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Screening for Novel Regulators of Embryonic Stem Cell Identity

Vidy Subramanian, Carla A. Klattenhoff, and Laurie A. Boyer

Embryonic stem cells (ESCs) are characterized by their ability to self-renew and to differentiate into any cell type in the organism. This potential could be used to develop treatments for a variety of human diseases. Elucidating the molecular mechanisms responsible for maintaining ESC identity will lead to a better understanding of both development and disease, and will contribute to advances that enable therapeutic use of these cells.

Two recent studies, including one in this issue of Cell Stem Cell, have identified novel regulators of embryonic stem cell (ESC) self-renewal using genome-wide RNAi screens in mouse ESCs (Ding et al., 2009; Hu et al., 2009) and have further expanded the repertoire of factors that regulate ESC identity.

RNAi screens have proven to be a powerful genetic method for the identification of pathway components in a variety of systems. The success of these screens relies not only on an appropriate reporter system but also on stringent validation criteria. In the current studies, both groups used the same reporter cell line to monitor Oct4 expression levels and hence the differentiation status of ESCs (Figure 1A). Importantly, Oct4 is an essential pluripotency regulator that functions in a transcriptional circuit with Sox2 and Nanog as well as within a larger network that includes a growing list of transcription factors (Loh et al., 2008). While these studies each confirmed the roles of several previously known genes in self-renewal, Ding et al. (2009) identified various components of the Paf1C complex from 296 candidates, whereas Hu et al. (2009) focused their efforts on the characterization of Cnot3 and Trim28 from their initial list of 104 high-confidence target genes. The targets in each study were similarly validated using independent siRNAs and by analysis of cell morphology and of the expression of ESC markers including Oct4 and Nanog upon depletion of these factors.

The screen by Ding and colleagues yielded members of the highly conserved Paf1C complex (Ctr9 and Rtf1), and the authors then tested that additional components (Paf1, Leo1, Cdc73) of this complex were also required to maintain ESC identity (Ding et al., 2009). While the role of Paf1C in mammalian development is not known, prior studies in yeast suggest that it plays a role in transcription initiation and elongation by recruitment of the H3K4 methyltransferase to RNApol2 promoters as well as in mRNA processing (reviewed in Rosonina and Manley, 2005). Consistent with a role in early lineage decisions, the levels of Paf1C subunits decreased during ESC differentiation, and overexpression of Ctr9 blocked exit from self-renewal, maintaining the population in an undifferentiated state. The authors also found that Paf1C occupied the promoters of pluripotency genes and is required to maintain H3K4me3 patterns and expression of its target genes. Paf1C depletion led to gene expression changes similar to losses of Oct4 or Nanog and, surprisingly, to lineage-restricted differentiation, as only endoderm differentiation was impaired in these cells. These data suggest that Paf1C may coordinate signals from the core regulatory network and provide further clues as to how this network may be robustly maintained in an active state.

The work by Hu et al. also identified several transcriptional regulators, including Cnot3 and Trim28 (Hu et al., 2009). Cnot3 (Ccr4-Not transcription complex, subunit 3) is a component of the Ccr4-Not complex, which has been shown to regulate gene expression at both transcriptional and posttranscriptional levels (Collart and Timmers, 2004). Because other members of the complex were not identified in this screen, the authors propose that Cnot3 might have functions independent of the Ccr4-Not complex in ESCs. It is interesting to note that the screen by Ding and colleagues detected Cnot1, suggesting that perhaps other components of the complex are also involved in ESC regulation. Thus, a systematic analysis of Ccr4-Not complex will be required to determine the role of each of these factors in ESC self-renewal. Trim28/Kap-1 (tripartite motif-containing 28) was previously identified as important for self-renewal in an independent screen for regulators of ESC identity (Fazzio et al., 2008).

Consistent with their proposed role in self-renewal, both Cnot3 and Trim28 are highly expressed in ESCs and downregulated during differentiation. Chromatin immunoprecipitation revealed that these factors co-occupied a similar set of target genes and shared many common targets with two other components of the ESC regulatory circuitry, namely c-Myc and Zfx (Wang et al., 2006), but not with Oct4, Sox2, and Nanog. Interestingly, the common target genes include regulators of cell cycle and cell survival, suggesting that these factors function cooperatively to maintain self-renewal. The authors propose that Cnot3, Trim28, c-Myc, and Zfx form a unique module distinct from the Oct4-Sox2-Nanog network to maintain self-renewal through...
the regulation of genes involved in cell cycle and cell survival. It has recently been suggested that the Ccr4-Not complex functions to facilitate histone methylation by recruitment of the PAF complex (Mulder et al., 2007), indicating that the transcription modules identified in the two independent screens may converge to orchestrate ESC self-renewal. Collectively, these findings uncover a new set of factors that may influence transcriptional and chromatin states, cell-cycle progression, and the execution of cell-fate decisions in ESCs.

While the two genome-wide screens were similarly designed to identify regulators of self-renewal and pluripotency, based on the maintenance of Oct4 expression, these studies led to the identification of a different set of factors (Figure 1A). It is notable that the RNAi libraries used in each study were generated differently. While the esiRNA library in the Ding study relies on the processing of long dsRNA into siRNAs, Hu et al. (2009) used in vitro-synthesized siRNA pools. The siRNAs generated by these different methods might target similar as well as unique pathways, and this distinction may account for the lack of concordance between data sets. Moreover, the presence of unique hits in each case also suggests that neither screen has reached saturation and that many additional factors remain to be discovered. Other recent RNAi screens have also identified self-renewal regulators in ESCs. Ivanova and colleagues targeted transcription factors that resulted in the identification of four genes (EsrRb, Tbx3, Tcl1, and Dppa4) with previously unrecognized roles in self-renewal (Ivanova et al., 2006). While the current studies from Ding and Hu and their respective colleagues each confirmed some of these targets, neither detected components of the Tip60-p400 chromatin-remodeling complex, as was identified in a screen for chromatin-related proteins (Fazzio et al., 2008). Notably, Tip60/p400 depletion resulted in a decrease in Nanog but not Oct4 levels. It is likely that these regulators were not observed in the Buckholz and Elledge studies, given that the current screens were based on Oct4 expression.

Genome-wide RNAi screens have proven useful in identifying genes whose roles in the maintenance of the ESC state had not been previously established (Figure 1B). Further characterization of these regulators will help to provide a more comprehensive view of the molecular mechanisms that govern the balance between self-renewal and lineage commitment. This advance may lead to improved protocols for directing the differentiation of ESCs toward particular lineages and for the generation of ES-like cells from somatic cells. It will be essential to integrate the new components into the broader network and to understand how signaling pathways communicate with these factors to control cell fate. Importantly, regulatory networks controlling self-renewal in stem cells could also be active in certain cancers (Ben-Porath et al., 2008; Wong et al., 2008), and so their further characterization may uncover novel therapeutic targets or diagnostic markers.

REFERENCES


