Bhimrao Ambedkar University
Room 6	P-11	Shoichiro Tani	The University of Tokyo
Room 7	P-13	Takafumi Ichikawa	ASHBi, Kyoto University

19:30-20:00 Poster Session 2

Room #	Poster #	Name	Affiliation
Room 1	P-2	Shinya Oki	Kyoto University Graduate School of Medicine
Room 2	P-4	Zhe Wang	Institute for Frontier Life and Medical Sciences, Kyoto University
Room 3	P-6	Masaya Hagiwara	Cluster of Pioneering Research, RIKEN
Room 4	P-8	Kenji K. Kojima	Genetic Information Research Institute
Room 5	P-10	Takahiro Suezawa	Graduate School of Medicine, Kyoto University
Room 6	P-12	Shruti Bhagat	ASHBi, Kyoto University

20:00-20:30 Break

Organoid biology/Cancer genome (Chair: **Ryo Yamamoto)**

20:30-20:55 **S-17**
Design principles of multicellular systems
Prisca Liberali (Friedrich Miescher Institute for Biomedical Research)

20:55-21:20 **S-18**
Modeling human gastrointestinal diseases using organoid technology
Toshiro Sato (Keio University)

21:25-21:45 **S-19**
Molecular classification and risk stratification of colorectal cancer
Seishi Ogawa (ASHBi, Kyoto University)

Wednesday, November 10 (Day 3)

Development/Neural development (Chair: **Cantas Alev**)

17:00-17:25 **S-20**
Self-organisation in mammalian development
Takashi Hiiragi (ASHBi, Kyoto University / Hubrecht Institute)

17:25-17:50 **S-21**
The Dynamics of Spinal Cord Development
James Briscoe (The Francis Crick Institute)

17:50-18:15 **S-22**
Tracing and perturbing lineages during human brain organoid development
Barbara Treutlein (ETH Zurich)

18:15-18:45 Break

Session 4 Germ cells: Genetic and epigenetic regulation (Chair: **James Turner**)

18:45-19:10 **S-23**
Oogenesis *in vitro*: what we can and cannot reconstitute.
Katsuhiko Hayashi (Kyushu University)

19:10-19:35 **S-24**
Genomic imprinting, human development and the 'immortal' germline
Azim Surani (Wellcome Trust/CRUK Gurdon Institute, University of Cambridge)

19:35-20:00 **S-25**
The selfish testes of ageing men and de novo mutations
Anne Goriely (University of Oxford)

20:00-20:25 **S-26**
Mechanism and In Vitro Reconstitution of Mammalian Germ-Cell Development
Mitinori Saitou (ASHBi, Kyoto University)

Closing Remarks
20:25-20:35 Takashi Hiiragi

Posters

P-1 **MODELING LIMB SKELETOGENESIS IN ORGANOID CULTURE DERIVED. FROM MOUSE EMBRYONIC LIMB BUD AND HUMAN ES CELLS**
Rio Tsutsumi (ASHBi, Kyoto University)

P-2 **Data-driven and technical approaches to understand spatial gene regulation**
Shinya Oki (Department of Drug Discovery Medicine, Kyoto University Graduate School of Medicine)

P-3 **Modeling disease-specific gene regulation of facio-scapulo-humeral muscular dystrophy (FSHD)**
Mitsuru Sasaki-Honda (CiRA, Kyoto University)

P-4 **Hydrogel for precise manipulation of human pluripotent stem cells microenvironment**
Zhe Wang (Institute for Frontier Life and Medical Sciences, Kyoto University)

P-5 TBA
Hanna Lu (The Chinese University of Hong Kong)

P-6 **Organoids Platform: Control and Design the microenvironments to achieve organoid architecture**
Masaya Hagiwara (Cluster of Pioneering Research, RIKEN)

P-7 Cancelled
~~Shino Shimada (NHGRI)~~

P-8 **Human Transposable Elements in Repbase: Genomic Traces of Human Evolution**
Kenji K. Kojima (Genetic Information Research Institute)

P-9 **Brain Energy Dynamics with integration of functions**
Anjani Kumar Tiwari (Babasaheb Bhimrao Ambedkar University)

P-10 **Disease modelling of pulmonary fibrosis using human pluripotent stem cell-derived alveolar organoids**
Takahiro Suezawa (Department of Drug Discovery for Lung Diseases, Graduate School of Medicine, Kyoto University / Watarase Research Center, Kyorin Pharmaceutical Co. Ltd.)

