

## MEETING REVIEW

## Human development, heredity and evolution

Ryuichi Nishinakamura<sup>1,\*</sup> and Minoru Takasato<sup>2</sup>**ABSTRACT**

From March 27–29 2017, the RIKEN Center for Developmental Biology held a symposium entitled ‘Towards Understanding Human Development, Heredity, and Evolution’ in Kobe, Japan. Recent advances in technologies including stem cell culture, live imaging, single-cell approaches, next-generation sequencing and genome editing have led to an expansion in our knowledge of human development. Organized by Yoshiya Kawaguchi, Mitinori Saitou, Mototsugu Eiraku, Tomoya Kitajima, Fumio Matsuzaki, Takashi Tsuji and Edith Heard, the symposium covered a broad range of topics including human germline development, epigenetics, organogenesis and evolution. This Meeting Review provides a summary of this timely and exciting symposium, which has convinced us that we are moving into the era of science targeted on humans.

**KEY WORDS:** Human development, Single-cell sequencing, Epigenetics, Organoid, Evolution

**Introduction**

Developmental biology research has a long history of studying model organisms such as the fruit fly *Drosophila melanogaster*, focusing mainly on early embryogenesis. One major revolution in the field came in the early 1990s, when knockout mouse technology led to a focus on embryogenesis and organ development in mammals. Now, recent advances in human embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) technology have enabled the generation of human organoids (miniature versions of human tissues and organs grown in culture) of a variety of tissues, which can at least partially mimic human diseases and potentially be used in drug discovery platforms. Genome editing is further accelerating these research activities. In addition, single-cell sequencing is becoming a powerful tool for developmental biology, revealing the states of individual cells during embryogenesis and hence allowing investigation of the dynamics of tissue and organ development in greater detail. This technology is now being applied to human embryos, as well organoids generated *in vitro*. Human embryos are obtained and analyzed routinely at some institutions to study human development, not only for RNA sequencing but also for live imaging. These technological advances made for a very timely symposium, although the scope of this meeting was in fact much broader than our expectations. Rapid progress in next-generation sequencing has enabled comparative studies between humans and other mammals that are revealing the evolutionary processes that led to modern humans. Thus, we are now in the era of human developmental biology. In this review of the recent RIKEN Center for Developmental Biology (CDB)

symposium ‘Towards Understanding Human Development, Heredity, and Evolution’ (Fig. 1), we summarize some of the prominent talks and take-home messages from the symposium, beginning with the investigation of early human embryogenesis and germline development, before considering talks focused on the epigenetic regulation of development, organogenesis and, finally, heredity and evolution of the human lineage.

