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Breathing Chromatin in Pluripotent Stem Cells

Only a few factors controlling stem cell pluripotency have been identified to date, and we do not yet fully understand how they act to maintain pluripotency and control differentiation. A report in this issue of *Developmental Cell* (Meshorer et al., 2006) describes a new trait of pluripotent cells: hyperdynamic or "breathing" chromatin. According to this report, hyperdynamic chromatin is both specific and functionally relevant for pluripotent cells.

Although embryonic stem cells (ES cells) were first isolated over a quarter century ago (Evans and Kaufman, 1981; Martin, 1981), the biology of pluripotency received little attention until a few years ago, when the isolation of human ES cells sparked new interest (Thomson et al., 1998). ES cells represent a fascinating biomedical research tool; derivatives of these cells could eventually be used for drug testing, disease models, or transplantation medicine. In addition, nuclear transplantation experiments (Wilmut et al., 1997) and cellular fusion experiments (Cowan et al., 2005) have demonstrated that almost any cell type can be regressed to an early embryonic stage, indicating that the epigenetic status of cells can be profoundly altered or even reprogrammed.

Since all cell types are derived from pluripotent cells, a deeper understanding of these cells will impact our understanding of nearly all areas of molecular and cellular biology. A number of genes exclusively expressed in pluripotent cells have been identified, including the preeminent transcription factors, Oct4 and Nanog. At the protein level, a number of signaling pathways have been identified as important for pluripotency, including the LIF-STAT and WNT pathways. None of the pathways identified to date are specific to pluripotent cells, which is perhaps not surprising given that most signaling pathway components are responsible for multiple cellular functions, with specificity conferred by fine-tuning of individual pathway components. However, it is generally accepted that a great many components of pluripotent cell biology remain yet undiscovered.

Thus, it is quite exciting to welcome a new player into the game, the concept of hyperdynamic chromatin, which is introduced in this issue of *Developmental Cell*. In their paper Meshorer et al. (2006) report their investigation of chromatin-associated protein mobility in pluripotent cells (Figure 1). Unexpectedly, the results of their work revealed that pluripotent cells appear to share a unique state of dynamic chromatin that the authors call "breathing chromatin."

It has been known for some time that chromatin fibers are quite dynamic. For example, nucleosomes are constantly remodeled by ATP-dependent chromatin remodeling complexes (e.g., ISWI, SWI2/SNF), which modulate the strength of histone-DNA interactions and allow the exchange of chromatin-associated proteins. In addition, other regulatory complexes continuously modify specific amino acids in the histone tails and nucleic acid bases in the DNA. This flexibility is required for most cellular functions, including cell cycle control, differentiation, and programmed cell death. It was initially assumed that the exchange rates of core histones and other chromatin-associated proteins were relatively low, but recent reports have shown that some chromatin-associated proteins may be much more dynamic than originally thought.

In the present paper, Meshorer et al. (2006) measured this exchange rate and the affinity of chromatin-associated proteins to DNA in ES cells, by fluorescent recovery after photobleaching (FRAP) technology and biochemical means. They then compared the results between undifferentiated and differentiated cells. Previous work has shown that linker histone H1 remains positioned on chromatin for up to several minutes in most cell types (Misteli et al., 2000), while core histones like H2B and H3 are replaced within hours (Kimura and Cook, 2001). In contrast, Meshorer et al. (2006) showed that in ES cells, the H1 subtype $H1^0$ may be replaced within seconds, while the H2B and H3 core histones are replaced within slightly more than a minute. In differentiated cell types, less than 3% of the cellular pool of H2B and H3 core histones are loosely bound to chromatin; in ES cells, this binding reaches up to 25%. The authors posit that the quickly replaced (or "hyperdynamic") histones are not simply soluble components of the nucleoplasm, but instead are loosely bound to chromatin.

This begs the question of whether hyperdynamic or "breathing" chromatin is found in other stem cells, i.e., progenitor and pluripotent cells. Indeed, Meshorer and colleagues tested other pluripotent and committed progenitor cells and found that truly pluripotent cells (i.e., ES, P19 and C3H/10T1/2) all had hyperdynamic chromatin, whereas this was not seen in lineage restricted (non-pluripotent) progenitor cells. Thus, it appears as though hyperdynamic chromatin is a hallmark of pluripotency.