P-11 **Elucidating dynamic gene regulatory networks in human skeletal development using single cell analyses on a human pluripotent stem cell-based model of endochondral ossification**

Shoichiro Tani (Center for Disease Biology and Integrative Medicine, Graduate School of Medicine, The University of Tokyo / Sensory & Motor System Medicine, Graduate School of Medicine, The University of Tokyo)

P-12 **Elucidating the genetic and evolutionary differences between promoters and enhancers at high resolution**

Shruti Bhagat (Institute for the Advanced Study of Human Biology (ASHBi), Kyoto University / RIKEN Center for Integrative Medical Sciences / Department of Biosciences and Nutrition, Karolinska Institutet)

P-13 **An *ex vivo* system to study cellular dynamics underlying mouse peri-implantation development**

Takafumi Ichikawa (European Molecular Biology Laboratory (EMBL) / Institute for the Advanced Study of Human Biology, Kyoto University)

S-1

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.

S-2

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.

S-3

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.

S-4

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.

S-5

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.

S-6

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.

S-7

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.

S-8

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.
Supported by the NIMH, NINDS, the Allen Frontiers Program through the Allen Discovery Center for Human Brain Evolution, and the HHMI.

S-9

Elucidating the genetic and evolutionary differences between promoters and enhancers at high resolution

Shruti Bhagat[1,2,3], Akiko Suga[4], Akiko Oguchi[2,5], Naoki Hirose[1,2,6], Shuichiro Komatsu[2], Shigeki Hirabayashi[7], Juha Kere[3], Yoshihide Hayashizaki[8], Hideya Kawaji[2,6,8], Chikashi Terao[2], Michael P. Fautsch[9], Takeshi Iwata[4#] and Yasuhiro Murakawa[1,2,8,10#]

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[6]Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan
[7]Department of Hematology and Oncology, Kyoto University, Kyoto, Japan
[8]RIKEN Preventive Medicine and Diagnosis Innovation Program, Wako, Japan
[9]Department of Ophthalmology, Mayo Clinic, Rochester, MN, USA
[10]IFOM, The FIRC Institute of Molecular Oncology, Milan, Italy
[#]Co-corresponding authors

Promoters and enhancers are cis-regulatory elements that are instrumental in driving gene expression. We sought to identify human retina-specific promoters and transcribed enhancers at nucleotide resolution to elucidate which features differentiate these elements. We profiled transcription start sites of mRNAs and enhancer RNAs generated bidirectionally from functionally active enhancers in retina samples in humans, rhesus macaque, and mice at an unprecedented depth. We show that cell-type-specific transcription factors such as the homeobox family are specifically enriched in retinal enhancers but not in promoters. While sequences of many enhancers were conserved until mouse, a larger fraction of enhancers is conserved only in rhesus macaque (but not in mouse) when compared with promoters. Additionally, transcriptional events are also preserved for sequence conserved enhancers. We also identified SNPs associated with macular thickness, which were located in transcribed enhancers potentially disrupting transcription factor binding. Our study establishes a general framework for the functional interpretation of enhancers providing insights into understanding the role of enhancers in genomic evolution and gene regulation.

