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From Mother or Father: Uniparental Embryos Uncover Parent-of-Origin Effects in Humans

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In mammals, both parents make unique contributions to the offspring and maternal and paternal genomes are required for development. Two recent papers in *Cell Stem Cell* (Leng et al., 2019; Sagi et al., 2019) study uniparental embryos and uniparental embryonic stem cells to interrogate parent-of-origin effects in human embryogenesis.

Organisms with sexual reproduction possess two sets of chromosomes, which are inherited from the mother and the father. Therefore, two copies of each gene exist in every cell. In mammals, a subset of genes is expressed only from either the maternal or paternal chromosome. These imprinted genes carry epigenetic modifications, including parental specific patterns of DNA methylation (Tucci et al., 2019), marking their parental origin. Evidence for the non-equivalence of parental genomes initially came from pronuclear transfer experiments using mouse embryos (McGrath and Solter, 1984; Surani et al., 1984). Uniparental embryos that were reconstituted by combining two maternal or two paternal pronuclei failed to complete embryogenesis, whereas reconstituted biparental embryos could develop into mice. These experiments demonstrated that parent-of-origin effects abrogated uniparental embryo development in mice.

In this issue of *Cell Stem Cell*, Leng et al. (2019) generate human uniparental embryos to analyze gene expression and DNA methylation. This work together with a related study in another recent issue of *Cell Stem Cell* (Sagi et al., 2019) now provides extensive characterization of parent-of-origin effects in human embryogenesis (Figure 1). These insights are important for extending earlier observations that were largely limited to mice. An understanding of parent-of-origin effects on phenotypes is important as genomic imprinting has implications for human diseases. A well-known example of the effect of genomic imprinting on disease development is found on human chromosome 15. Loss of the paternally inherited region on chromosome 15 causes Prader-Willi Syndrome, whereas loss of the maternally inherited region leads to Angelman Syndrome (Tucci et al., 2019). The new uniparental embryos and cells provide mechanistic insights into the ontogeny of parent-of-origin effects in early human development. The hope would be that these early changes can inform us about the root causes of diseases by allowing us to make observations at a time when most of the diverse symptoms have not yet manifested themselves.

Uniparental embryos have a complete imbalance of genomic imprinting as all their chromosomes are derived from one parent. Nevertheless, in both humans and mice, uniparental embryos develop to some extent. Earlier work on mouse embryos has shown that androgenetic embryos preferentially develop extraembryonic and placental structures at the expense of embryo development. Conversely, parthenogenetic embryos have poor development of placental lineages and arrest in development possibly due to extraembryonic defects.

Leng et al. and Sagi et al. generate parthenogenetic human embryos by artificially activating oocytes and follow preimplantation development in embryo culture dishes. Androgenetic embryos are constructed by removal of the maternal chromosomes and injection of paternal chromosomes from sperm. Although methodical details differ, both groups succeeded in generating a number of uniparental human embryos and characterize their development by comparing them to biparental embryos that were obtained through fertilization. They find that in humans, androgenetic development displays a predisposition for extraembryonic differentiation. This is an important result showing that parent-of-origin effects on development have similar consequences in human and mouse embryos, suggesting a conservation of genomic imprinting.

Leng et al. perform single-cell transcriptome analysis to gain a comprehensive view of gene expression in the cells of uniparental embryos at early stages of development. This allows them to observe when the embryonic genomes are activated and when genes begin to be transcribed. The first and minor wave of genome activation is clearly affected in androgenetic embryos, which show a delay in the expression of several genes, although they seem to catch up to some degree at the second and major wave of genome activation. Indeed, the initial differences in transcription of the parental chromosomes lead to the shifts in overall expression profiles. At the 4-cell stage, androgenetic embryos appear delayed and are more similar to 2-cell stage embryos. The opposite is observed for parthenogenetic embryos, which show similarities to 8-cell embryos at this stage. In parallel, differences in the timing of cell division are also observed. Androgenetic embryos divide faster than biparental embryos, whereas parthenogenetic embryos have slower cell divisions. This is an interesting observation as imprinted genes have been associated with embryonic growth and include cell-cycle inhibitors such as p57KIP2, which is expressed from maternal chromosomes only (Matsuoka et al., 1996). Although the role of p57KIP2 is not clear in this case, it is