**Germline development and early embryogenesis**

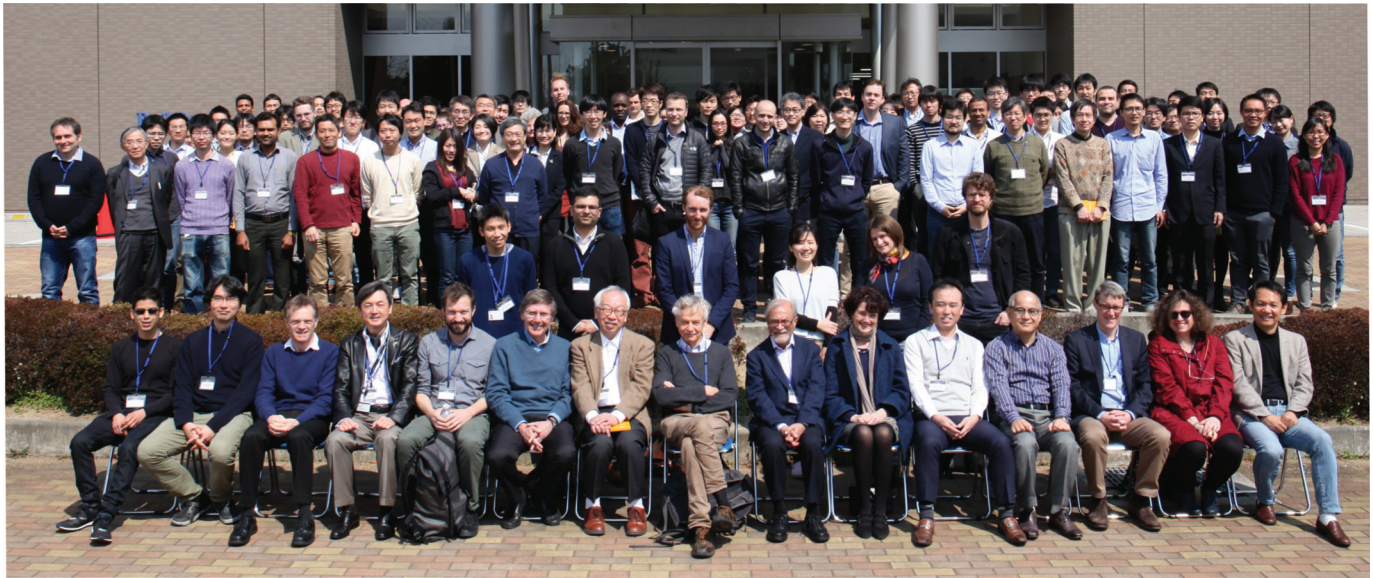
Mitinori Saitou (Kyoto University, Japan) previously succeeded in inducing mouse ESCs (mESCs) toward primordial germ cell (PGC) fate, generating cells that could contribute to spermatogenesis and oogenesis and to fertile offspring (Hayashi et al., 2011, 2012; Nakaki et al., 2013). He also reported PGC-like cells from human iPSCs (hiPSCs) (Sasaki et al., 2015). In his presentation, he discussed single-cell sequencing of pre- and post-implantation embryos of cynomolgus monkey, setting up a reference of normal early embryogenesis in non-human primates. This revealed that monkey ESCs and hiPSCs are transcriptionally similar to post-implantation late stage epiblasts, whereas mESCs correspond to the pre-implantation epiblasts (Nakamura et al., 2016). He further showed that primate PGCs do not appear in the epiblast but rather in the amnion as SOX17<sup>+</sup> TFAP2C<sup>+</sup> BLIMP1<sup>+</sup> cells and then migrate toward the epiblast (Sasaki et al., 2016). The inductive roles of Wnt and BMP in PGC specification are conserved from mouse, but their source is different: Wnt is provided from cytotrophoblast and BMP4 from amnion. This illustrates important species-specific differences and highlights the limitations of the mouse as a model of human development. Reconciling this finding – that human PGCs (hPGCs) first appear in the amnion – with the fact that hPGCs can be induced from hiPSCs through an incipient mesoderm-like state will require further investigation. Azim Surani (University of Cambridge, UK) also reported that the mechanisms of PGC specification between human and mice are not conserved. He showed that, unlike in mouse where SOX2, BLIMP1 (PRDM1) and PRDM14 are required for PGC specification, SOX17 and BLIMP1 could induce hPGCs from ESCs *in vitro*. Equal dosage of these two factors is important for hPGC specification; dominant expression of SOX17 over BLIMP1 produces aberrant cells with expression of some definitive endoderm genes. Furthermore, Surani obtained human embryos to investigate the *in vivo* development of hPGCs. In these samples, human-specific ‘escapee’ loci that evade germline reprogramming could be identified, revealing potential for transgenerational epigenetic inheritance that may have phenotypic consequences (Tang et al., 2015).

Patrick Chinnery (University of Cambridge, UK) studies mitochondrial DNA (mtDNA) heteroplasmy within the germline. Heteroplasmic mtDNA mutations cause inherited metabolic diseases when the proportion of mutant mitochondria exceeds a critical threshold. Interestingly, the phenotype varies widely even within the same family depending on the level of mtDNA heteroplasmy. Such variation between individuals can be explained by the ‘bottleneck theory’, in which the number of

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**Fig. 1. Participants at the RIKEN CDB symposium 'Towards Understanding Human Development, Heredity, and Evolution'.** Official symposium group photo reproduced with permission from RIKEN CDB.