Keyword(s): promoters, transcribed enhancers, gene regulation, transcription factors, evolution

S-10

Analyzing human peri-implantation development using naïve pluripotent stem cells

Yasuhiro Takashima

CiRA, Kyoto University

We aim to understand early human development by focusing on the pre-implantation and post-implantation stages and their transition. Most knowledge about peri-implantation development is based on mouse models. Recent single-cell RNA sequencing (scRNA-seq) analysis of pre-implantation embryos, however, has revealed critical differences between humans and rodents. In contrast, differences at the peri-implantation stage are obvious even morphologically when comparing mouse and human embryos. For example, in humans, epiblast and primitive endoderm (hypoblast) make a bilaminar disc, but in mouse, one sees a cylindrical structure. In addition, human embryos at this stage contain the amnion and extraembryonic mesenchyme, which do not exist in rodents before gastrulation. Therefore, to understand human development, human models are ideal, but ethical limitations restrict access to human uteri during peri-implantation.
To overcome these issues and achieve our objective, our group has established a series of methods and human cells that correspond to human peri-implantation embryos. The blastocyst contains three different cell types, epiblast, hypoblast, and trophectoderm. We successfully induced hypoblast and trophectoderm from naive human pluripotent stem cells (PSCs) that share features with pre-implantation embryos. In this presentation, I will present the specification in vitro of naive human PSCs to extraembryonic lineages and embryonic development during peri-implantation.

S-11

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.

S-12

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.

S-13

Reconstituting human somitogenesis *in vitro*

Cantas Alev

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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.

S-14

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

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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.

S-15

Kidney organoids for modeling human development and disease

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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.

S-16

Modelling human pancreas development with organoids

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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.

S-17

Design principles of multicellular systems

Prisca Liberali

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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.

S-18

Modeling human gastrointestinal diseases using organoid technology

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Recent advances in sequencing technology delineated the genetic abnormality of human cancers. However, there remains a gap between genetic abnormalities and clinical cancer phenotypes due to a paucity of disease models that can recapitulate a variety of biological aspects of clinical cancers. Organoid technology was originally developed to regenerate tissue-like structures from pluripotent stem cells or tissue stem cells. Recent works revealed that organoids could be derived from patient cancer tissues without losing their original biological traits, such as drug sensitivity, pathohistology, cancer stem cell hierarchy, and metastatic potentials. Despite this progress, the limited spatiotemporal resolution has precluded us from understanding the biological behavior of patient-derived cancers at a single-cell level. We have recently developed a live genetic fate-mapping system that allows longitudinal tracking of individual cells in xenotransplanted human colorectal cancer organoids and identified a dormant LGR5+ cancer stem cell subpopulation that repopulates the tumors after chemotherapy. Organoid-based biological analysis revealed the molecular mechanism of how cancer tissues maintain the dormancy of cancer stem cells, providing a feasible therapeutic strategy targeting dormant cancer stem cells. In this symposium, I would like to share our recent findings and discuss the approach to override the drug resistance of cancer cells.

S-19

TBA

S-20

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.

S-21

The Dynamics of Spinal Cord Development

James Briscoe

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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.

S-22

Tracing and perturbing lineages during human brain organoid development

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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.

S-23

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

Katsuhiko Hayashi

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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.

S-24

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.

S-25

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

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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.

S-26

Mechanism and In Vitro Reconstitution of Mammalian Germ-Cell Development

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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.

P-1

MODELING LIMB SKELETOGENESIS IN ORGANOID CULTURE DERIVED FROM MOUSE EMBRYONIC LIMB BUD AND HUMAN ES CELLS

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Approximately 1 in 2000 infants are bone with Congenital limb deficiencies. Although developmental genetic studies have contributed to our detailed understanding on molecular mechanisms which control limb morphogenesis, in vitro model of 3-dimensional limb morphogenesis has not yet been established.

Limb skeletons are derived from limb bud mesenchymal cells during embryonic development. Shapes the limb skeletons are different depending on their positions in the limb. For example, stylopod (upper arm and thigh) has one bones, zeugopod (lower arm and shin) has two bones, and autopod (hand, foot) has many small bones including phalanges. After formation of limb skeletons, those bone grow differently depending on their position. For example, human thigh and shins grow relatively longer than arms. This pattern of differential bone growth is one of the specific features of bipedal human body as extant non-human apes shows longer arms than legs.

In order to establish in vitro model of position-specific cartilage morphogenesis and growth, we isolated limb bud mesenchyme from mouse embryo and differentiate those cells into limb cartilage in 3-dimensional culture. Interestingly, after manipulation of positional identities of those cells by cytokines, those aggregates showed different patterns of cartilage formation and its growth. Those differences might reflect position-specific cellular properties which contribute to position-specific morphogenesis during limb development.

