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Different Species Choose Their Own Paths to Pluripotency

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Pluripotency is well defined functionally but ambiguously defined at the molecular level. In this issue of *Developmental Cell*, Boroviak and colleagues (2015) use a multi-species approach to differentiate between fundamental features of pluripotency in mammals and those that exhibit evolutionary plasticity.

Since pluripotent mouse embryonic stems (ESCs) were first derived in the early 1980s (Evans and Kaufman, 1981; Martin, 1981), attempts to derive similar cells from other species have been far less fruitful. Although human ESCs were also derived almost two decades later (Thomson et al., 1998), they differ markedly from mouse ESCs and for obvious ethical reasons cannot be exploited and characterized to anywhere near the same extent as mouse ESCs. Applying similar methods has yielded ESC-like cells from many other mammalian species, but they too have fallen short of the manipulability and usefulness of mouse ESCs. The mouse is thus somehow specially predisposed to life in the laboratory but conversely is a rather atypical mammal in many aspects of its biology.

In fact, mammals exhibit an amazing degree of variation in their reproductive strategies, with corresponding heterochrony in key early developmental events, differences in the timing of implantation, and diversity in modes of placentation (reviewed by Wimsatt, 1975). A major challenge is therefore to identify those features of early development that are shared, and thus fundamental to all mammals, versus those that are taxon-specific. Chief among these is the regulation of pluripotency, the property of certain early embryonic cells that enables them ultimately to differentiate into any cell type of the adult body. Now, to better understand how pluripotency mechanisms in the mouse compare to those of other mammalian species, Boroviak et al. (2015) report a large-scale gene-expression analysis comparing different embryonic stages in mouse and in marmoset.

Although pluripotent stem cells can self-renew in vitro, pluripotent cells in vivo tend to be in a constant state of flux. Capturing pluripotency in culture thus depends on identifying developmental stage(s) at which self-renewal happens to be possible. Two distinct pluripotent states have been defined based on the properties of isolated mouse stem cells. “Naive pluripotency” is characteristic of conventional mouse ESCs and of the E4.5 preimplantation epiblast shortly after it has segregated from the hypoblast (the epiblast and hypoblast are both derived from the inner cell mass, but the hypoblast contributes to extraembryonic tissues, whereas the epiblast forms the embryo proper). “Primed pluripotency,” on the other hand, is characteristic of later, post-implantation epiblast-derived stem cells and differs from naive pluripotency in the signaling pathways on which it depends and the lack of germline competency (reviewed by Kalkan and Smith, 2014). The definition of pluripotency has traditionally depended on functional tests of cultured cells, but an alternative is to describe the molecular properties of pluripotent cells in the embryo—in vivo—in as much detail as possible.

Now, Boroviak et al. (2015) do just that. By applying single-cell RNA-seq methods to pooled groups of up to 20 embryonic cells, they explore the progression of pluripotency in the early mouse conceptus and compare it with ESCs. They use a *Pdgfra::GFP* knockin reporter mouse line to separate epiblast from hypoblast and target four main developmental stages of pluripotency: the 8-cell morula; whole inner cell mass (ICM) of the early E3.5 blastocyst; isolated epiblast of the late E4.5 blastocyst, just after segregation from the hypoblast; and the epiblast of the implanted E5.5 egg cylinder. The ESC transcriptome was most similar to that of the E4.5 epiblast, supporting previous suggestions that these two cell types share an essentially equivalent naive pluripotency. A number of genes were downregulated during the transition from pre- to post-implantation epiblast, including *Esrrb*, *Nr0b1*, *Klf2*, *Klf4*, *Klf5*, *Lifr*, *Il6st*, *Spp1*, *Tcl1*, *Zfp57*, and *Zfp42*. Other genes were upregulated, including *Foxd3*, *Lef1*, *Ccnd1*, *Zscan10*, *Phc1*, and *Nr216*, and thus represent candidate specific markers of primed pluripotency.

