Published in final edited form as:
*Science*. 2009 January 9; 323(5911): 220–221. doi:10.1126/science.1166261.

# Chromatin and the pluripotent ground state

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## Abstract

Recent studies highlighting the primacy of the transcription factor network have led some researchers to question what role, if any, chromatin plays in pluripotent cells. Part of the answer may lie in its now well-established function in buffering transcriptional noise.

Their unique ability to self-renew *in vitro* while retaining the capacity to develop into essentially any cell type has made embryonic stem (ES) cells an essential model for developmental biology and a promising substrate for cell transplantation therapy. How ES cells establish and maintain this suspended state of pluripotency is the subject of intense investigation. ES cell identity is specified by a regulatory network that principally involves the transcription factors Oct4, Sox2, and Nanog (1). The primacy of this network is reinforced by recent demonstrations that adult somatic cells can be reprogrammed to pluripotent ES-like cells by over-expression of defined transcription factors (reviewed in (1)). It has been suggested in recent literature that the ES cell represents a ‘ground state’ for a mammalian cell predicated solely on the activities of these ‘pluripotency’ factors and a ‘freedom’ from extrinsic differentiation signals (2, 3).

As fundamental regulators of development, chromatin components could also be presumed to make important contributions to a pluripotent ground state. Indeed, chromatin in ES cells is distinguished from that in lineage-restricted cells by a number of characteristic features. ES cell chromatin is distinctly decondensed with major architectural chromatin proteins loosely bound and exhibiting hyperdynamic binding kinetics (4). High levels of Polycomb-group repressors and the associated histone H3 lysine 27 trimethyl mark are present, most notably in association with silent developmental gene loci (5, 6, 7). These target loci also exhibit features of active chromatin, including the trithorax-associated histone H3 lysine 4 trimethyl mark, which may poise them for subsequent activation (8, 9).

Despite its unique properties, the functional significance of chromatin in ES cells remains uncertain. It is possible, although often difficult, to establish ES cell lines that lack critical chromatin regulators, including DNA methyltransferases, histone methyltransferases, chromatin remodeling proteins, and Polycomb-group proteins (reviewed in (10)). These

lines tend to exhibit defective differentiation which may reflect roles for these regulators in differentiation-associated remodeling and/or the subsequent engagement of lineage-specifying epigenetic controls. Yet the viability of the mutants implies that chromatin regulation may paradoxically be dispensable for maintaining ES cell identity.

Nonetheless, an expanding body of research highlights an essential function for chromatin – namely, the buffering of noise in the transcriptional network – that is almost certainly an indispensable feature of the ES cell ground state. ES cells sit in a precarious position ready to respond to differentiation signals with dramatic state switches marked by extensive transcriptional changes. To maintain their pluripotent state, they must minimize stochastic extrinsic and intrinsic signals that create variability (noise) in gene expression and may thereby trigger differentiation.

Evidence that chromatin can act as a buffer to transcriptional noise has emerged from biochemical, genetic and computational studies (11, 12, 13, 14, 15). At its most basic level, chromatin consists of DNA and histones intimately wrapped into nucleosomes. Transcription factors must compete with histones for access to the DNA, and the binding energy of several factors is typically required to displace one nucleosome. This ‘collaborative competition’ between transcription factors and chromatin has profound consequences at the level of gene regulation, including combinatorial control and threshold responses (11). Accordingly, chromatin may prevent gene induction due to weak activating signals and limit noise-induced errors.

Current models of nucleosomal regulation have been developed largely through studies in yeast. Focusing on the classical *PHO5* promoter, O’Shea and colleagues demonstrated that nucleosomal occupancy of regulatory protein binding sites can decouple the threshold of promoter induction from expression capacity. These investigators studied a panel of *PHO5* promoter mutants that varied in their exposure of high- and low-affinity Pho4 motifs. Under intermediate inducing conditions, gene expression was a function of accessible Pho4 sites and unaffected by occluded sites. In extreme conditions, nucleosome eviction exposed the full complement of regulatory sites enabling maximal expression (12).

Direct links have also been established between nucleosomal regulation and transcriptional noise. Studies from the O’Shea and Kornberg labs have shown that *PHO5* expression noise is due to stochastic chromatin remodeling events involving removal and reformation of nucleosomes on the gene promoter (13, 14). Remarkably, the extent of noise fluctuation could be altered *in cis* by mutations affecting nucleosome stability at the *PHO5* promoter or *in trans* by mutations affecting chromatin-remodeling complexes.

The basic elements of transcriptional noise and buffering are almost certainly general to eukaryotes. Mammalian cell models exhibit stochastic bursts of gene activation that can generate large variations in expression within a clonal population (16). The nucleosomal buffering mechanisms established in yeast may be particularly applicable to ES cells whose dynamic chromatin environment and relative lack of restrictive heterochromatin is reminiscent of the model organism. Their generally accessible chromatin state appears to render ES cells prone to transcriptional leakiness (17). This may confer an added

dependency on buffering, especially at lineage-specifying developmental genes whose promoters must integrate complex signals and whose inappropriate expression risks triggering differentiation.

How might ES cells achieve a higher level of transcriptional safeguarding without compromising plasticity? As the yeast mutant studies indicate, noise buffering can be tuned by varying the activities of chromatin complexes. Polycomb-group repressors are attractive candidates for fulfilling such a role in ES cells as they are expressed at uniquely high levels and target many essential developmental genes. Although the functional significance of Polycomb-group proteins in pluripotency has been called into question by the viability of corresponding knock-out ES cell lines (10), the phenotype of these mutants – propensity to differentiate and weak up-regulation of target loci (5, 18) – is entirely consistent with a role in buffering noise through minimization of transcriptional variation.

Hence, we suggest that by reinforcing the repression of critical gene targets, Polycomb complexes filter noise and thereby fulfill one of the hallmark requirements of the pluripotent ‘ground state’ – minimization of extrinsic perturbations (2). Other chromatin components, including several disproportionately up-regulated nucleosome remodeling complexes (17), may also contribute to a specialized ES cell chromatin network that neutralizes extrinsic noise by buffering transcriptional fluctuations while remaining permissive to appropriate differentiation programs.

The compelling implication is that chromatin plays distinct regulatory roles that vary along the developmental axis. Its buffering function, perhaps most essential in early development, is distinct from the epigenetic functions typically ascribed to chromatin in lineage-committed cells. The latter is associated with stable chromatin compaction and silencing of non-utilized genomic loci. The widespread changes in chromatin dynamics and expression of key chromatin components that occur during differentiation may reflect a functional re-prioritization of chromatin from a buffer to a preserver of lineage fidelity.

The noise control concept invokes interesting questions about the existence of other unrecognized roles for chromatin. Recent studies indicate a role for transcriptional noise in lineage-determination (19), raising the intriguing possibility that chromatin may influence lineage decisions during development by modulating the amplitude of transcription fluctuations. Thus, fundamental principles gleaned through biochemical and model organism studies highlight an underappreciated role for chromatin in pluripotency and suggest new experimental directions that may ultimately lead to greater understanding of chromatin regulatory mechanisms in mammalian development.

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20. We are grateful to Jon Widom, Eric Mendenhall and Alex Meissner for helpful discussions and comments.