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Snapshots of Pluripotency

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SUMMARY

Pluripotency is a unique developmental state that lays the foundation upon which the entire embryo is built. Pluripotent cells possess the unique capacity to generate, in an exquisitely defined sequence, all the distinct cell types comprising the fetal and adult organism. The discovery of pluripotent stem cells and now the ability to generate them from differentiated cells has had a profound impact on a vast array of scientific disciplines. In addition to their clinical potential as a source of therapeutic cell types, pluripotent stem cells provide scalable access to otherwise experimentally inaccessible development- and disease-associated biology. Here I provide my perspective on the continuum of pluripotency in the early mammalian embryo. I also discuss how novel genomic technologies are now enabling the capture of molecular "snapshots" of the several distinct pluripotent states that stem cells undergo during this pivotal developmental period.

The Continuum of Pluripotency in the Mouse Embryo

The development of placental mammals is unique in that embryos are nourished by interfacing with the mothers' reproductive tract, in contrast to embryos of other classes of vertebrates that develop outside the womb. Thus, mammalian embryos must generate extra-embryonic cell types to mediate their implantation into the uterus, while at the same time maintain a distinct population of unspecified pluripotent cells to form the embryo proper. To do so, within 5 days of fertilization, the mouse zygote partitions itself into three separate cell populations, the trophoectoderm, primitive endoderm, and epiplast, which carry out these diverse tasks in concert. The developmental potential of each of these cell populations was defined by seminal experiments in which chimeric embryos were generated by cell transplantation into host blastocysts (Gardner, 1968; Gardner and Rossant, 1979; Rossant et al., 1978). These studies, among others, showed conclusively that trophectoderm cells form the bulk of the fetal portion of the placenta, primitive endoderm cells generate the parietal and visceral yolk sac endoderm, and epiblast cells generate the entire embryo proper as well as additional extra-embryonic tissues such as the amnion and allantois.

Several pioneering studies have shown that epiblast cells in the pre- and post-implantation epiblast function to maintain pluripotency until the onset of gastrulation. During implantation, the trophectoderm invades the maternal uterine tissue to provide sustained access to nutrient and waste exchange for the remainder of gestation. At this point, the post-implantation epiblast changes from a small cluster of cells into a pseudostratified epithelium that must remain unspecified while it prepares to differentiate into all of the early somatic and germ cell fates that appear during gastrulation. Evidence that that the post-implantation epiblast is capable of generating cell fates from each of the three primary germ layers was provided by experiments in which it was transplanted to ectopic sites in adult mice (Diwan and Stevens, 1976). These data were later supported by fate-mapping studies revealing that individual cells of the post-implantation epiblast were not lineage restricted and could contribute to all three germ layers, even when transplanted from one spatial region of the post-implantation epiblast to another (Lawson et al., 1991; Tam and Zhou, 1996).

Historically, pluripotency was considered a single state, yet it was clear quite early on that epiblast cells before and after implantation were morphologically and functionally dissimilar. In contrast to cells of the pre-implantation epiblast, cells of the post-implantation epiblast did not readily incorporate back into host blastocysts or contribute to the developing embryo in standard chimera assays (Gardner et al., 1985). In retrospect, this observation demonstrated a clear developmental distinction between pre- and post-implantation epiblast cells and provided the first indication that more than one shade of pluripotency might exist.

The Two Dominant Pluripotent Attractor States

It only became possible to study pluripotency and its properties when, in 1981, two groups concurrently reported that they had derived pluripotent cells from mouse pre-implantation blastocyst stage embryos, and that these cells could be expanded indefinitely in culture in an undifferentiated state (Evans and Kaufman, 1981; Martin, 1981). Remarkably, these mouse embryonic stem cells (mESCs), later shown to originate from the pre-implantation epiblast (Brook and Gardner, 1997), could be induced to differentiate into a plethora of functional cell types spanning all three germ layers. Later, stringent in vivo assays confirmed the pluripotency of mESCs by showing that when injected into host blastocysts, they integrated into the developing embryo and contributed to all cell types of the resulting chimeric mice, including the germline (Bradley et al., 1984; Nagy et al., 1993). Since then, pluripotent stem cell lines have been derived from earlier