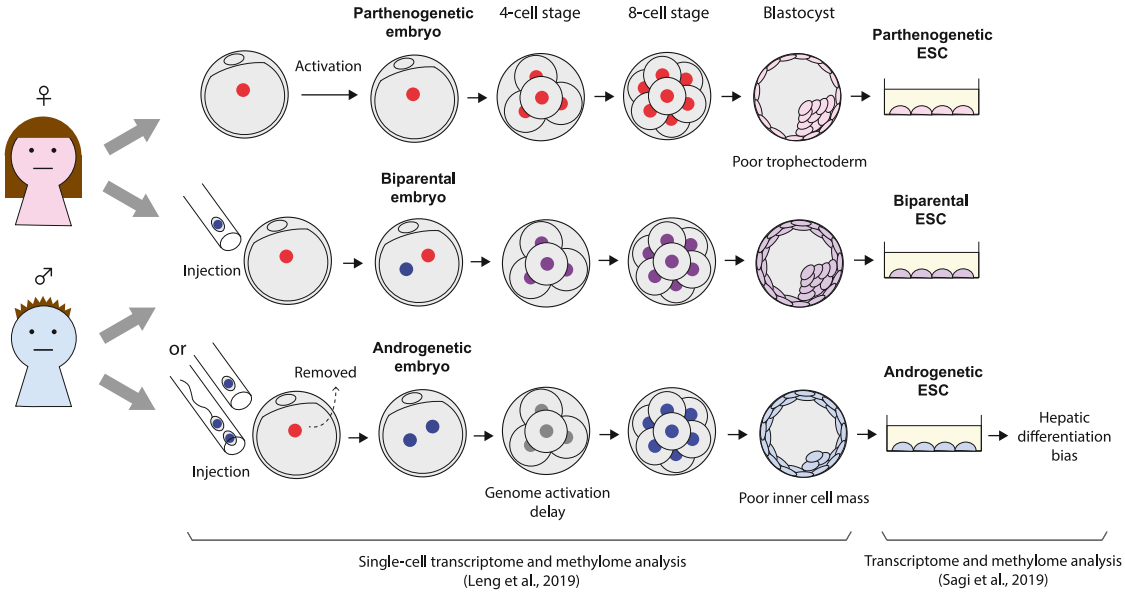

Figure 1. An Outline of the Techniques Used by Leng et al. and Sagi et al. for Generating Human Uniparental Embryos
The micromanipulation for generating androgenetic embryos by injection of sperm chromosomes is shown (left). Parthenogenetic embryos are generated by activation of oocytes. Key differences in the development of uniparental embryos from biparental embryos are marked along with the developmental stages and ESC cultures (right).

plausible to assume that cell-cycle differences might be linked to imprinted gene expression.

The study by Sagi et al. extends observations of uniparental human embryos further in development. This is made possible by their derivation of embryonic stem cells (ESCs) from blastocyst-stage embryos. ESCs can be induced in culture to differentiate into most of the cell types of human embryos. This allows for characterizing parent-of-origin effects on the development of cell lineages that contribute to tissues of organs in humans. From these studies, it appears that differentiation of liver cells is affected by the imprinted expression of the paternally expressed *IGF2* gene. Thus, ESCs provide a model for studying parent-of-origin effects on human post-implantation development.

The first mammalian imprinted genes were initially described in 1991. *Igf2r* (Barlow et al., 1991), *Igf2* (DeChiara et al., 1991), and *H19* (Bartolomei et al., 1991) were identified by genetic approaches in mice. Today, the list of imprinted genes has been extended well into the hundreds. Studying the mechanism of imprinting has shown a close association with parental-specific patterns of DNA methylation (Tucci et al., 2019). Leng et al. perform analyses of DNA methylation in human uniparental embryos and can associate DNA methylation with paternal-biased expression of a subset of genes. This result is consistent with a mechanism by which DNA methylation represses expression of these genes from the maternal chromosomes.

