Wnt/β-catenin target gene *conductin* were detected in many human SCCs. Following knockdown of β-catenin expression by short hairpin RNA, tumor growth of human SCC13 cell lines was reduced in xenografts, suggesting that β-catenin signaling is required for tumorigenesis. However, as noted by the authors, nuclear β-catenin expression is not strictly a marker of CSCs, and CD34 is not expressed in human SCCs, precluding a CSC analysis of human tumors. In the human hair follicle, CD34 immunoreactivity is found below the bulge; however, there is no evidence that this marker enriches for stem cells. Several markers have been reported to enrich for human interfollicular stem cells, including high expression of β1 integrin, high expression of α6 integrin (in combination with low levels of CD71), and expression of melanoma-associated chondroitin sulfate proteoglycan or Lrig1. In the human hair follicle, CD200-positive bulge cells have a high clonogenic-forming capacity in vitro, a characteristic associated with stem cells (Ohyama et al., 2006). Therefore, in future studies it will be useful to determine whether any of these markers identify CSCs within human epidermal tumors.

In summary, Malanchi and colleagues (Malanchi et al., 2008) elegantly demonstrate that murine cutaneous tumors contain a subpopulation of CSCs that can be enriched by selection for the follicular bulge cell surface marker, CD34. They provide convincing evidence that β-catenin signaling is required for growth of murine squamous cell carcinomas, most likely via the maintenance of CSCs, although the mechanism by which this occurs is still unclear. Finally, they demonstrate that the Wnt/β-catenin pathway also is activated in human malignant SCCs. However, the challenge remains to identify CSCs within human epidermal tumors. These studies are reminiscent of the role that the Wnt/β-catenin pathway plays in modulating stem cell maintenance and tumorigenesis in the intestinal tract, suggesting that therapeutic interventions targeting this pathway may be beneficial in multiple epithelial tissues (Reya and Clevers, 2005).

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Open Chromatin and Hypertranscription in Embryonic Stem Cells

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In this issue of *Cell Stem Cell*, Efroni et al. (2008) use tiling microarrays to reveal genome-wide hypertranscription in mouse embryonic stem cells (ESCs), including expression of normally silent, noncoding regions. Hypertranscription may reflect the unusual "open" structure of ESC chromatin.

By several criteria, ESC nuclear DNA is packaged in an unusual form of chromatin that appears to be more "open" than that in differentiated cells. Histones and nonhistone proteins exchange more rapidly, i.e., are more loosely bound to DNA, in ESCs (Meshorer et al., 2006), their constitutive heterochromatin is more dispersed, or at least less prominent microscopically (Meshorer et al., 2006; Spivakov and Fisher, 2007), and the positioning of genes and chromosome territories changes markedly on ESC differentiation (Giadrossi et al., 2007). ESCs also exhibit global enrichment of histone modifications associated with transcriptional activity as well as depletion of modifications associated with silent chromatin (Spivakov and Fisher, 2007). Meshorer, Misteli, and colleagues (Efroni et al., 2008) now provide further evidence for the open structure of ESC chromatin and go on to ask whether it might be associated with altered transcription.

In their analyses, Efroni et al. (2008) utilize whole-genome tiling arrays to compare transcript levels in the R1 ESC line to those present in their differentiated progeny. In ESCs, transcription was detected in intronic, intergenic, and exonic regions, with over-threshold readings from just over one-million probes. In contrast, the number of positive probes was

reduced ~20% when R1 cells were differentiated for 7 days in culture to yield neural progenitor cells (NPCs). A less extensive reduction in positive probes was detected after only 24 hr and occurred while the population was still proliferating rapidly, an important consideration as the unusual cell cycle of ESCs (most are in S phase) differs markedly from that of NPCs. Importantly, changes detected during differentiation were not limited to complete loss of expression of selected regions. Comparing relative change in the expression level of all individual probes that remained positive after differentiation revealed that far more were downregulated in differentiated cells than were elevated during this process. The authors note that the net change in transcription (measured by relative numbers of down- and upregulated probes) varied widely from one chromosome to another. Some chromosomes (e.g., 6, 7, X) exhibited a several-fold excess of downregulated probes, whereas others showed a much smaller difference or even a slight excess of upregulated probes (e.g., 1, 12). By eye, the relative numbers of upregulated and downregulated probes within intergenic and exonic regions of any given chromosome appear correlated, suggesting that differences observed between individual chromosomes may not be due entirely to different numbers of tissue-specific genes. Could these findings reflect, in part at least, differences in the stage at which individual chromosome territories are remodeled as differentiation proceeds?