mitochondria per cell is reduced from  $\sim 100,000$  in an egg to  $\sim 200$  in PGCs shortly after their specification (Cree et al., 2008; Freyer et al., 2012). The bottleneck leads to different proportions of mutant and wild-type mtDNA in different oocytes, and thus explains the variation in inherited mutation load among the offspring of a single mother. He suggested that PGCs with too many mtDNA mutations might not be able to survive further, whereas others with lower loads can develop and pass inherited phenotypes to the next generation. Tomoya Kitajima (RIKEN CDB, Japan) analyzed chromosome segregation (meiosis I) of mouse and human oocytes by automated high-resolution live imaging, and showed that premature separation of bivalents into univalents is a major cause of age-related chromosome segregation errors (Sakakibara et al., 2015). It is likely that weakly cohered bivalents between sister chromatids in aged oocytes as a consequence of reduced amounts of cohesin proteins cause these defects, which might also explain why smaller chromosomes are affected in disorders such as Down's syndrome.

Austin Smith (University of Cambridge, UK) proposed that a state of formative pluripotency (Smith, 2017) exists between the naïve and primed states of ESCs, characterized by a different transcription factor network. Using a *Rex1::GFP* mESC reporter line, he demonstrated that the *Rex1::GFP*-low population shows loss of naïve factors and gain of peri-implantation gene expression, without induction of lineage-specific markers, as well as accelerated exit from the pluripotency state upon differentiation cues, as compared with *Rex1::GFP*-high naïve cells. He also described the ERK targets downstream of FGF that are crucial for the transition to formative pluripotency; deletion of these causes delayed exit from the pluripotent state and impaired expression of peri-implantation genes. This intermediate formative phase of pluripotency might be especially important for understanding the development of primate embryos with a much longer peri-implantation period than mouse, as also shown by Saitou.

X-chromosome inactivation (XCI) plays an important role in early embryogenesis. Rickard Sandberg (Karolinska Institutet, Sweden) has established a sensitive single-cell RNA sequencing technology (Smart-seq2) that he used for allele-resolution analyses of transcription in early pre-implantation embryos. Whereas the paternal X chromosome is inactivated from the 4-cell stage in the

mouse, a gradual dosage compensation of the X chromosome occurs during human preimplantation development, taking until mature blastocyst stage and, surprisingly, maintaining expression from both X copies (Petropoulos et al., 2016). Moreover, the single-cell analyses revealed that transcription of autosomal genes is characterized by pronounced allelic fluctuations, leading to random monoallelic expression over time in cells, and to cellular heterogeneity (Reinius and Sandberg, 2015).

Edith Heard (Institut Curie, France) also discussed XCI. Although the *Xist* non-coding RNA is believed to be crucial for both imprinted and random XCI during mouse development, the exact reason for early lethality in the absence of *Xist* was not known. Through single-cell RNA sequencing, Heard showed that lack of paternal *Xist* leads to dosage compensation failure and genome-wide transcriptional misregulation as early as the blastocyst stage (Borensztein et al., 2017). She also discussed the rather different regulation of *XIST* in humans and other mammals such as rabbits. In these species, both X chromosomes show *XIST* RNA expression in early embryos, but only one ultimately retains *XIST* and becomes inactive. Finally, Heard described recent work on the reactivation dynamics of XCI in the mouse inner cell mass and the potential role of a histone demethylase in facilitating reprogramming of some genes. The talk revealed the remarkable diversity of X-chromosome silencing and reactivation during mammalian development, from mouse to human.

### Epigenetics and heredity

Despite advances in human development research, the mouse still remains a useful model organism, especially in the field of epigenetics, with likely relevance to the human system. Rudolf Jaenisch's lab (Massachusetts Institute of Technology, USA) has developed a method to monitor DNA methylation status at single-cell resolution. In this technique, a GFP reporter is fused to the *Snrpn* promoter, which is neutral but is sensitive to methylation of adjacent sequences, and this cassette can then be inserted into differentially methylated regions. Jaenisch reported an example in which the cassette was integrated at both alleles of an imprinted locus. The methylated paternal allele was negative for GFP expression, whereas the unmethylated maternal allele expressed