Next, we aimed to establish a model of human limb morphogenesis in 3-dimensional culture. We drove differentiation of human embryonic stem cells towards limb bud mesenchyme and derived cells which express typical limb mesenchyme marker genes. Those cells were able to form cartilage in 3-dimensional culture.

We expect our study would establish novel model system to assess causality of human congenital limb deficiencies. Furthermore, we recently reported the derivation of limb bud cells from mouse embryonic stem cells. Establishment of self-organizing system of limb skeletal morphogenesis from pluripotent stem cells of multiple species would open up venues to understand cellular mechanisms of species-specific limb morphologies.

P-2

Data-driven and technical approaches to understand spatial gene regulation

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Multicellular organisms are composed of a variety of tissues and cell types, and their dynamic changes are organized by spatiotemporal gene expression. Although numbers of genes have been characterized to be expressed in specific tissues and time points, underlying mechanisms of the spatiotemporal gene regulation are poorly understood. To elucidate the mechanisms with a data-driven framework, we have developed ChIP-Atlas, an epigenomics database fully integrating public ChIP-seq, ATAC-seq, and Bisulfite-seq data (> 240,000 experiments; Oki, S., et al. *EMBO Rep* 2018). By taking advantage of the huge amount of data, we identified multiple transcription factors pivotally involved in cellular differentiation, genetic diseases, and drug actions. In addition, to understand spatial gene expression in a high-resolution manner, we established a transcriptome profiling method coupled with photo-isolation chemistry (PIC) that allows the determination of expression profiles specifically from photo-irradiated regions of interest (Honda, M., et al. *Nat Commun* 2021). By using this method, we identified area-specific transcripts not only from microtissues in mouse embryos and brains but also from subcellular and subnuclear microstructures (stress granules and nuclear speckles, respectively). In this symposium, I would like to talk about the regulatory mechanism of spatial gene expression revealed by the combination of data-driven and technical approaches, and further discuss the application for the research of developmental biology and pathological processes.

Keyword(s): gene regulation, epigenomics database, spatial transcriptomics

P-3

Modeling disease-specific gene regulation of facio-scapulo-humeral muscular dystrophy (FSHD)

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Facio-scapula-humeral muscular dystrophy (FSHD) is a skeletal muscle genetic disorder caused by patient-specific structural variants on 4q35 region characterized by shortened macrosatellite D4Z4 repeats (<10, FSHD type 1) or relatively short D4Z4 repeats (basically 10~20) with chromatin regulators' mutations (FSHD type 2). These abnormal variants are supposed to relax otherwise heterochromatin structure to allow toxic DUX4 gene expression in a sporadic manner in muscle cells. How DUX4's stochastic expression is regulated is critical to understand pathology and a key to drug targets for cures, but still remains unclear. Combining our FSHD patient-iPSC muscle cells model with patient-specific DUX4 activation, a DUX4-sensitive fluorescence reporter system and FACS sorting, we established a model of the sporadic pattern of DUX4 gene expression in FSHD cells, which showed distinct transcriptome and chromatin structure in DUX4 positive cells compared to negative population or genetically modified cells, indicating the rare population with high DUX4 expression is the main source of DUX4 expression in FSHD muscle. Moreover, for treatment, we developed a method to achieve prolonged DUX4 suppression by transient activity of dCas9-mediated DNA methylation editing, which could work for both types of FSHD cell models beyond difference in genetic backgrounds and seemed sufficient to target rare DUX4 high population. This method hopefully will provide a clue to avoid potential risks around typical gene therapy strategy and drug delivery system.