It can be expected that the detailed characterization of parental-specific gene expression and genomic imprinting will inspire further studies. Recent studies in mice might provide ideas on how to proceed. Considerable progress has been made to identify the functional differences between the parental epigenetic states of chromosomes. Initial studies have shown that deletions of the imprinting control regions in the *H19-Igf2* and *Dlk1-Gtl2* loci are sufficient to change a maternal genome of non-growing oocytes in a way that allows the modified genome to be used as a substitute for a paternal genome (Kawahara et al., 2007). Furthermore, a recent issue of *Cell Stem Cell* reported the use of androgenetic and parthenogenetic haploid ESCs from mice for identifying a set of seven mutations that change imprinted expression of paternal chromosomes to approximate the maternal chromosomes, albeit the conversion is not fully complete yet (Li et al., 2018). It will be exciting to see if future studies can identify similar mutations that cause developmental aberrations and diseases in humans. These studies in *Cell Stem Cell* provide a strong advance toward this aim.

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The Princess and the P: Pluripotent Stem Cells and P-Bodies

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In this issue of *Cell Stem Cell*, Di Stefano et al. (2019) identify the RNA helicase DDX6 as a regulator of stem cell pluripotency and differentiation. DDX6 depletion leads to P-body disruption, which, in turn, inhibits or promotes stem and progenitor cell differentiation, depending on their cellular context.

Processing bodies ("P-bodies") are membraneless cytoplasmic mRNP granules that form via liquid-liquid phase separation. P-bodies were originally reported as cytoplasmic sites of mRNA degradation that contain the key enzymes responsible for decapping and 5′ to 3′ degradation of mRNA molecules (Sheth and Parker, 2003). However, since their original discovery, P-bodies have been found to carry out additional important functions including storage sites for mRNAs (Brengues et al., 2005), cell signaling, stress regulation, and protein homeostasis (Zhang and Herman, 2019). The observation that P-bodies control both mRNA decay and long-term storage raises interesting questions regarding the regulation of these processes (Wang et al., 2018) and whether these functions might also be temporally separated (e.g., during different stages of development or cell differentiation).

In this issue of *Cell Stem Cell*, Di Stefano et al. (Di Stefano et al., 2019) report that P-bodies control stem cell exit from pluripotency, as well as adult progenitor cell differentiation, in a context-dependent manner. To search for potential regulators of pluripotency, the authors analyzed previous datasets of RNA binding proteins and identified the RNA helicase DDX6, an essential P-body component, as a key factor in the exit from pluripotency. Among the identified candidates, they found DDX6's involvement in regulating pluripotency to be the most prominent, as well as conserved in mouse and human pluripotent cells. In human pluripotent cells, DDX6 depletion resulted in the complete dissolution of P-bodies, which caused the cells to be entrapped in a "hyper-pluripotent," naive-like state and prevented differentiation (Figure 1A). Further analyses revealed the identity of several RNA transcripts that are kept translationally repressed inside the P-bodies, many of which encode for chromatin regulators.

Chromatin, composed of DNA, histone proteins, and additional structural molecules, regulates essentially all cellular processes including pluripotency. This occurs by changes in DNA methylation, post-translational modifications of histones, and chromatin compactness, all of which ultimately regulate gene expression. In pluripotent cells specifically, chromatin was shown to assume a unique, more open conformation, which contributes to stem cell function and potency (Schlesinger and Meshorer, 2019). Once liberated upon P-body disruption, mRNAs encoding for chromatin regulators are then free to produce functional proteins.

To test the functional consequences of increased translation of these chromatin regulators, Di Stefano et al. analyzed several chromatin features. Using ATAC-seq, they first probed chromatin accessibility across the genome and found over 7,400 sites that gained accessibility versus ~4,000 where accessibility was lost. Not only does this demonstrate a global chromatin reconfiguration, but also a shift into a globally more open chromatin structure, supporting the idea that the cells become hyper-pluripotent. Consistent with the observation that loss of DDX6 and the ensuing P-body disruption lead to a hyper-pluripotent state, sites