Paul Tesar received his undergraduate degree from Case Western Reserve University (CWRU) in Cleveland, Ohio, in 2003 and went on to earn his D.Phil. from the University of Oxford as a recipient of a prestigious scholarship from the NIH. During his graduate and postdoctoral studies, under the tutelage of Professor Sir Richard Gardner and Dr. Ron McKay, Paul investigated how to harness stem cells as a tool to understand early mammalian development. Paul's early studies culminated in a landmark paper in *Nature* in which he described the isolation of a new type of pluripotent stem cell termed epiblast stem cells. Paul returned home to join the CWRU School of Medicine faculty in 2010 and his laboratory has continued to develop cutting-edge stem cell technologies for better understanding and treatment of nervous system disorders including multiple sclerosis, pediatric leukodystrophies, and brain cancer. Paul is currently an Associate Professor and the Dr. Donald and Ruth Weber Goodman Professor of Innovative Therapeutics at CWRU and a Robertson Investigator of the New York Stem Cell Foundation. In 2015, Paul was honored with the International Society for Stem Cell Research Outstanding Young Investigator Award at the annual meeting in Stockholm, Sweden.

blastomere and morula stage mouse embryos, as well as primordial germ cells (Matsui et al., 1992; Resnick et al., 1992; Tesar, 2005). Later, in a series of groundbreaking studies, it was shown that even adult somatic cells can be "reprogrammed" to a pluripotent state by forcing expression of what are now known as core pluripotency transcription factors, yielding induced pluripotent stem cells (iPSCs) (Takahashi and Yamanaka, 2006). Pluripotent cells derived from each of these methods are subtly different in terms of their epigenome, yet remarkably, they show almost identical functional properties. Collectively, these studies support the notion that a single pluripotent state, referred to as naive pluripotency, can be captured in vitro from mouse embryos or reprogrammed from mouse somatic and germ cells.

In the meantime, pivotal advances were being made in defining pluripotency in human cells. In 1998, human ESCs (hESCs) were isolated for the first time from human blastocyst stage embryos and, like their mouse counterparts, could be renewed indefinitely in culture while maintaining their pluripotent state (Thomson et al., 1998). This achievement marked the beginning of the path for advancing pluripotent stem cells into the clinic for regenerative medicine. In a remarkably short time, this goal has been realized, with a number of clinical trials using hESCs to regenerate damaged or diseased tissues and organs now underway.

Yet despite being derived from identical blastocyst stages, hESCs were perplexingly distinct from their murine counterparts in their morphology, molecular profiles, and their need for different signaling molecules to maintain them in an undifferentiated state (Daheron et al., 2004; James et al., 2005; Smith et al., 1988; Vallier et al., 2005; Ying et al., 2003, 2008). Initially, these differences were largely overlooked and attributed to minor species-specific variation, as opposed to reflecting true significance in the inherent developmental origin and capacity of each cell type.

Then, in 2007, two studies reporting a new type of mouse stem cell type transformed the understanding of pluripotency (Brons et al., 2007; Tesar et al., 2007). The cells, termed epiblast stem cells (EpiSCs), were initially isolated from early post-implantation mouse and rat embryos just prior to gastrulation. In contrast, mESCs characterized in earlier studies were derived from pre-implantation embryos. However, although mouse EpiSCs showed striking similarity to native post-implantation epiblast cells, they did not readily incorporate into the developmentally earlier blastocyst in mouse chimeric embryos. Yet EpiSCs were clearly pluripotent, as demonstrated by in vitro differentiation, teratoma generation, and transplantation into the peri-gastrulation epiblast of in vitro cultured whole mouse embryos (Brons et al., 2007; Huang et al., 2012; Tesar et al., 2007).

These data led to a major shift in how pluripotency is defined. What had previously been defined as the pluripotent state based on the earlier mouse ESC studies represented only a common attractor state inherent to pluripotent cells derived from the mouse pre-implantation epiblast, referred to as naive pluripotency (Nichols and Smith, 2009). EpiSCs, on the other hand, represent a distinct pluripotent state, referred to as primed pluripotency, based on morphological, molecular, and functional criteria. Most strikingly, EpiSCs derived from mice shared defining properties with hESCs. This observation led to the current understanding that standard hESC lines