A reasonable working hypothesis is that global hypertranscription in ESCs is a consequence of their unusually open chromatin structure, thus allowing easier access to transcription factors and the transcriptional machinery. ESC chromatin may be in a "ground state" that permits progression toward multiple distinct differentiation pathways, as directed by intrinsic or extrinsic signals (Silva and Smith, 2008). However, transcript abundance (the primary measure of transcription used by Efroni et al. [2008]) may also be influenced by posttranscriptional events, including RNA processing, transport, and even ribosome loading, all of which will change during differentiation (e.g., Sampath et al., 2008, this issue of *Cell Stem Cell*). The relative contributions of these various processes to regulation of transcript levels require further exploration.

ESC differentiation involves not only altered gene expression patterns but also remodeling and repositioning of chromosome territories and changes in replication timing (Azuara et al., 2006). These events are all likely to be facilitated by an initially open, "plastic" chromatin state, raising the possibility that hypertranscription is simply a byproduct of a necessary chromatin conformation and of no specific consequence in itself. To explore this, Efroni et al. (2008) analyzed expression of the annotated genes on the tiling array. Expression of 54% of these genes was elevated in ESCs relative to NPCs, 38% were reduced, and only 8% were classed as unchanged. Of all the relatively overexpressed genes in ESCs, the expression level of general transcription factors and chromatin remodeling proteins was significantly elevated. Could the relatively high expression of chromatin remodelers in undifferentiated ESCs be responsible for their unusual chromatin conformation and persistence of the pluripotent state? To test this, three highly expressed remodelers, *Brg1*, *Chd1l*, and *Smarcd2*, were knocked down individually. The effects were mixed. *Smarcd2* knockdown produced no phenotype, whereas diminution of *Chd1l* and *Brg1* expression reduced growth rate. However, only loss of *Brg1* also compromised differentiation capacity of ESCs into nexin-positive neural progenitors. Further work is needed to address the potential roles of chromatin remodeling proteins, initially by determining whether altered transcript levels generate altered amounts of functional protein product.

A recent publication provides a good illustration of how chromatin modifying enzymes can exert both global and local gene-specific effects (Loh et al., 2007). Knocking down the histone demethylases Jmjd1a and Jmjd2c in mouse ESCs globally increased the levels, respectively, of H3 di- and trimethylated at lysine 9. These histone modifications are typically associated with silent chromatin. In addition to their global effects, Jmjd1a and Jmjd2c were shown to target, and regulate, specific genes, including *Tcl1*, a potential regulator of self-renewal, and *Nanog*, a key determinant of pluripotency. Thus, we can see the beginnings of a network in which key chromatin modifying enzymes exert both global and gene-specific effects, which in turn influence differentiation. Further, both demethylase genes were themselves positively regulated by the transcription factor Oct4, providing an example of how a transcription factor can trigger genome-wide epigenetic changes by altering the expression of histone modifying enzymes. It will be interesting to see whether differentiated cells induced to become pluripotent by the combined transduction of several transcription factors, including Oct4 (Jaenisch and Young, 2008), also exhibit an open chromatin structure and hypertranscription.