GFP. The reporter revealed heterogeneous GFP expression and DNA methylation in Purkinje cells of the cerebellum as well as in other tissues at single-cell resolution, suggesting selective changes in imprinting state. Contrary to expectation, the paternally transmitted (initially silent) reporter was expressed in muscle of males but not females, suggesting a non-cell-autonomous effect of sex hormones on imprinted allele methylation. He also introduced editing tools for DNA methylation: fusion of Tet1 or Dnmt3a to inactive Cas9 (dCas9) to either demethylate methylated or *de novo* methylate unmethylated genomic sequences (Liu et al., 2016). He showed that demethylation of the MyoD locus could lead to fibroblast to myoblast conversion, that *de novo* methylation at CTCF sites could disrupt CTCF-mediated chromatin looping, and that, in an hiPSC model of fragile X syndrome, modulating methylation could restore normal FMR1 expression. He even showed that this technique is applicable *in vivo* in mice, demonstrating its wide utility for functional studies of epigenetic regulation. It remains to be examined whether the effects of this manipulation of methylation status are transient or long-lasting.

Suppression of transposons is crucial for embryonic development and heredity. Deborah Bourc'his (Institut Curie, France) reported a new member of the DNA methyltransferase (Dnmt) family, Dnmt3c, which selectively targets the promoter of evolutionarily young retrotransposons and only during spermatogenesis. This gene specifically evolved in rodents by tandem duplication of *Dnmt3b* (Barau et al., 2016). This raises the question of how the male germline of humans and other non-rodent mammals controls retrotransposons. On a related theme, Anne Ferguson-Smith (University of Cambridge, UK) discussed the impact of transposons and their methylation status on non-genetic inheritance of certain traits in mice (Miska and Ferguson-Smith, 2016).

### Organogenesis

Readers will be aware that the late Yoshiki Sasai opened up the organoid research area, taking advantage of the self-organizing capacity of pluripotent stem cells. His groundbreaking achievements initiated new avenues of research worldwide. Mototsugu Eiraku (RIKEN CDB, Japan), a former member of the Sasai laboratory involved in these pioneering experiments, talked about the groundbreaking optic cup formation *in vitro* from mESCs and hiPSCs. He showed the size and growth speed differences between mouse and human organoids, and also demonstrated that even ventral fissures can be formed *in vitro*, which is likely to be due to Wnt activation on the dorsal side that generates dorsal-ventral polarity in the organoid (Hasegawa et al., 2016). In addition, he pointed out some limits to the self-organizing strategy. Since an asymmetric distribution of cells and factors is involved in complex structure formation, reproducibility can be low. To overcome this disadvantage, Eiraku described some of his recent trials employing a device to locally manipulate morphogen signaling.

An alternative cause of low reproducibility might be that induction is incomplete in organoid cultures. Rather than letting an organoid self-organize, as demonstrated for the kidney by Takasato et al. (2015), Ryuichi Nishinakamura (Kumamoto University, Japan) is trying to induce precursor populations of embryonic kidney separately and then combine them to generate the higher order structure of the kidney. He used the gene expression profiles of the mouse embryonic kidney as a reference for the cell type produced *in vitro*, and succeeded in generating three-dimensional kidney tissues both from mESCs and hiPSCs

(Taguchi et al., 2014). However, we are still faced with a problem in that we do not know how similar the induced human organoids are to their *in vivo* counterparts. Thus, revealing the characters and gene expression profiles of human embryonic tissues will be crucial to generate genuine human organs, instead of 'organoids'.

In this sense, the talk of Arnold Kriegstein (University of California, San Francisco, USA) should have a particular impact for organoid research. He utilized human fetal brain tissue for live imaging, lineage tracing, and single-cell sequencing (Hansen et al., 2010; Nowakowski et al., 2016; Pollen et al., 2014, 2015). Unlike the situation in mouse, the developing human brain contains an enormous number of outer subventricular zone radial glia (oRG) cells that are likely to contribute to the formation of the bigger human brain. He showed that oRG cells undergo a characteristic movement – mitotic somal translocation – when they divide, and that they divide asymmetrically to generate transient amplifying cells. Moreover, a single oRG cell can produce hundreds of upper and lower layer neurons by establishing a LIF/Stat3-dependent self-renewal niche. This niche is discontinuous with the conventional ventricular proliferative zone, and forms the primary scaffold for migration of upper cortical layer neurons to the cortical plate, emphasizing the unique nature of human brain development. He also provided evidence that glioblastoma, lissencephaly, and Zika virus infection are also associated with oRG cell defects. A clear take-home message of this and other talks was that a detailed understanding of how a tissue develops *in vivo* will be important to improve the authenticity of organoid models of human development and disease.

