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# The Molecular Harbingers of Early Mammalian Embryo Patterning

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**Random or not, the mechanisms at play during the first cell-fate determination in mammalian embryos have been debated for years. Studies by Goolam et al. and White et al. shed new light on the molecular mechanisms underlying the intrinsic biases that lead to non-random lineage segregation in early mammalian development.**

In many non-mammalian species, asymmetrically localized maternal factors in the fertilized egg determine which cells become embryonic and extraembryonic lineages. In mammals, however, these "egg determinants" have not yet been identified, leaving open the question of when and how cellular heterogeneity first arises in a mammalian embryo and whether it has any bearing on ensuing cell-fate determinations. Efforts to address this question have resulted in recurring debates over the timing and mechanism underlying the first lineage choice: the specification of the trophectoderm (TE) versus the inner cell mass (ICM). Two seemingly contradictory models have been proposed (Graham and Zernicka-Goetz, 2016). The "stochastic" model argues that early embryonic cells are equal in their developmental potency and that lineage segregation arises as a consequence of stochastic processes leading to differential cell position and polarity. Contrary to this view, the "biased heterogeneity" model asserts that some developmental bias exists in early pre-implantation development, which predisposes uncommitted blastomeres to choose either TE or ICM fates. With the aid of single-cell transcriptomics and quantitative fluorescence analyses, in this issue of *Cell*, Goolam et al. (2016) and White et al. (2016) provide long-awaited molecular evidence in support of the biased heterogeneity model of early cell-fate commitment in mammals.

Mice, like other mammals, have a highly regulative early development that can compensate for a variety of natural or unnatural invasive perturbations. All blastomeres in early development display indistinguishable morphology and are thus believed to be equal in their generative capability. Unexpectedly, accumulating evidence derived from time-lapse studies, functional interrogations, epigenetic regulations, and kinetic behavior of a key pluripotent protein (OCT4) raises an intriguing possibility that developmental bias is present among blastomeres at as early as the 4-cell stage (Graham and Zernicka-Goetz, 2016; Burton et al., 2013; Torres-Padilla et al., 2007; Plachta et al., 2011). However, until now, the significance of these studies was undermined by lack of sufficient understanding at the molecular level of this early stage of embryogenesis, which in part can be attributed to the technical challenges involved. Recent advances in single-cell "omics" and fluorescence imaging of molecular dynamics have spurred new interests in identifying this "missing link" between early lineage specification and the observed heterogeneity in cleavage-stage mouse blastomeres.

In one of the studies, Goolam et al. perform a detailed comparative analysis of transcriptomes of all individual cells from mouse pre-implantation development. They find that OCT4/SOX2 target genes are, in part, responsible for the intra-embryonic heterogeneity of the 4-cell embryo and identify *Sox21*, a SOX2 downstream target, among the most highly variable genes in all 4-cell embryos analyzed. Through RNA interference (RNAi) experiments and live-cell tracking, the authors demonstrate that clonal reduction of *Sox21* levels leads to elevated expression of *Cdx2*, a master regulator of TE fate, and results in a greater contribution to the extra-embryonic progeny. In the accompanying study by White et al., the authors examine molecular heterogeneity in live 4-cell mouse embryos from the angle of transcription factor-DNA binding dynamics, a key process for gene regulation. By quantifying binding in single cells in vivo using photo-activatable fluorescence correlation spectroscopy, the authors reveal that transcription factors display lineage-specific binding properties to DNA in early blastocysts where ICM and TE have been segregated. Interestingly, they find that the long-lived SOX2-DNA interaction varies among uncommitted 4-cell blastomeres. Moreover, blastomeres that retain more long-lived SOX2-DNA-bound fraction yield more pluripotent progeny. Taken together, these results highlight that heterogeneous gene transcription dynamics mediated by SOX2-DNA binding plays an important role in cell-fate bias in 4-cell embryos.

Importantly, both studies link their findings to heterogeneous histone H3 arginine 26 methylation (H3R26me2) mediated by CARM1, previously implicated in cell-fate determination of 4-cell blastomeres (Torres-Padilla et al., 2007). By using a CARM1 inhibitor that reduces H3R26me levels, Goolam et al. notice a complete loss of *Sox21* expression at 4-cell stage; *Carm1* overexpression, on the other hand, upregulates *Sox21* mRNA at 8-cell stage. Likewise, White et al. find that blastomeres having larger long-lived SOX2-DNA-bound fraction display higher H3R26me2 and CARM1 levels. *Carm1* downregulation via RNAi leads to the reduction of long-lived bound fraction of SOX2 as well as the expression levels of SOX2 target genes (*Sox21* was