As an added bonus, Boroviak and colleagues also compared the above samples with the epiblast of the diapausing blastocyst. Embryonic diapause is a dormant phase of development used as a reproductive strategy by at least 100 mammalian species (including many rodents, carnivores, and marsupials) to delay breeding until environmental or physiological conditions are optimal (reviewed by Wimsatt, 1975). In the mouse, it is induced by lactation or artificially by ovariectomy. A recent study suggested that the capacity for diapause is an evolutionarily conserved property of the blastocyst, at least among eutherian mammals, and thus only the maternal regulatory mechanism has evolved independently in different taxa (Ptak et al., 2012). Counter to this notion, diapause in the mouse was proposed to explain the amenability of blastocysts from this species to ESC derivation (Boroviak and Nichols, 2014), suggesting that the mouse blastocyst has properties not conserved in those of most other species. Thus, a better understanding of the mechanisms of diapause is likely to provide valuable insights into how pluripotent cells choose between self-renewal, differentiation, proliferation, and quiescence.

Previous studies compared transcriptomes or proteomes of diapausing versus

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non-diapausing blastocysts (reviewed by Hondo and Stewart, 2005) but used whole blastocysts rather than dissected epiblasts. The data produced by those studies were thus confounded by other tissues and developmental events, such as differentiation of the extraembryonic endoderm and preparation of the trophoblast for implantation. As would be expected, Boroviak and colleagues identified a large number of genes with roles in metabolism that were differentially expressed between diapausing and non-diapausing blastocysts. However, when they focused only on genes that were dynamically expressed during normal (non-diapause) development, two signaling pathways stood out. Profiles suggested activation of PPAR signaling and repression of mTOR signaling specifically in dormant epiblasts. In addition, upregulation of *Wnt4* suggested a specific role for WNT signaling in maintaining pluripotency in the diapausing epiblast, consistent with known roles for the pathway in ESC self-renewal (reviewed by Merrill, 2012).

Human ESCs are more similar to mouse late epiblast-derived pluripotent stem cells than to conventional mouse ESCs, in both culture requirements and expression profiles. To determine the extent to which mouse studies are applicable to human, it is necessary to characterize a species that can be used as a proxy for human. To this end, Boroviak and colleagues also performed transcriptome profiling of marmoset early, mid, and late blastocysts and compared these data to the mouse transcriptome data. Although only whole marmoset blastocysts could be used in the marmoset studies, rather than dissected epiblasts, several key features were still apparent. Marmoset blastocysts differed from mouse blastocysts by an absence of expression of some naive pluripotency-associated genes, such as *Klf2*, *Nrob1*, *Fbxo15*, *Gbx2*, and *Bmp4*, but importantly were similar to previously published expression patterns from human blastocysts (e.g., epiblast-specific expression of *KLF17*, *LEFTY1*, and *NODAL*), showing that the marmoset is likely to be an appropriate model for early human development. Boroviak et al.'s mouse-marmoset comparative analysis also revealed differences in FGF, WNT, and TGFβ/NODAL signaling components. By performing treatments of cultured blastocysts with small-molecule pathway inhibitors, the authors demonstrated that whereas FGF/ERK signaling is the principle driver of epiblast-hypoblast segregation in the mouse, WNT signaling also contributes to this process in the marmoset, with a somewhat reduced role for FGF/ERK signaling. This may help to explain data from previous studies on an apparently minimal role for FGF/ERK signaling in human hypoblast differentiation, as well as a greater role for WNT signaling in differentiation of human ESCs compared with mouse ESCs. It is possible that species differences in the signaling pathways involved in hypoblast specification are closely linked with differences in the mechanisms regulating pluripotency.

The study by Boroviak and colleagues highlights the importance of a multi-species approach for understanding early development of mammals. Signaling pathways identified by their study, including PPAR, mTOR, and WNT4, should be functionally tested for potential roles in diapause and ESC maintenance. With the aid of recently developed tools such as CRISPR, functional studies of naive and primed pluripotency-specific markers in marmoset or other non-murine models could provide much-needed insights into the most conserved and thus fundamental mechanisms of early development and its evolution in mammals.

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