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Grounded: Transcriptional Pausing in Naive mESCs

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Growing mouse pluripotent stem cells in defined media with signaling inhibitors establishes a naive ground state different from that of cells grown in serum. Recently in Cell, Marks et al. (2012) reported differences in the transcriptional and epigenomic landscapes of naive and serum-exposed mouse stem cells that improve our understanding of the pluripotent ground state.

Embryonic stem cells (ESCs) provide a powerful model system for studying early development because of their capacity to differentiate into any cell type or tissue in the adult organism, a property known as pluripotency. Murine ESCs (mESCs) were originally explanted and cultured in the presence of “feeder” embryonic fibroblasts, fetal calf serum, and other growth supplements to produce cells that self-renew indefinitely in culture and retain pluripotency (Evans and Kaufman, 1981; Martin, 1981). Heterogeneity in cellular morphology and in expression of key pluripotency factors suggested that mESCs grown in serum may be in a metastable and more developmentally advanced state that is not representative of the ground state of cells in the inner cell mass of the preimplantation embryo. Erk pathway stimulation by serum exposure is thought to predispose these cells to lineage commitment and prevent them from acquiring a naive pluripotent state (reviewed in Nichols and Smith, 2009). Propagation of mESCs in the presence of Mek/Fgf pathway and Gsk3 inhibitors and in the absence of serum factors, a condition known as 2i, effectively blocks differentiation signals and allows capture of the naive pluripotent state. Thus, mESCs grown in the 2i condition may more accurately represent the naive state of pluripotent cells in the inner cell mass of the blastocyst.

In a recent issue of Cell, Marks et al. (2012) compared the transcriptional and chromatin profiles of serum-grown and 2i-grown cells to gain insights into the naive pluripotent state. They found that many lineage-specification genes expressed in serum-grown mESCs are repressed in 2i conditions. Interestingly, they also found that reduced expression of lineage-specification and other genes in 2i cells is associated with enhanced promoter-proximal pausing (Figure 1). Transcription initiation and promoter-proximal pausing by RNA polymerase II (Pol II) has previously been shown to occur at genes that encode lineage-specific regulators in embryonic cells, and is a mechanism that allows these silent genes to be precisely and synchronously induced in response to developmental cues or extrinsic stimuli (reviewed in Levine, 2011).

mESCs grown in serum and 2i conditions have similar levels of transcripts for genes encoding key pluripotency factors (Pou5f1, Nanog, Sox2, Esrrb, Klf2, Klf4, and Tbx3) and exhibit similar proliferation behavior and differentiation potential, but cells propagated in the 2i condition exhibit more uniform expression of key pluripotency factors and cellular morphology. When Marks et al. (2012) examined genes that are differentially expressed in the two conditions, they found that cells grown in the 2i condition have increased levels of transcripts for genes encoding metabolic functions and decreased levels of transcripts for many ectodermal and mesodermal specification genes. The precocious transcription of such genes in ESCs, which has been called lineage priming, might poise stem cells for differentiation. However, as the authors note, the repression of lineage-specification genes in 2i conditions suggests that multilineage priming is not an essential feature of the pluripotent state.

Trithorax Group and Polycomb Group protein complexes, which catalyze histone H3K4me3 and H3K27me3 modifications, play prominent roles in development. Nucleosomes with H3K4me3 occur at sites of Pol II initiation and those with H3K27me3 occur at repressed lineage-specification genes, among others. Marks et al. (2012) mapped the genome-wide locations of H3K4me3- and H3K27me3-modified nucleosomes in ESCs grown in serum and 2i conditions, and found that H3K4me3 profiles were highly similar, but that the levels of H3K27me3 were reduced somewhat in the 2i condition. The decrease in H3K27me3 occupancy in the 2i condition was associated with limited transcriptional changes, but the threshold for functional occupancy is unknown, so the biological repercussions of the different levels of H3K27me3 await further study.

In embryonic cells from a broad range of species, many genes experience transcription initiation and promoter-proximal pausing by Pol II (Guenther et al., 2007; Zeitlinger et al., 2007; Levine, 2011). Embryonic cells employ promoter-proximal pausing as a mechanism to regulate genes for lineage-specific regulators that are silent in the early embryo, but must be activated in a precise, reliable, and coordinated fashion in response to developmental cues. Pol II molecules recruited to promoters by transcriptional activators can initiate synthesis of a short RNA species, but are subjected to promoter-proximal pausing until phosphorylated by the elongation factor P-TEFb, which is recruited by various factors including Mediator and c-Myc (Rahl et al., 2010; Takahashi et al., 2011). Marks et al. (2012) found that promoter-proximal pausing by RNA Pol II is more prevalent in ESCs grown in the 2i condition than in serum, and that paused Pol II was more evident at many lineage-specification genes in the 2i condition. Their results suggest that increased promoter-proximal pausing, which involves lineage-specification genes, among others, may be especially important for establishing...
and maintaining the ground state of naive pluripotent ESCs. 

Marks et al. (2012) found that the transcriptional and chromatin states of mESCs were interconvertable when cells were switched from one growth condition to the other. This observation indicates that whatever molecular memory mESCs have of one state is rapidly lost when exposed to the other condition.

Cultured human ESCs have features that indicate that they are more developmentally advanced than mESCs, and there is considerable interest in isolating human pluripotent stem cells in a more naive state (reviewed in De Los Angeles et al., 2012). This is challenging because the simple application of 2i conditions to cells isolated from preimplantation human embryos or from reprogramming has thus far failed to produce naive human ESCs/iPSCs. It seems likely that naive human ESCs will eventually be isolated, and it will then be interesting to determine if enhanced Pol II pausing and other features of the transcriptional and chromatin landscape described by Marks et al. (2012) are also found in these human cells.

REFERENCES


Autoimmune T Cells Lured to a FASL Web of Death by MSCs

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Achieving immune tolerance through cell transplantation is a promising approach for treating autoimmune disease. In this issue of Cell Stem Cell, Akiyama et al. (2012) demonstrate that human and mouse mesenchymal stem cells can induce immune suppression by attracting and killing autoreactive T cells, which stimulates TGFβ production by macrophages and generates regulatory T cells.

Mesenchymal stem cells/marrow stromal cells (MSCs) are perhaps the cell type most commonly investigated for tissue repair therapies. However, MSCs appear capable of doing much more than simply facilitating tissue repair. These interesting cells, derived most often from the bone marrow or fat, can serve as “paramedics” to help heal tissue through local and

Figure 1. Transcriptional Differences between 2i and Serum-Grown mESCs
Schematic diagram of mESC growth conditions (top), transcriptome (middle), and RNA Polymerase II pausing differences (bottom, Pol II) in mESCs grown in 2i (left) or serum (right). Reads indicate ChIP-Seq reads. Models for lineage-specification genes are shown below the graphs in black, and start sites are indicated by an arrow. The transcriptional state of mESCs grown in either condition is readily interconverted by changing the growth media.