Editorial Commentary

Stem Cell INVESTIGATION

Tuning the chromatin landscape of embryonic stem cells

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During implantation, cells of the embryo dramatically increase their proliferation rate. This developmental transition involves increased demand for the synthesis of building blocks needed to support the growth of post-implantation epiblast cells. Embryonic stem cells (ESCs) cultured in serum closely resemble cells of the post-implantation epiblast and similarly exhibit high proliferation rates (1). Consistent with the need for biosynthetic precursors, ESCs and pluripotent cells of the blastocyst have high transcriptional output (2), often referred to as hypertranscription, or transcriptional amplification, which is associated with a highly permissive chromatin landscape marked by a lack of condensed heterochromatin and high levels of active histone modifications (3,4). However, it is not known how stem cells tune the chromatin landscape to cellular needs or the molecular basis for this feedback. In a recent issue of *Cell Stem Cell*, Bulut-Karslioglu *et al.* performed a genome-wide screen to establish a role for cell growth pathways, and specifically translation, in promoting the open chromatin state of ESCs (5). Further analysis demonstrated that several key chromatin modifiers are unstable proteins that are stabilized by high translational output. These data provide a model by which chromatin state is tuned to the transcriptional, translational, and growth needs of stem cells during implantation and inform future studies on the coordination of these processes in stem/progenitor cells and cancer.

In order to screen for positive regulators of ESC euchromatin, the authors developed a live-cell reporter based on the direct and specific binding of the Chd1 ATP-dependent chromatin remodeler to histone 3 trimethylated at lysine 4 (H3K4me3), which is highly abundant in ESCs (3) and associated with active transcription. Specifically, they fused the double chromodomains of Chd1, which are sufficient to bind H3K4me3 (6), to enhanced green fluorescent protein (Chd1chr-EGFP), thereby using EGFP fluorescence as a surrogate measure of H3K4me3 levels. Performing a genome-wide RNAi screen in ESCs, the authors identified 461 genes whose knockdown resulted in lower Chd1chr-EGFP fluorescence, 303 of which are also highly expressed in ESCs. Several previously reported regulators of ESC chromatin were recovered, including histone acetyltransferases Tip60, p400 (7), the histone methyltransferase Mll4 (8), and Chd1 itself (9), validating the screen conditions. In addition, genes involved in cellular growth and protein synthesis, ribosomal proteins, translation factors, RNA processing, and RNA Polymerase I components were highly enriched among factors required for Chd1chr-EGFP fluorescence. Mammalian target of rapamycin (mTOR), a sensor of cellular nutrient availability and regulator of translation, was the top hit among positive regulators of Chd1chr-EGFP. Consistent with these findings, cycloheximide (CHX), an inhibitor of translational elongation, resulted in reduced Chd1chr-EGFP fluorescence, as did treatment with mTOR and Myc inhibitors. These data suggested the surprising finding that biosynthetic pathways regulating translational output could be directly coupled to the maintenance of the euchromatic state in ESCs.

Biosynthetic pathways regulating hypertranscription have previously been implicated in the developmental transition regulating growth of the embryo during blastocyst implantation and diapause, when development of the blastocyst is suspended temporarily in unfavorable

conditions. Specifically, mTOR inhibitors were found to promote a reversible, paused pluripotent state resembling diapause, in which blastocysts could be maintained up to 22 days under culture conditions that typically cause blastocyst collapse after 24–48 h (10). mTOR inhibition was associated with global transcriptional suppression, mimicking the gene expression program of diapause. Similarly, treatment of ESCs with Myc inhibitors resulted in a reversible, dormant state resembling diapause in which translation and proliferation were reduced due to downregulation of RNA processing, RNA biogenesis, and translation genes (11), albeit not as profoundly as mTOR inhibitors (10). Like Chd1, Myc was shown to be a global amplifier of transcription in ESCs (12) and cancer cell lines (13). These results reinforce the notion that the positive regulation of cell growth and translation through hypertranscription of biosynthetic pathway genes is essential to maintain proliferation of ESCs and promote development of the blastocyst. Reduction in these processes results in cells transitioning to a developmental dormancy until translational output is re-established. However, it is not known how these processes are coupled to the establishment and maintenance of permissive chromatin landscapes that enable high transcriptional output in the context of embryonic development.