Keyword(s): skeletal muscle disease, FSHD, DUX4, epigenome editing

P-4

Hydrogel for precise manipulation of human pluripotent stem cells microenvironment

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Mammalian cells sense the cellular microenvironment and adjust their behavior based on the surrounding chemical/mechanical/topological conditions. Culture substrates that allow precise manipulation of these properties would help us better recapitulate the in vivo environment and benefit tissue engineering. Hydrogels have been used intensively in biomedical research as cell culture substrates due to their mechanical and chemical similarity with the extracellular matrix3. However, current well-used hydrogels whether do not allow human pluripotent to attach and growth or do not fully recapitulate in vivo microenvironment, in terms of their physical, chemical, and topological features. To overcome this problem, we developed a polyvinyl alcohol/Polyethylene glycol composite hydrogel (PVA-PEG gel) which allows us to spatially control the stiffness and the geological pattern at the micrometer level. Laminin 511 conjugated PVA-PEG gel supports human pluripotent stem cell maintenance and differentiation. Furthermore, by spatially control the stiffness of the patterned gel, we could differentiate cells towards different fates on the same patterned gel, thus generate a tissue with complex structure. Generated patterned gel with millimeters thickness allows cultured cells to form aggregate and achieve complicated tissue transformation. Taken together, our hydrogel provided a culture platform with well controlled microenvironment for human pluripotent stem cell research.

Keyword(s): Hydrogel, Stem cell, Microenvironment, Culture substrate

P-5

TBA

P-6

Organoids Platform: Control and Design the microenvironments to achieve organoid architecture

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In vitro 3D and organoid culture methods that mimic the rich complexity of cell population and extracellular matrix (ECM) components of *in vivo* tissues contribute greatly to furthering our understanding of various biological phenomena. Nevertheless, it remains a challenge to exert control over the repeatability, shape and architecture, as well as tissue-tissue interactions of cultured organoids. Engineering principles, on the other hand, are great tools that enable us to tailor the design, composition, and construction of organoids according to the intended purpose of the study. On the other hand, the technologies tend to be too complicated to be used when the multiple-factors need to be controlled simultaneously.

In this research, we will present how our simple cube culture device, which comprises a polycarbonate frame with rigid agarose walls and an inner ECM hydrogel, can be used to (i) control the spatial distribution of cells by employing 3D-printed moulds to create cell seeding pockets in the ECM hydrogel, (ii) design tissues with localized ECM by isolating ECM hydrogels of varying the composition or stiffness in separate compartments, and (iii) facilitate easy integration with microfluidics for regulating the concentration gradient of morphogens to direct cell growth and differentiation, applying shear stress to cells, as well as connecting organoids for tissue-tissue interactions. The simple organoid platform attains easy integration with other technologies to achieve complex environmental control.
Thus, it is envisioned that increasingly sophisticated and interconnected organoids will be achievable in the future.

Keyword(s): Organoid culture, Tissue engineering, ECM, Repeatability

P-7

Cancelled

P-8

Human Transposable Elements in Repbase: Genomic Traces of Human Evolution

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Repbase is a comprehensive database of eukaryotic transposable elements (TEs) and repeat sequences, and contains over 1300 human repeat sequences. Recent analyses of repeat sequences have accumulated evidences for the contribution of repeat sequences to the human evolution through becoming functional elements such as protein-coding regions or binding sites of transcriptional regulators. However, resolving the origins of these repeat sequences is a challenge because of their age, divergence and degradation. Ancient repeats have been continuously classified as TEs by characterizing similar TEs from other organisms. Here the most comprehensive picture of human repeat sequences is presented. Human genome contains traces of 10 clades (*L1, CR1, L2, Crack, RTE, RTEX, R4, Vingi, Tx1* and *Penelope*) of non-long terminal repeat (non-LTR) retrotransposons (long interspersed elements, LINEs), 3 types (*SINE1/7SL, SINE2/tRNA*, and *SINE3/5S*) of short interspersed elements (SINEs), 1 composite retrotransposon (*SVA*), 4 classes (*ERV1, ERV2, ERV3* and *Gypsy*) of LTR retrotransposons, and 11 superfamilies (*Crypton*, *Harbinger*, *hAT, Helitron, Kolobok, Mariner, Merlin, MuDR, P, piggyBac* and *Transib*) of DNA transposons. These footprints of TEs reveal an evolutionary pass of the human genome.