Human pluripotent stem cell-derived tissues/organoids can be used as platforms to test biological hypotheses about human development instead of using real human embryos. In this vein, Cantas Alev [Center for iPS Cell Research and Application (CiRA), Japan] established an *in vitro* model capable of mimicking somitogenesis by the directed differentiation of mESCs and hiPSCs. In this system, utilizing HES7-luciferase reporter lines, oscillatory gene expression was visualized to measure key properties of the segmentation clock. He also reported an example of abnormal oscillatory gene expression activity when selective oscillatory genes were knocked out using CRISPR/Cas9 genome editing technology. Yoshiya Kawaguchi (CiRA, Japan) showed the phenotype of pancreatic exocrine cell-specific *Pdx1* conditional mutant mice (*Elastase-Cre* × *Pdx1*<sup>fllox/fllox</sup>) (Kodama et al., 2016). In the mutant mice, the wild-type endocrine cells (insulin-producing cells) were somehow affected in maturation, expansion and differentiation, eventually resulting in symptoms of diabetes. This provided a good example of the complexity of organogenesis, which always involves interaction between multiple tissues and cell types.

Bioengineering is also an important approach for generating organs *in vitro*. Takashi Tsuji (RIKEN CDB, Japan) introduced bioengineered organ germs by combining mouse embryonic mesenchyme and epithelia in a tiny droplet in collagen gel and transplanting these into host animals. He reported successful generation of teeth, hair and salivary glands (Ogawa et al., 2013; Takagi et al., 2016; Yamamoto et al., 2016). Importantly, these tissues have functional nerve connections to the host animals. This type of approach will accelerate our understanding of the complexities of organ formation. Zev Gartner's (Stanford University, USA) research also focuses on tissue engineering, and he presented work looking at how the initial configuration of cells in organoids affects their outgrowth and ultimate three-dimensional geometry, with a focus on how cell dynamics in the mesenchyme affect the architecture of the overlying epithelium.

## Human evolution

Advances in sequencing technology have enabled analyses of human evolution and helped characterize genetic differences between species, differences between human races and even individuals, and adaptation to environments. The presentations at this meeting on this topic were eye-opening to developmental biologists, giving a broader view of human-focused scientific research. Guillaume Bourque (McGill University, Canada) presented the results from collaborating efforts of the International Human Epigenome Consortium. Using data from ENCODE (<https://www.encodeproject.org/>), he showed that 44% of open chromatin regions, as determined by DNase hypersensitivity assay, are in transposable elements (TEs). This figure reaches 63% for primate-specific regions, suggesting that TEs have been a major contributor to the evolution of gene regulation in the human genome (Jacques et al., 2013). TEs also serve as major contributors to the origin and diversification of long non-coding RNA (lncRNA). Indeed, TEs are found in 75% of lncRNAs, and lncRNAs from the human endogenous retrovirus H (HERVH) subfamily are required for pluripotency in human ESCs (Lu et al., 2014) and are conserved between primates (Ramsay et al., 2017). Thus, TEs have contributed significantly to human genome evolution.

The Icelandic company deCODE Genetics has collected DNA samples from just under half of the Icelandic population. These data provide a powerful platform to examine the association of sequence variants with human traits, helped by the relative homogeneity of Icelanders. Agnar Helgason from the company reported the reconstruction of genomes of long-dead ancestors from living descendants, focusing on the unusual case of Hans Jonatan, who was born to an enslaved African mother and a European father in the Caribbean and migrated to Iceland at the beginning of the 19th century. Using extensive single-nucleotide polymorphism (SNP) and whole-genome sequence data from his descendants, 38% of Hans Jonatan's African genome was reconstructed, and the geographical origin of his mother in Africa was determined. Although Hans Jonatan is an unusual case, ancestor reconstruction can in principle be extended to other individuals and populations, thereby adding power to studies in both evolutionary and medical genetics.

