Alternative pre-mRNA splicing switch controls hESC pluripotency and differentiation

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Alternative splicing (AS) of pre-mRNAs is a ubiquitous process in mammals that is tightly regulated in a cell type- and cell state-dependent manner. However, the details of how splicing is regulated to impact specific cell fate decisions remains incompletely understood. A study by Yamazaki and colleagues (pp. 1161–1174) in this issue of *Genes & Development* provides exciting new insight into the role and regulation of splicing in the maintenance of pluripotency of human embryonic stem cells (hESCs). In brief, they show that AS of several genes is robustly regulated upon differentiation of hESCs. One of these genes, T-cell factor 3 (*TCF3*), is regulated at least in part through the activity of heterogeneous nuclear ribonucleoproteins H1 and F (hnRNP H/F) to control the mutually exclusive expression of the encoded E12 and E47 transcription regulators. The investigators demonstrate that reduced expression of hnRNP H/F favors expression of E47, which in turn decreases E-cadherin expression to promote hESC differentiation. In contrast, high levels of hnRNP H/F induce expression of E12 to maintain pluripotency. Thus, this work provides at least one new link between AS and control of human stem cell fate and suggests a broader role of splicing in pluripotency.

Although alternative splicing (AS) is widely regulated across mammalian tissues, direct connections between particular splicing events and downstream physiologic consequences are still lacking in many cases. In this issue of *Genes & Development*, Yamazaki et al. (2018) identify the transcription factor T-cell factor 3 (TCF3) as a key regulator of human embryonic stem cell (hESC) self-renewal and differentiation.

The investigators begin the study by using RNA sequencing (RNA-seq) to identify changes in splicing between pluripotent hESCs and differentiated cells. They first carried out RNA-seq and splicing quantification on RNA isolated from undifferentiated H9 hESCs as well as two differentiated hESC-derived cell types. Additionally, they performed the same analysis on RNA-seq data from H1 hESCs generated by Xie et al. (2013) that had higher sequencing depth. The analysis of each data set yielded 73 and 1996 significantly changing AS events, respectively, that were consistent within each cell line. Moreover, 35 AS events were consistent among all of the differentiation pathways and cells, some of which they further validated by RT–PCR.

One of the most robustly regulated AS events that they identified was within the *TCF3* gene, which encodes two related transcription factors—E12 and E47—through the mutually exclusive use of alternative exons 18a and 18b, respectively. Previous studies have implicated TCF3 in development (Merrill et al. 2001), but differential activity of E12 and E47 had not been addressed. The investigators demonstrated that E12 expression predominates in all pluripotent cells tested, while expression of E47 is high in differentiated and transformed cells. To link TCF3 isoform expression to differentiation, Yamazaki et al. (2018) investigated the differential impact of E12/E47 on expression of *CDH1* mRNA that encodes E-cadherin. E-cadherin has been implicated previously in pluripotency maintenance, and its expression is known to be repressed by E47 (Perez-Moreno et al. 2001; Yi et al. 2011). Notably, using siRNAs to selectively deplete E12 or E47, Yamazaki et al. (2018), demonstrated that *CDH1*/E-cadherin is repressed only by E47 and not E12. Together, this suggests that the switch from exon 18a to 18b of *TCF3* supports differentiation by promoting expression of E47 to repress *CDH1* (Fig. 1).

In order to facilitate the identification of *cis*-elements and *trans*-acting factors that regulate endogenous *TCF3* exon 18a and 18b splicing, the investigators generated a minigene that contained only the sequences from exon 17 to exon 19 of the *TCF3* gene. Importantly, they showed that the minigene recapitulated endogenous splicing

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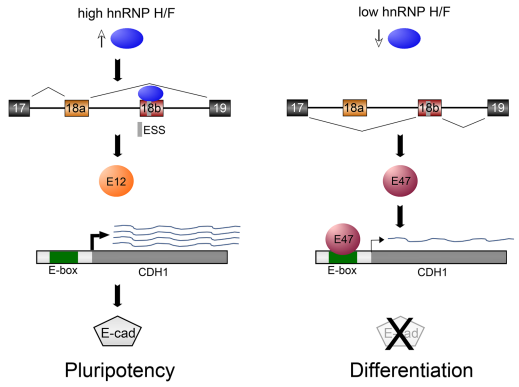

Figure 1. Model of regulation of pluripotency through heterogeneous nuclear ribonucleoprotein H1 and F (hnRNP H/F)-mediated repression of *TCF3* exon 18b. Differential abundance of hnRNP H/F in pluripotent and differentiated cells controls occupancy of an exonic splicing silencer (ESS) in *TCF3* exon 18b, thereby controlling expression of E12 versus E47. E47, but not E12, represses transcription of the *CDH1* mRNA encoding E-cadherin.

patterns in the context of pluripotent and differentiated hESCs, demonstrating that the required regulatory pre-mRNA sequences were included in the simplified model. After a series of systematic sequence deletions and swaps of sequence between exons 18a or 18b, the investigators determined that exon 18b harbors an exonic splicing silencer (ESS) sequence that is sufficient to shift the balance between 18a/18b inclusion and E12/E47 protein expression ratios.

Analysis of the sequence of this ESS highlighted a G-rich element reminiscent of the known binding motif for heterogeneous nuclear ribonucleoproteins H1 and F (hnRNP H/F), two highly related RNA-binding proteins that have been shown previously to regulate splicing changes of the neuronal differentiation factor TRF2 and during the epithelial–mesenchymal transition (EMT) (Grammatikakis et al. 2016; Huang et al. 2017). Of note, Yamazaki et al. (2018) found that hnRNP H/F protein levels decrease ~50% upon hESC differentiation, which correlates with increased exon 18b usage. Consistent with a direct role of hnRNP H/F in the repressive activity of the exon 18b ESS, the investigators confirmed the binding of hnRNP H/F to the exon 18b ESS by RNA affinity and demonstrated that overexpression of hnRNP H/F resulted in suppression of exon 18b inclusion. Conversely, siRNA knockdown of these proteins resulted in increased *TCF3* exon 18b inclusion and E47 expression. Most interestingly, siRNA depletion of hnRNP H/F also decreased expression of *CDH1* and increased differentiation of hESCs. These data thus provide a direct link between splicing regulation of *TCF3* and hESC differentiation (Fig. 1) as well as a molecular explanation for the identification of hnRNP H1 in a previous siRNA screen for determinants of hESC identity (Chia et al. 2010).

Importantly, *TCF3* is unlikely to be the only AS event that controls the switch between pluripotency and differentiation. Indeed, distinct isoforms of Foxp1 have already been shown to have differential effects on the induction of key pluripotency genes (Gabut et al. 2011). Moreover, Yamazaki et al. (2018) identified six genes in addition to *TCF3* that exhibit robust isoform differences between pluripotent cells and all differentiation pathways tested, with additional AS events that differ between stem cells and specific differentiation fates. It will be interesting in the future to determine how these additional splicing events contribute to the maintenance of pluripotency and whether hnRNP H/F also regulate these events. The investigators also were careful to point out that the contribution of *TCF3* AS to stem cell differentiation is likely specific to human cells, leaving open the possibility for additional mechanisms in other organisms. In sum, the study by Yamazaki et al. (2018) provides important new information regarding the molecular mechanisms by which hESC pluripotency is achieved and maintained and opens the door toward a deeper understanding of the role that regulated splicing plays in stem cell biology.

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