Tex10: A New Player in the Core Pluripotency Circuitry

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Revealing how the core pluripotency circuitry is orchestrated to maintain the ground state of embryonic stem cells (ESCs) is fundamental for understanding self-renewal and early lineage specifications. In this issue of *Cell Stem Cell*, Ding et al. (2015) identify a new Sox2-interacting protein, Tex10, which, together with Tet1 and p300, regulate super-enhancers to sustain pluripotency.

The core pluripotency circuitry of embryonic stem cells (ESCs) encompasses a small number of very potent key master transcription regulators that bind proximal and distal regulatory elements of transcription regulators, signal transduction components, and chromatin-modifying enzymes that facilitate ESC self-renewal (Young, 2011). Alongside promoters and typical enhancers, key master transcription regulators occupy, together with the Mediator complex, densely packed enhancer domains, which are termed super-enhancers (SEs), at many genes that regulate the ESC ground state (Whyte et al., 2013). SEs are characterized by large size, high transcription factor density, sensitivity to perturbation, and a robust ability to activate transcription (Whyte et al., 2013).

The trio Oct4, Sox2, and Nanog (OSN) were among the first to be identified as key components of the core pluripotency circuitry. Chromatin-immunoprecipitation (ChIP) experiments showed that OSN co-occupy a considerable portion of their target genes, including their own regulatory elements, suggesting that OSN collaborate to form regulatory circuitry consisting of autoregulatory and feedforward loops (Young, 2011). In recent years other key master transcription regulators, such as Tcf3 (Cole et al., 2008), Sall4 (Lim et al., 2008), Tbx3 (Niwa et al., 2009), Klf4 (Niwa et al., 2009), and Esrrb (Martello et al., 2012) have been suggested to be part of the core pluripotency circuitry. In this issue of *Cell Stem Cell*, Ding et al. (2015) describe the identification of a potential new member of the core pluripotency circuitry, Testis expressed 10 (Tex10), which, together with Sox2, Tet1, and p300, positively regulates ESC SEs via modulating DNA methylation and histone acetylation to promote ESC self-renewal.

The authors started their journey by searching for bona fide Sox2-interacting proteins using a combined approach of IP followed by mass spectrometry (MS) to shed light on the Sox2 interactome in ESCs. They identified 67 high-confidence Sox2 partners, out of which three, Wdr18, Tex10, and Las1L, were of particular interest, due to the fact that they are often co-purified together as a part of a complex and because of their known role in transcriptional regulation, ribosome biogenesis, and cell cycle regulation. In addition, the authors noticed that, like Sox2, all the three factors are enriched in the undifferentiated state of ESCs and are upregulated late in reprogramming during the hierarchical/deterministic stage (Buganim et al., 2013). Intrigued by Tex10 and its interaction with Sox2, Ding and colleagues decided to explore its role and interactions during embryonic development, ESC maintenance, and somatic cell reprogramming. Initially, they knocked down Tex10 in ESCs and noted that the cells start to express multiple lineage-specific markers and downregulate ESC markers. In accordance with that, when they depleted Tex10, they also observed a significantly reduced formation of induced pluripotent stem cells (iPSCs) and a partial blockage in embryo development from the morula to the blastocyst stage. Tex10 depletion generated a strong effect on cell cycle as well, where p53 and its target gene p21 were significantly upregulated. This strong effect on cell cycle regulation thus raises the question of how much of the observed phenotype can be attributed to true loss of pluripotency and how much of it is actually due to cell cycle defects. In support of a direct role in regulation of pluripotency, the authors highlight a close correlation of the transcriptomic changes upon Tex10 depletion with those upon reduction of other pluripotency factors including OSN, Esrrb, and Tbx3. This correlation gave the first hint that Tex10 most likely plays an important role in the acquisition of the core pluripotency circuitry.

The authors went on to search for the mechanism by which Tex10 establishes and maintains pluripotency. ChIP-seq experiments demonstrated that 46% of Tex10 targets are also occupied by Sox2 and that Tex10 binding regions are also enriched for OSN and Med1/12, a component of the Mediator complex, as well as for the active enhancer marks H3K4me1 and H3K27Ac, suggesting that Tex10 may play a role in regulating enhancer activity in ESCs. Accordingly, Tex10 depletion reduced enrichment of H3K27ac modifications in the SE regions of *Oct4*, *Nanog*, and *Esrrb* loci in the presence of overall unchanged basal expression levels of H3K27ac, suggesting that Tex10 may use histone acetylation to regulate the activity of SEs. Indeed, the authors found that p300, an H3K27 acetyltransferase that is enriched at enhancer regions in ESCs, is also overrepresented at Tex10 bound regions, suggesting that Tex10 may regulate SE activity, in part, by recruiting p300 to the enhancer regions to establish H3K27 acetylation (Figure 1A).