Keyword(s): Transposable elements (TEs), retrotransposons, DNA transposons, Repbase

P-9

Brain Energy Dynamics with integration of functions

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Glucose is one of the most important substrate of human brain that is responsible for different functions at cellular level. Glucose molecule is metabolized at different node points for neuronal/microglial/astrocytic signaling. It has been assumed that the rate of glucose consumption is one Glucose/synapse/ms (millisecond) for approximate 170 biochemical/metabolic processes occurring in different brain region. But the next question is……..Is this rate is similar to all region….and answer is no. The speed of glucose metabolism is heterogeneous throughout the brain. One of the major flux consumption is Neuron–astrocyte cycling of glutamate and glutamine in glutamatergic neurons (approximately 80 % of glucose metabolism in brain). However, many important insights of the energetics and their functions remain to be firmly established for in vitro/in-vivo conditions.

The next question is about the regulation of this metabolism which is complex due to involvement of various enzymes/membrane transporters of the related energy dynamics. Lactate, is one of the most important marker which is shuttled in between glycolytic/oxidative cells. Now its transportation through monocarboxylate transporters (MCTs) and its rapid equilibrium is equally important for astrocytes and cancer cells.

How much quantification of cellular glucose and other related substrate in resting, activated state studied using [^{18}F]FDG -positron-emission tomography (studying CMRglc) and [^{13}C/31P]glucose/magnetic resonance spectroscopy (^{13}C-MRS: for neuroenergetics & neurotransmitter cycling &^{31}P-MRS: for energy induction & redox state) has helped to increase our insight.

Keyword(s): Metabolism, Brain, PET, MRS

P-10

Disease modelling of pulmonary fibrosis using human pluripotent stem cell-derived alveolar organoids

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Epithelial-mesenchymal interactions have long been reported to play important roles in various fibrosis-related disorders, and in site-specific tissue regeneration. Although alveolar epithelial cell injury followed by mesenchymal cell activation play a critical role in the pathogenesis of pulmonary fibrosis, there are few practical in vitro models to study the alveolar epithelial-mesenchymal interaction, because of the difficulty of culturing alveolar epithelial type 2 (AT2) cells which are lung epithelial stem cells that can self-renew and differentiate into alveolar epithelial type 1 (AT1) cells. Here, we established a novel in vitro pulmonary fibrosis model using alveolar organoids consisting of human pluripotent stem cell-derived alveolar epithelial cells and primary human lung fibroblasts. In this human model, bleomycin (BLM) treatment induced phenotypes such as epithelial cell-dependent organoid contraction, cellular senescence of AT2 cells, and presence of abnormally differentiated alveolar epithelial cells, which have been observed in the pulmonary fibrosis lung. Chemical screening identified that inhibition of TGFβ signaling could therapeutically ameliorate these epithelial cell phenotypes. Furthermore, treatment of BLM-stimulated alveolar organoids with a TGFβ signaling inhibitor increased the expression of AT1 markers and induced thinner morphology of epithelial cells, suggesting that inhibition of TGFβ could be therapeutic for the pathogenic phenotypes, such as cellular senescence and abnormal differentiation states, with maintaining or protecting the differentiation states of alveolar epithelial cells. In this model, the injured epithelial cells specifically expressed integrin αVβ6, a major activator of latent TGFβ, indicating that epithelial cell-dependent activation of TGFβ signaling occurred in the organoids. In addition, organoid contraction and extracellular matrix accumulation, which could depend on activated fibroblasts, were also suppressed by inhibiting TGFβ signaling. This human model may therefore accelerate the discovery of effective therapeutic agents for the otherwise incurable pulmonary fibrosis by targeting alveolar epithelial cells and epithelial-mesenchymal interactions.