Genome sequencing of large numbers of human individuals is also revealing natural selection in contemporary populations. Molly Przeworski (Columbia University, USA) developed a method to identify genetic variants that affect viability in large cohorts. She applied it to two large datasets and found *APOE* e4 (a known risk factor for Alzheimer's disease) and *CHRNA3* (a nicotinic receptor associated with smoking quantity), as well as a number of other quantitative traits that affect viability in men. Because there are so few common variants that only affect survival late in life, others may have been weeded out of the population by natural selection.

Lluis Quintana-Murci (Institut Pasteur/CNRS, France) hypothesized that natural selection should act strongly on host defense genes, and examined expression quantitative trait loci (eQTL) in response to Toll-like receptor activation and influenza infection. Indeed, he found that population differences affect the response of eQTLs and that immune-related genes are privileged targets of natural selection in humans (Quach et al., 2016). He is also interested in the degree of genetic and epigenetic variation in the human population, and analyzed SNP data and genome-wide DNA methylation at 450,000 sites in various African populations. He showed that historical lifestyle affects DNA methylation under genetic control at genes associated with development, such as bone density and height, whereas methylation variation associated with

recent changes in habitat prominently affects loci involved in immune processes (Fagny et al., 2015). As Michael Snyder (Stanford University, USA) showed in his talk about personal 'omics' data analyses for managing and predicting diseases, these integrative approaches – using population genetics, functional genomics and big data relating to human lifestyles – represent a powerful means to understand (epi)genotype-phenotype correlations and mechanisms of adaptation/response to environmental stresses.

Gen Suwa (University of Tokyo, Japan) provided an overview of his work on human evolution through the investigation of hominid fossils. Fossils provide insights into morphological and behavioral attributes such as body size and locomotor behavior that have changed over human evolution. Using this information in combination with genomic data on other primates, we can speculate a timeline of human evolutionary history. Finally, Rasmus Nielsen (University of California at Berkeley, USA) gave some examples of human physiological adaptation to the environment. Tibetans, who have adapted to high altitude, have genetic variants in *EPAS1* (*HIF2A*) and *PHD2* (*EGLN1*), both of which regulate the hypoxic response (Huerta-Sánchez et al., 2014; Yi et al., 2010). Interestingly, the *EPAS1* haplotype was transferred into humans by introgression from an archaic hominin group. Another example is adaptation of the Inuit in Greenland to life in the Arctic (Fumagalli et al., 2015). This population has experienced natural selection on genetic variants in *TBX15*, a gene expressed in heat-producing brown adipose tissue, and in the genes encoding enzymes that catalyze rate-limiting steps in the synthesis of long-chain omega-3 and omega-6 polyunsaturated fatty acids. These changes have strong effects on many phenotypes including body mass index, plasma cholesterol and triglyceride levels. Inuit have a diet that is very rich in fatty acids from fish and marine animals, and the selection in these genes is thought to compensate for the altered dietary intake.

## Summary and future directions

This symposium clearly showed that humans are now realistic targets for research in developmental biology and evolution. Increased access to human embryos combined with single-cell RNA sequencing and *in vitro* culture techniques is opening the door to human developmental biology. Common and distinct mechanisms exist between humans and other animals, as exemplified in this symposium by both germ cell and brain development. A precise understanding of human development is also essential for organoid research, which requires many more breakthroughs before we can claim to have generated truly functional organs. Of course, ethical issues should be carefully addressed when dealing with human embryos; one case of careless handling would hamper the scientific progress of the entire research community. Genome sequencing of large numbers of human samples has turned humans into an experimentally amenable population, with various mutations on various genetic backgrounds. In this sense, not only interspecies but also intraspecies comparisons, especially between individuals/populations of humans, present new areas of focus where the target is to collect more examples showing phenotypic differences caused by genetic changes, which will help us further understand human development and physiology. While genomic information of individuals should be handled carefully for ethical reasons, a huge amount of information is being deposited in databases, and it will be increasingly important to interpret the flood of information, sometimes automated by artificial intelligence to process these

amounts of data. More researchers proficient both in laboratory and computational approaches, and collaboration between researchers from different disciplines will be necessary to advance the science of human developmental biology.

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#### Competing interests

The authors declare no competing or financial interests.

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