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Lost but Still Missed: A New Chapter about TBX3 in Pluripotency and Fate Decision

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Introduction

The network defining pluripotency comprises a fine tuned pattern of factor activity with Tbx3 believed to be indispensable for the induction and maintenance of pluripotency. In this issue of Stem Cell Reports Russell et al. delineate novel facets of Tbx3 action contradicting a series of previous reports.

A gene expression program coupled with a complex signalling circuitry allows pluripotent cells to self-renew, yet remain poised towards differentiation into essentially all cell types in response to differentiation cues. The pluripotent state is largely under the control of a small set of core transcription factors namely Oct4, Sox2, and Nanog [1]. Ancillary regulators of gene expression and transcription factors are known to collaborate with Oct4, Sox2, and Nanog to control the pluripotency gene expression program, either supporting or reinforcing the network. These factors comprise e.g. Smad1, Stat3, Klf4, Tcf3, c-Myc, Zfx, as well as factors mediating BMP, Lif, Wnt signalling, which modulate proliferation and self-renewal, respectively [2]. A great effort of research has been done on the complex signalling circuitry and the fine-tuned pattern of factor activity required for a pluripotent state. Recently, Russell and colleagues uncovered a new role for one of these factors that was thought to be indispensable for pluripotency, namely Tbx3. Tbx3 is the sole member of the T-Box family being expressed in the ICM. It regulates the expression of key factors of pluripotency and during reprogramming [3,4], while knockdown of Tbx3 leads to differentiation [5]. Contrastingly, it also drives the onset of mesendodermal differentiation [6]. In an elegant study, Russell et al. show a dynamical and heterogeneous expression pattern of Tbx3 in mouse embryonic stem cells (ESCs) [7], resembling previous knowledge on heterogeneous expression of Nanog. By using two different reporter systems, they were able to characterize the diverse expression states in the developing embryo *in vivo* but also in ESCs *in vitro*, showing that varying TBX3 expression levels correlate with distinct developmental potential. Although many researchers question the relevance and functional significance of transcription factor heterogeneity, the authors clearly point out a link between Tbx3 expression and developmental potency: Tbx3-high cells resemble the pluripotent inner cell mass, while Tbx3-low cells seem to be poised toward an epiblast fate as shown by rigorous assays like chimeric embryo formation and strict comparison of transcriptional profiles. The observation that the Tbx3-low cells do not lose their pluripotency suggests the possibility of a pluripotent Tbx3-null state (Figure 1).

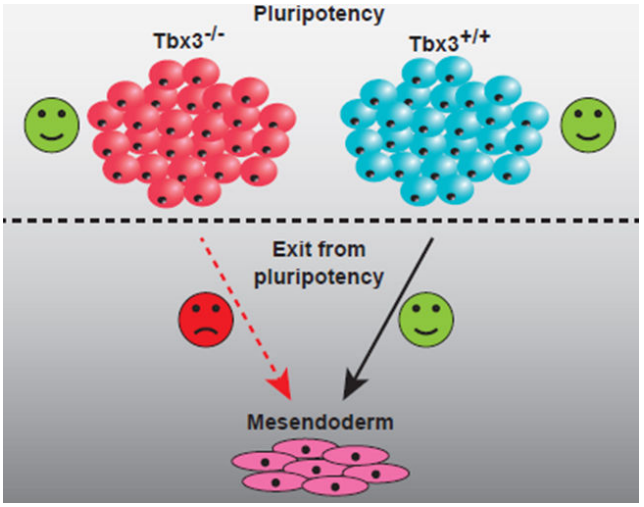

Figure 1: Proposed model of Tbx3 during cell fate determination.

Indeed, contradicting a series of previous reports, Russell and colleagues were able to generate Tbx3-null pluripotent stem cells showing all hallmarks of pluripotency, thereby demonstrating that Tbx3 is dispensable for both the induction and maintenance of pluripotency. The Tbx3-null phenotype even outperformed the wildtype controls in terms of self-renewal and exit from pluripotency. As was previously affirmed for Oct4 haploinsufficiency, a Tbx3-null state leads to a pluripotency arrested state with increased self-renewal and a curbed differentiation capacity. Surprisingly, Oct4 haploinsufficiency stabilizes the pluripotency network on a transcriptional and epigenetic level [8,9]. These findings on Oct4, as well as the reported data on loss of Tbx3, a factor formerly believed to be a key player to maintain pluripotency, might suggest that pluripotency networks are more stable and adaptable than we thought after all, and not doomed to collapse upon diminutive changes of factor activity. Though, the fact that a pluripotent Tbx3-null state is possible suggests an alternate pluripotency network being installed upon loss of Tbx3 with a number of compensating factors, as suggested by the authors' investigation of the underlying mechanism of this process. The question of why the complete loss of Tbx3 leads to a strengthening of the pluripotency network, while Tbx3-low expressing cells are poised towards differentiation, needs to be addressed by epigenetic research, according to the authors, having shown that genes associated with DNA methylation are differentially regulated just in the null but not in the Tbx3 low cells. Although seeing the same

reciprocal connection of Tbx3 and Dppa3 as Waghray et al., Russell and colleagues state that the status of Dppa3 as a compensating factor for the loss of Tbx3 seems to be not exclusive, suggesting that it might be down to more than just one factor, rather a complex network, to nullify Tbx3 ablation. Tbx3 might represent more a safeguard of the pluripotency network, with overexpression enhancing the reprogramming efficiency and stabilizing in case of loss of other factors such as LIF-Stat3 signalling. It may play a more important role in differentiation, as suggested by the loss of Tbx3 hampering the differentiation. Interestingly, overexpression during differentiation promotes mesendodermal lineage [6], while overexpression prior to differentiation prevents mesoderm formation [10]. Also, Waghray et al. could show that Tbx3 is responsible for blocking the premature exit from pluripotency by directly repressing mesoderm and Wnt pathway genes. As a knockdown of Tbx3 leads to an upregulation of mesoderm genes like T, Wnt8a, or Hes7, as well as Wnt pathway genes, Tbx3 seemingly tweaks Wnt signalling, maintaining a balanced state between pluripotency and differentiation towards mesoderm [10]. Much is still in the dark about the role of Tbx3 and its connection to Dppa3 and other possible compensating factors but nevertheless new insights were provided into Tbx3 action. These studies illustrate that every single factor associated with pluripotency should be checked on its own to clearly demonstrate its crucial role in the pluripotency network or, in case of Tbx3, the complete dispensability. The example illustrates the necessity to conduct detailed experiments under varying conditions to investigate the molecular mechanisms controlling pluripotency and lineage commitment. Undoubtedly, Tbx3 still plays many important roles in the developing embryo, but being an indispensable factor for pluripotency isn't one of them.

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