Keyword(s): alveolar epithelial cell, pulmonary fibrosis, epithelial-mesenchymal interaction, pluripotent stem cell, contraction

P-11

Elucidating dynamic gene regulatory networks in human skeletal development using single cell analyses on a human pluripotent stem cell-based model of endochondral ossification

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Elucidating dynamics of gene regulatory networks is an essential step to understand the physiology and pathology in target organs. Although human pluripotent stem cells (hPSCs) can differentiate into any embryonic cell type, it is challenging to recapitulate a whole process of skeletal development, from hPSCs to 3D bone tissues. To recapitulate human bone formation, we induced the in vitro differentiation of hPSCs into sclerotome and implanted them beneath the renal capsules of immunodeficient mice. RNA-seq analysis demonstrated cell type-specific gene expression, indicating a stepwise differentiation of hPSCs into the sclerotome. In vivo micro-CT analysis after implantation showed the growth of mineralized tissues over time. Histological analyses revealed structures similar to endochondral bones with specific marker expression patterns: columnar structures of chondrocytes, bone collar, and bone marrow. The induced bone tissues were then analyzed by single-cell RNA-seq, and the obtained data were integrated with the publicly available dataset of human embryonic long bones at 8 weeks post conception. Clustering analysis revealed that multiple skeletal cell types with distinct gene expression signatures, including embryonic skeletal progenitors, were commonly present in both datasets. Pseudotime analysis predicted a bifurcating trajectory from skeletal progenitors to osteoblasts or chondrocytes. By integrating differential gene expression analysis, gene regulatory network analysis, and ligand-receptor analysis, we extracted novel transcriptional factors that may regulate human osteogenesis. In situ hybridization of the hPSC-derived bone tissues showed a partial co-expression of the identified regulators with *RUNX2* and *SP7*, master transcriptional regulators of osteoblasts. The knockdown of the identified regulators downregulated osteoblast marker genes in a human osteosarcoma cell line and human mesenchymal stromal cells, indicating the involvement of these regulators in human osteogenesis. Collectively, our research may provide a valuable framework based on a well-recapitulated human skeletal development to investigate detailed mechanisms underlying its dynamic processes.

Keyword(s): pluripotent stem cells, human skeletal development, single-cell analysis, gene regulatory networks

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Elucidating the genetic and evolutionary differences between promoters and enhancers at high resolution

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Promoters and enhancers are cis-regulatory elements that are instrumental in driving gene expression. We sought to identify human retina-specific promoters and transcribed enhancers at nucleotide resolution to elucidate which features differentiate these elements. We profiled transcription start sites of mRNAs and enhancer RNAs generated bidirectionally from functionally active enhancers in retina samples in humans, rhesus macaque, and mice at an unprecedented depth. We show that cell-type-specific transcription factors such as the homeobox family are specifically enriched in retinal enhancers but not in promoters. While sequences of many enhancers were conserved until mouse, a larger fraction of enhancers is conserved only in rhesus macaque (but not in mouse) when compared with promoters. Additionally, transcriptional events are also preserved for sequence conserved enhancers. We also identified SNPs associated with macular thickness, which were located in transcribed enhancers potentially disrupting transcription factor binding. Our study establishes a general framework for the functional interpretation of enhancers providing insights into understanding the role of enhancers in genomic evolution and gene regulation.

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An *ex vivo* system to study cellular dynamics underlying mouse peri-implantation development

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Upon implantation, mammalian embryos undergo major morphogenesis and key developmental processes such as body axis specification and gastrulation. However, limited accessibility obscures study of these crucial processes. Here, we develop an *ex vivo* Matrigel-collagen-based culture to recapitulate mouse peri-implantation development from E4.5 to 6.0. Our system not only recapitulates embryonic growth, axis initiation, and overall 3D architecture in 49% of cases, its compatibility with light-sheet microscopy enables study of cellular dynamics through automatic cell membrane segmentation. We find that upon implantation, release of the increasing tension in the polar trophectoderm is necessary for its constriction and invagination. The resulting extra-embryonic ectoderm plays a key role in growth, morphogenesis and patterning of the neighbouring epiblast, which subsequently gives rise to all embryonic tissues. This 3D-*ex vivo* system thus offers an unprecedented access to peri-implantation development for *in toto* monitoring, measurement and spatio-temporally controlled perturbation, revealing a mechano-chemical interplay between extra-embryonic and embryonic tissues.

Keyword(s): Peri-implantation development, Egg cylinder formation, Epiblast morphogenesis, Lumen formation, *In toto* live-imaging

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