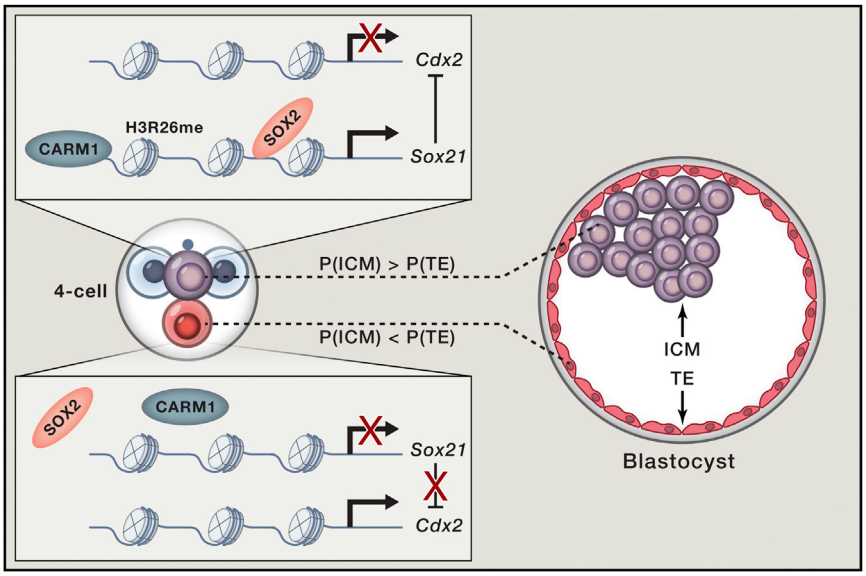


**Figure 1. Molecular Heterogeneity Underlies Biased Lineage Segregation in 4-Cell Mouse Embryo**

Heterogeneous gene expression and transcription factor-DNA binding dynamics pre-pattern the 4-cell mouse embryo for biased fate contribution to either inner cell mass (ICM) or trophectoderm (TE) in the blastocyst. *Sox21* is among the most highly heterogeneous genes at the 4-cell stage. *Sox21* expression is likely controlled by dynamic SOX2-DNA binding, which is also different among 4-cell blastomeres. *Sox21* suppresses *Cdx2* expression and TE fate and predisposes blastomeres toward ICM. P(ICM), probability of ICM fate; P(TE), probability of TE fate.

not examined in this context). When these data are strung together, a model emerges suggesting a CARM1-SOX2-SOX21 axis in pre-patterning 4-cell blastomeres that accounts for their biased fate choices in subsequent cell divisions (Figure 1). Intriguingly, while Goolam et al. demonstrate intercellular differences of *Sox21* mRNA levels among all blastomeres within the 4-cell embryos examined, with regard to long-lived SOX2-DNA bound fraction, White et al. show half of the 4-cell blastomeres with comparable higher levels. If a link between SOX2-DNA binding dynamics and *Sox21* expression can be established, a couple of puzzling questions arise. Does heterogeneous expression of *Sox21* also exist in blastomeres with larger long-lived SOX2-DNA-bound fraction? And if so, would this provide further bias toward subsequent fate decisions?

Recently, another epigenetic modifier, *Prdm14*, has also been shown to be heterogeneously expressed in 4-cell stage embryos (Burton et al., 2013). PRDM14 interacts with CARM1 and regulates H3R26me2 levels in vivo, thereby promoting a fate bias toward ICM. In this context, it will be interesting to examine the relationship between PRDM14 and SOX21/SOX2-DNA binding. A remaining question is how does CARM1 become heterogeneous at the 4-cell stage in the first place? Some evidence from the previous work links this observation with the cell division pattern of the 2-cell embryo (Torres-Padilla et al., 2007). Indeed, White et al. find that long-lived SOX2-DNA-bound fraction also correlates with the orientation of cell cleavages during the 2- to 4-cell transition. Future investigations are needed to further explain this correlation, as well as to look for other yet-to-be identified factors involved in this process.

Both *Sox21* and *Sox2* knockout mouse embryos form normal blastocysts, arguing for their non-essential roles during the first lineage segregation (Kiso et al., 2009; Wicklow et al., 2014). It is important to note that partial and temporal reduction in gene function mediated by RNAi, in many cases, can unveil important biological processes otherwise masked by a complete and permanent gene ablation. A case in point is the observation that, and in contrast to *Sox2* knockout, RNAi-mediated *Sox2* knockdown affects TE development and leads to embryo arrest at the morula stage (Keramari et al., 2010). This paradox could potentially be explained by the highly regulative capacity of early mammalian embryos where redundant mechanism(s) would be in place for separating TE and ICM lineages. It will be of interest to extend the analyses here presented by both Goolam et al. and White et al. to embryos deficient in *Sox2* or *Sox21* to identify other possible pathways involved in this process.