To understand the direct effects of translational inhibition on euchromatin, the authors performed a short 3 h CHX treatment to reveal global reductions in active histone modifications H3K4me3, histone 4 lysine 16 acetylation (H4K16ac), and histone 3 lysine 27 acetylation (H3K27ac) in ESCs and E4.5 blastocysts. Nascent transcription was dramatically reduced in the presence of CHX, with concomitant reduction in elongating RNA Polymerase II, which was especially evident at ribosomal protein genes. Genome-wide profiling revealed that the largest reductions in H4K16ac were observed at the most highly transcribed genes, indicating this mark may be an important regulator of hypertranscription. The effect of CHX on ESCs and blastocysts was relatively specific as ESCs differentiated with retinoic acid, mouse embryonic fibroblasts, and neural stem cells were overall less sensitive with respect to reductions in active histone modifications and nascent transcription. Notably, inhibition of mTOR similarly resulted in reduction in H4K16ac and nascent transcription in blastocysts, which was also observed in diapaused blastocysts (10).

Finally, the authors undertook a SILAC-based mass spectrometry approach to identify proteins that were rapidly depleted in 1–3 h of CHX treatment of ESCs, and thus, potential candidates for the regulation of permissive chromatin in ESCs. They identified 2,309 proteins depleted upon CHX treatment, many of which were associated with cell cycle, transcription, or chromatin modification. After overlapping with positive regulators of Chd1chr-EGFP reporter activity, they found 60 proteins that were both unstable and positive regulators of euchromatin in ESCs. Among these, they identified Chd1 itself, which the authors had previously showed is required to maintain the euchromatic state of ESCs as knockdown of Chd1 in ESCs and peri-implantation embryos results in accumulation of heterochromatic foci (9). In addition, Tip60-p400 histone acetyltransferase complex (7), the BRD1 subunit of the MOZ/MORF histone acetyltransferase complex (14), and several key pluripotency transcription factors (Klf5, Zic1) were found to be unstable euchromatic regulators. Cell fractionation experiments revealed a reduction in the chromatin-bound levels of these proteins following CHX treatment, but not heterochromatin regulators HP1a, EZH2, or G9a. Surprisingly, global reductions in acetylated H3/H4 in CHX treated ESCs did not result in extensive changes in chromatin accessibility as measured by Assay for Transpose-accessible Chromatin followed by high-throughput sequencing (ATAC-seq). However, losses in chromatin accessibility were observed at 454 developmental enhancers associated with Klf5/Zic1 motifs, presumably due to loss of Klf5/Zic1 protein expression. These results suggest that hypertranscription is largely a function of histone modifications enriched at promoters that recruit the binding of epigenetic reader proteins and transcription factors involved in transcriptional amplification, such as Myc (12,13). Indeed, like Myc, Chd1 binding is correlated with the levels of H3K4me3 and elongating RNA Polymerase I/II at the promoters of actively transcribing genes (9,15). In the absence of Chd1, the overall transcriptional output of ESCs is decreased (15), similar to the effects of Myc depletion or inhibition (12). The relative contribution of other specific histone modifications (H4K16ac) and epigenetic readers (bromodomain-containing proteins) to hypertranscription remains to be established.

With these data, Bulut-Karslioglu *et al*. are the first to establish a mechanistic link between the regulation of cell growth and biosynthetic pathways and the permissive chromatin modifications necessary to maintain high transcriptional output. Specifically, they implicate protein instability of key chromatin modifying enzymes and epigenetic reader proteins as being an important

mechanism by which translational output positively feeds back on chromatin to sustain high levels of active histone modifications. Thus, in the context of environmental stressors that inhibit translation, decline in the levels of chromatin modifiers may result in lower transcriptional output, particularly for the most highly expressed genes, including ribosome biogenesis and ribosomal RNA genes. This in turn could lead to less biosynthetic building blocks to support high rates of cell proliferation and growth. Future studies are needed to dissect the mechanism by which protein instability of chromatin modifiers is regulated, for example through inherent protein sequences or post-translational modifications regulating protein turnover. It is worth noting that the authors identified several proteins involved in ubiquitination and SUMOylation, which could regulate the turnover of a handful of protein substrates in a context-specific fashion. Other pathways clearly contribute to the coordination of transcription, translation, and cell division during development. For example, glucose availability contributes to high acetyl-CoA levels that promote histone acetylation in ESCs (16). Additionally, histone acetylation promotes the expression of cell cycle genes and the efficient firing of replication origins during cell proliferation. Thus, the coordination of these cellular processes is intricately wired to ensure proper execution of developmental transitions only under favorable conditions. Chemical inhibition of mTOR activity results in diapause (10), and deletion of many chromatin modifiers such as Chd1 is embryonic lethal (15). Future studies are necessary to understand how natural environmental stresses such as nutrient deprivation, viral infection, exposure to toxins, or hypoxia may affect mTOR-dependent feedback or other pathways that ensure the coordination of these cellular processes in pluripotent cells of the blastocyst. Additionally, the authors provide a compelling framework with which to study the regulation of other developmental transitions in stem and progenitor cells that similarly require high biosynthetic output.

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None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

Ethical Statement: The author is accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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