A chromatin state in which gene expression controls are relaxed and levels of adventitious transcription increased would seem likely to disrupt the orderly sequence of epigenetic events necessary for development. Thus, it is important that hypertranscription and open chromatin do not bring with them a complete loss of transcriptional control. Transcription of many genes is effectively suppressed in ESCs, often by PcG proteins with a consequent increase in H3K27me3, but this can be reversed by depleting the relevant methyltransferase, demonstrating that plasticity is retained (Azuara et al., 2006). Also, the endogenous pluripotent epiblast cells from which cultured ESCs are derived exist for only a short developmental window, after which pluripotency is lost (Silva and Smith, 2008). Perhaps relaxed transcriptional control can be tolerated in vivo for this short period. We should also remember that ESCs derived from this transient pluripotent population are adapted to growth in tissue culture, a process that exerts strong selection pressure in favor of rapidly proliferating cells; it may be that the unusual chromatin structure of ESCs reflects, in part, their adaptation to culture. Indeed, initial comparisons of the epigenetic properties of a few silenced genes in ESCs and inner cell mass indicate that histone modifications associated with silencing are comparatively low in ESCs, consistent with a relaxation in the silencing signal (O'Neill et al., 2006). A more detailed comparison of the transcriptomes and epigenetic properties of ESCs and the epiblast population from which they are derived will help answer this question.

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Translational Control: A New Dimension in Embryonic Stem Cell Network Analysis

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Systems biology studies have revealed transcriptional networks and proteomic signatures critical for embryonic stem cell (ESC) function. In this issue of *Cell Stem Cell*, Sampath et al. (2008) demonstrate that translation is also differentially controlled in undifferentiated versus differentiated ESCs.

Pluripotent embryonic stem cells (ESCs) have the capacity to differentiate into all adult cell lineages and thus offer much hope for cell-based therapies of human disease. However, to devise and optimize protocols to engineer ESC-derived tissues requires a comprehensive understanding of the networks controlling ESC fate decisions. Because ESC differentiation involves changes in numerous transcriptional networks (Walker et al., 2007; Ivanova et al., 2006), protein signaling networks (Wang et al., 2006), and chromatin structure (Mikkelsen et al., 2007; Pan et al., 2007), a systems biology approach that integrates the transcriptome, proteome, and promoter occupancy data is needed to fully describe the process of stem cell differentiation. Underlying these networks are highly refined control mechanisms, such as miRNA that modulate the transcriptome and are important for ES cell self-renewal and differentiation (Lakshmipathy et al., 2007; Ivey et al., 2008).

Currently, there are unresolved discrepancies between the transcriptome and proteome profiles of undifferentiated ESCs and their differentiated progeny. Specifically, the level of gene expression does not always correlate with protein levels observed in the same populations. A similar disconnect between mRNA and protein expression has been observed in lineage-specific differentiation systems, such as hematopoiesis and myogenesis, and might arise via regulatory mechanisms that impact translational efficiency and protein degradation rates. To bridge this gap, some mRNA transcripts may be primed for translation and are termed "potentiated" in yeast studies. These mRNA can be identified by the isolation of ribosome-enriched transcripts coupled to microarray analysis, known as translational state array analysis (TSAA).

In this issue of *Cell Stem Cell*, Sampath et al. (2008) delve into the translational control of the mouse ESC transcriptome during differentiation. The authors first identified that during ESC differentiation into embryoid bodies (EBs) there is an increase in global mRNA and protein synthesis. Using microarray analysis, they verified that indeed there was an increase in global transcript abundance in differentiated ESCs. Interestingly, the vast majority of ribosomes isolated from ESCs were not bound to mRNA, whereas the elevated transcript and protein levels observed in differentiated cells were coupled with a significant increase in transcript loading of ribosomes. Thus, the question that arises is whether there is preferential loading of ribosomes with transcripts that are important for ESC differentiation. To address this possibility, the authors conducted TSAA, a transcriptome-wide method to identify polysome-bound transcripts. Comparing the microarray datasets of transcript expression with the ribosome loading results (the TSSA dataset), the authors distinguished four distinct groups: (1) transcripts that were differentially expressed and differentially loaded on ribosomes, (2) transcripts that were differentially expressed but exhibited no change in translational efficiency, (3) transcripts that were not differentially expressed but exhibited changes in translational efficiency, and (4) transcripts that did not change in abundance or ribosome loading. It is important to emphasize that "differential expression" of transcripts refers to the combined number of signals that were either elevated or reduced beyond a set threshold