While both studies place more emphasis on commitment to ICM driven by the putative CARM1-SOX2-SOX21 axis, whether a similar mechanism, either in parallel or interdependent, exists for the TE fate specification remains unexplored. To speculate further, it is possible that a precarious balance, maintained by antagonizing epigenetic forces exists in the 2-cell embryo. Upon second cleavage, this balance is tipped by intrinsic and/or extrinsic influences, which ultimately leads to heterogeneous transcription factor-DNA binding dynamics and gene expressions among 4-cell blastomeres. This notwithstanding, an important concept to be extracted from both studies is that heterogeneous epigenetic regulations driving differential transcription factor activities likely precede observable lineage commitment during mammalian development. The findings presented here can potentially be extended to lineage choices in later developmental stages and/or cell-fate alterations that may occur during disease initiation and progression.

These discoveries provide strong molecular evidence for supporting the biased heterogeneity model, although they probably cannot rule out the involvement of random processes in making embryonic/extraembryonic lineage choices. Intrinsic biases in early embryos do not diminish the importance of extrinsic constraints that help to shape the embryo axis and polarity (Kurotaki et al., 2007). Pre-determined or "fixed" cell fate in early development, a concept initially uncovered and extrapolated from non-mammalian species, may not be flexible enough to accommodate for the complexity of early mammalian embryogenesis. Indeed, cell-fate nudging by heterogeneous forces and stochastic patterning should not be necessarily mutually exclusive

but, instead, may co-exist and may be unavoidable to confer the robustness and, at the same time, plasticity observed in the very early stages of mammalian development.

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# CASTORing New Light on Amino Acid Sensing


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**The activation state of mTORC1, a master regulator of cell growth, is particularly sensitive to changes in the intracellular levels of the amino acid arginine, but the sensing mechanisms are poorly understood. In this issue of *Cell*, Chantranupong et al. identify CASTOR1 as a direct arginine sensor that acts through the GATOR2 complex to regulate mTORC1.**


The mechanistic target of rapamycin complex 1 (mTORC1) is a highly conserved effector of cellular growth signals with the capacity to integrate diverse cues from intracellular nutrients and exogenous growth factors and hormones. Pro-growth signals stimulate the protein kinase activity of mTORC1, and it regulates downstream processes to promote an acute switch from catabolic (e.g., autophagy) to anabolic (e.g., protein, lipid, and nucleotide synthesis) metabolism (Dibble and Manning, 2013). Given the high cost of this metabolic shift, in the form of cellular energy, reducing equivalents and nutrients required, it is not surprising that mTORC1 is under tight regulatory control by a complex network of growth factor signaling and nutrient sensing pathways. This upstream network is dysregulated in a diverse array of human diseases, including cancer, metabolic diseases, neurological disorders, and autoimmune diseases. As such, there has been intense interest in defining the key mechanisms by which mTORC1 senses changes in cellular growth conditions. In this issue of *Cell*, Chantranupong et al. (2016) report the identification and characterization of a new direct sensor of arginine required for the responsiveness of mTORC1 to this conditionally essential amino acid.

A breakthrough in understanding how mTORC1 integrates diverse growth signals came from the recognition that its activation occurs through the coordinated action of two sets of small GTPases, the Rag and Rheb proteins, on the cytosolic face of the lysosome. The presence of sufficient intracellular amino acids promotes mTORC1 translocation to the lysosomal surface through its interaction with a heterodimeric complex of two members of the Rag family of GTPases (RagA or B with RagC or D), which are bound to the lysosome through interactions with a protein complex dubbed the Ragulator (Bar-Peled and Sabatini, 2014). Amino acid depletion and refeeding influence the guanine nucleotide-binding status of the Rags with amino acids stimulating RagA/B to be in the GTP-bound state in complex with RagC/D in the GDP-bound state. Only these RagA/B$^{GTP}$-RagC/D$^{GDP}$ heterodimers are capable of binding to and recruiting mTORC1 to the lysosome. The guanine nucleotide-binding state of RagC/D is regulated by the GTPase activating protein (GAP) activity of the Folliculin-FNIP1 complex. A protein complex called GATOR1 plays an essential role in shutting off mTORC1 in response to amino acid depletion by acting as a GAP for RagA/B, promoting its conversion to the GDP-bound state, yielding Rag heterodimers that cannot bind to mTORC1 (Bar-Peled and Sabatini, 2014). Yet another protein complex, called GATOR2, is required for lysosomal recruitment of mTORC1 by amino acids. The molecular function of GATOR2 is unknown but genetic epistasis analyses suggest that it could be an upstream inhibitor of GATOR1. The collective action of the Rag proteins and these regulatory