Next, the authors tested whether other Tex10 partners may be recruited to ESC enhancers for transcriptional activation

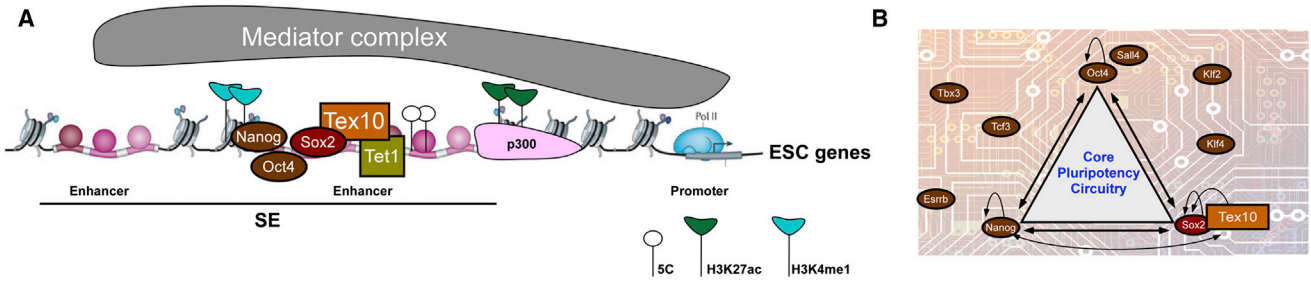

Figure 1. Tex10 in Pluripotency Regulation

(A) A schematic representation of the ground state of ESCs. In this stage the core pluripotent circuitry genes OSN bind to super-enhancers (SEs) of most ESC genes together with the Mediator complex and facilitate their expression. To maintain this state Sox2 interacts with Tex10 and recruits it to ESC-specific SEs. As a result Tex10 recruits the H3K27 acetyltransferase p300 to maintain histone acetylation by establishing H3K27ac marks at these regions. In parallel, Tex10 interacts with Tet1 to facilitate demethylation and transcription.

(B) A scheme depicting the various core pluripotent circuitry members and their potential cross-talks. White lines and nodes are for illustration only and emphasize complexity.

of enhancer-associated genes. Tex10-interacting proteins in ESCs biochemically purified via stable isotope labeling by amino acids in cell culture (SILAC) coupled with the IP-MS technique identified Tet1 as a Tex10-interating protein. The SILAC approach further showed that Tet1 is also abundant in SE regions and that Tex10, Sox2, and Tet1 co-binding targets are more overrepresented at enhancers than promoters and other regions. In agreement with the role of Tet1 in demethylation (Bagci and Fisher, 2013), Tex10 was found to be enriched in unmethylated regions. Remarkably, Sox2 and Tet1 shared peaks were enriched for either Tex10 or 5-hydroxymethylcytosines (5hmC), and Tet1-bound SEs had significantly lower 5-methylcytosine (5mC) and 5hmC when they were also bound by Tex10. These data led the authors to propose an exciting mechanism by which Tex10 facilitates Tet1 activity to demethylate ESC enhancers to retain their high activity (Figure 1A). Supporting this model, the authors found that depletion of Tex10 reduced the binding of Tet1 to the SE regions of *Oct4* and *Esrrb*, with a concomitant increase in 5mC enrichment at the same loci. Understanding how Tex10 expedites Tet1 activity requires further investigation. These results suggest a Tex10-dependent activity of Tet1 in binding to Tex10-occupied SEs and controlling methylation status of SEs. The Ding et al. study proposes Tex10 as an important factor that functionally interconnects with the core pluripotency circuitry of ESCs to promote ESC self-renewal (Figure 1B).

The authors start their story by validating the expression of Tex10 in the testis. Their proposed mechanism for Tex10 activity raises a very intriguing question of whether Tex10 executes the same function in germ cells. It has been shown that a very small fraction of Sox2-positive germ cells is present even in the adult testis (Arnold et al., 2011). Thus, it is tempting to speculate that Tex10 contributes to the self-renewal of adult germ cells as well by positively regulating SEs of genes that support stemness of germ cells. This study adds another brick to the multifaceted wall of the ground state of pluripotency and opens a new research direction for Tex10 as a new stemness factor.

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