To begin assessing the functionality of hyperdynamic chromatin in pluripotency, the authors used innovative thinking to experimentally increase and decrease the fractions of hyperdynamic chromatin in pluripotent cells. ES cells deficient for HirA, which is responsible for DNA synthesis-independent chromatin assembly, showed dramatically increased levels of histones H3 and H3.3. $HirA^{-/-}$ ES cells, grew normally when undifferentiated, but showed accelerated differentiation upon stimulation, suggesting that the availability of histones facilitated differentiation. Similarly, ES cells expressing a mutated form of histone $H1^0$ ($H1^0$cc, which binds almost irreversibly to chromatin and suppresses the dynamic exchange of histones) showed a significant delay in differentiation. Together, these findings suggest that hyperdynamic chromatin is necessary for ES cell differentiation.

To examine whether other chromatin-associated proteins move more quickly in pluripotent cells, Meshorer

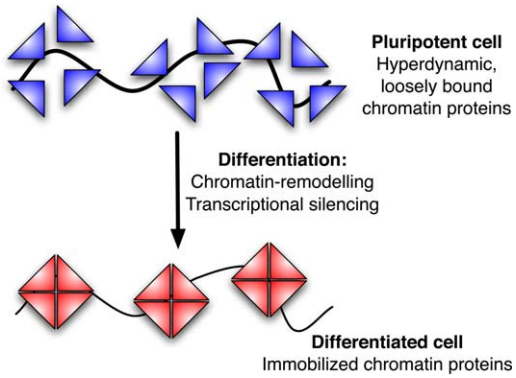

Figure 1. Chromatin-Associated Proteins in Blue Bind Only Loosely and Are Hyperdynamic in Pluripotent Cells

This loosely bound fraction of chromatin proteins plays a key role in the remodeling process during differentiation of pluripotent cells. Immobilization of these dynamic chromatin-associated proteins (red) could lead to higher-order silencing of portions of the genome during differentiation of pluripotent cells.

and colleagues examined two additional chromatin-associated proteins, heterochromatin protein 1 group (HP1) and histone variant H3.3. Their results revealed that HP1α moved much more rapidly in the heterochromatin of undifferentiated ES cells versus differentiated cells, whereas there was no significant difference in these dynamics in euchromatin. In contrast, H3.3, which is found in highly transcribed genomic regions, did not show significantly different dynamics in ES cells versus differentiated cells. These findings suggest that not all chromatin-associated proteins move faster in pluripotent cells, and that regional differences may exist within the nucleus. In addition, they suggest that ES cells may have a disproportionately high amount of transcriptionally active regions or regions that are primed for transcription.

The observations in Meshorer et al. (2006) support of the "stem cell priming" hypothesis. According to this hypothesis, the genome of stem cells may be generally subject to something like the "priming reaction" seen previously for example in the locus control region (LCR) adjacent to the globin gene cluster. During differentiation of hematopoietic progenitor cells, the chromatin structure of this region opens up before globin gene transcription, thus "priming" the transcription of lineage-specific genes (Jimenez et al., 1992). The new data fit this model nicely by indicating that the loosely bound fraction of chromatin proteins plays a key role in the remodeling process during differentiation of pluripotent cells. These findings have a number of interesting potential implications. For example, researchers could examine the pluripotency of stem cells by looking at chromatin mobility rather than gene expression profiles, an advantage because chromatin mobility may be more conserved across very different groups of pluripotent cells. In addition, it is possible (although experimentally challenging to address) that cells in the pre- and post-implantation embryo will show the same properties, potentially allowing the tracking of such pluripotent cells during development. Finally, this work prompts a number of fascinating questions. For example, which molecular mechanisms control the hyperdynamic state of ES cell chromatin, and what remodeling complexes are involved? What happens during cellular reprogramming after nuclear transfer into oocytes or fusion with pluripotent cells? Will modulation of the hyperdynamic pool of chromatin-associated proteins help us reprogram cells? As these and other questions are answered in the future, we will gain precious new insights into what pluripotency is.

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The Grapes of Incompatibility

When two colonies of the tunicate *Botryllus* contact each other, they either fuse into a single colony or mount a destructive reaction, which keeps them apart. Which of the two reactions takes place is determined by a single, highly polymorphic fusion/histocompatibility (FuHC) locus. Recent communications report the cloning and characterization of the FuHC locus and suggest that its function may be to protect against parasitism by conspecific stem cells.