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Investigations at the ‘Four-Front’ of Mammalian Development

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Abstract

Understanding how and when cells become different during embryogenesis is a goal that is at the forefront of investigations in mammalian development. Two recent studies from the laboratories of Nicholas Plachta and Magdalena Zernicka-Goetz present evidence that cellular heterogeneities detected in four-cell mouse embryos bias the process of cell fate acquisition thereafter.

Deciphering the genetic mechanisms that drive cell specialization during mammalian development is a goal that is at the forefront of research in reproductive and stem cell biology. To this end, gene knockout studies in mice have been invaluable for revealing when, where, and how genes regulate cell fates during embryogenesis. Knockout studies have shown that potent cell fate-specifying genes are required after the eight-cell stage, consistent with classical studies showing that cell fates are first specified after this stage [1].

While knockout studies can reveal the absolute requirement of genes, they do not reveal when a cell first begins to exhibit differences that could influence its path to maturation. The availability of single-cell RNA sequencing has enabled researchers to describe cell-to-cell gene expression differences originating earlier than the 16-cell stage [2–4]. This kind of study is joined by two more recent studies, from the laboratories of Nicholas Plachta and Magdalena Zernicka-Goetz, which describe molecular differences among cells of the embryo as early as the four-cell stage [5,6]. Together, these reports raise exciting questions for future investigations into early mammalian development.

One of the first goals of the mammalian embryo is to specify pluripotent epiblast cells, which are the progenitors of embryonic stem cells and the fetus. Therefore, an understanding of the mechanisms that drive cell fate specification in the early embryo could provide key insight into the origins of pluripotency.

Progenitors of the pluripotent epiblast are first morphologically evident at the 16-cell stage, when one or two cells come to occupy the inside of the ball of cleaving cells (Figure 1). At the 16-cell stage, pluripotency proteins, such as OCT4 and NANOG, are expressed in all

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cells, although SOX2 is specifically restricted to inside cells at this stage [7,8], indicating that it is one of the earliest markers of pluripotent progenitors.

Curiously, however, *Sox2* is not required for the formation of the inside cells at the 16-cell stage [7]. The two new reports present a model positing that SOX2 activity is regulated before the 16-cell stage, and describe genes acting upstream and downstream of SOX2 to regulate pluripotent cell fate. Using complementary approaches to investigate whether molecular differences are apparent among the cells of the four-cell stage mouse embryo, both groups arrive at the conclusion that the origins of pluripotent cells can be traced back to the four-cell stage, when heterogeneity in SOX2 activity is first apparent.

Using live imaging to track the behavior of fluorescently tagged transcription factors injected into early mouse embryos, White and colleagues report that SOX2-GFP expression exhibits cell-to-cell variation in nuclear localization dynamics at the four-cell stage. The authors interpret this observation to mean that the cells of the four-cell embryo exhibit differences in SOX2-DNA binding and, therefore, that SOX2-GFP acts as a sensor of chromatin differences among the cells of the four-cell embryo. By lineage tracing SOX2-GFP-overexpressing cells, the authors report a correlation between cells in which SOX2 dwells longer in the nucleus at the four-cell stage and acquisition of pluripotent cell fate at later stages. Although the nuclear dwell time of endogenous SOX2 was not measured at the four-cell stage, the report points to the existence of previously uncharacterized heterogeneity at the four-cell stage.

White and colleagues then focused on identifying genes acting upstream of SOX2 to regulate its nuclear dwell time in the four-cell embryo. The group examined the role of CARM1, a histone arginine methyltransferase, because it was previously reported to regulate cell fates as early as the four-cell stage [9].

Using siRNA to knock down CARM1 expression, White and colleagues showed that CARM1 levels correlate with SOX2-GFP nuclear dwell time and, ultimately, the acquisition of pluripotent cell fate.

The study by Goolam and colleagues investigated candidates acting downstream of SOX2 to predispose cells to adopt a pluripotent cell fate. Goolam and colleagues focused on the gene *Sox21*, which they proposed to be a target of SOX2 at the four-cell stage. Although this hypothesis has not been tested in mouse embryos, Goolam and colleagues note that *Sox21* is a target of SOX2 in other contexts. The authors reported that the levels of *Sox21* differ dramatically among the cells of the early embryo, and correlate with *Carm1* expression levels. Accordingly, mosaic siRNA knockdown of *Sox21* at the four-cell stage lowers the odds that knockdown cells will become pluripotent, consistent with a role for SOX21 in promoting pluripotent cell fate downstream of CARM1 and SOX2. This result is surprising, given that *Sox21*-knockout mice are reportedly born normally, exhibiting only an alopecia phenotype [10]. Therefore, *Sox21* is not required for pluripotency *in vivo*, but SOX21 could influence the likelihood of a cell adopting a pluripotent fate because cells are starting to choose their fates in the four-cell embryo.

Ultimately, these two reports propose that CARM1 is a key regulator of cell fate, and raise several interesting questions. First, why do *Carm1*-null embryos survive early embryonic development? Does oocyte-expressed *Carm1* function during the first few cleavages? Second, what causes the heterogeneous expression of *Carm1*, and is this mechanism and the *Carm1* expression pattern stereotyped among embryos? Finally, a question that applies to a broad range of biomedical investigations: does heterogeneity matter? Since pluripotent progenitors can form in the complete absence of many of the genes described above, this argues that heterogeneous gene expression is not important. While the heterogeneity may not be necessary for development, it may exist to create biases that influence the developmental trajectories during development. Either way, the field will be looking ‘fourward’ to future studies to address these exciting questions.

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Figure 1. Heterogeneities at the Four-Cell Stage Bias Cell Fate Allocation in the Early Mouse Embryo

Evidence presented in two recent studies leads to a model in which the CARM1 histone arginine methyltransferase increases access of SOX2 to its targets, such as *Sox21*, which then biases cells to adopt a pluripotent cell fate.