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Control of embryonic stem cell identity by nucleosome remodeling enzymes

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Abstract

Embryonic stem (ES) cells are pluripotent cells that can self renew indefinitely or be induced to differentiate into multiple cell lineages, and thus have the potential to be used in regenerative medicine. Pluripotency transcription factors (TFs), such as Oct4, Sox2, and Nanog, function in a regulatory circuit that silences the expression of key TFs required for differentiation and activates the expression of genes important for maintenance of pluripotency. In addition, proteins that remodel chromatin structure also play important roles in determining the ES cell-specific gene expression pattern. Here we review recent studies demonstrating the roles of enzymes that carry out one facet of chromatin regulation, nucleosome remodeling, in control of ES cell self-renewal and differentiation.

Introduction

Pluripotent ES cells are characterized by higher order chromatin structure that is generally dynamic and permissive to the transcriptional machinery [1]. This specialized chromatin state is unique to ES cells and is established in part by the activity of ATP-dependent nucleosome remodeling complexes. These complexes alter nucleosome-DNA contacts to affect the orientation of DNA around histone octamers, the position of nucleosomes on the DNA, or to exchange histones [2]. Because packaging of DNA into chromatin inhibits transcription by restricting DNA accessibility, the activity of nucleosome remodelers controls the extent to which specific DNA sites are utilized by TFs and other regulatory proteins.

The Swi2/Snf2 superfamily of ATPases is highly conserved throughout eukaryotes, with roles in gene regulation, DNA repair, and recombination, among others [3]. Swi2/Snf2 family members are modified helicase proteins that utilize the energy of ATP hydrolysis to alter the structure or positions of nucleosomes on DNA [4]. Usually, Swi2/Snf2 ATPases function within multisubunit complexes, in which accessory subunits target the complex to specific regions of chromatin, contribute to DNA or histone binding, or provide other regulatory roles [5,6]. In mammals, there are nearly 30 Swi2/Snf2 family members comprising an even larger number of ATP-dependent nucleosome remodeling complexes, the majority of which have not been thoroughly characterized. Recently, a number of ATP-

dependent nucleosome remodeling complexes have been shown to play important roles in both ES cell self-renewal and differentiation.

ES cell specific BAF complexes promote self-renewal

The BAF (Brg/Brahma associated factor) complexes contain either the Brg1 or Brm ATPases as their catalytic subunit, with different combinations of accessory subunits. Previously, homozygous deletions of genes encoding BAF subunits *Smarca4 (Brg1)*, *Smarcc1 (Baf155)*, and *Smarcb1 (Baf47, Snf5)* were found to be early embryonic lethal in mice, consistent with a possible role for BAF complexes in ES cell self-renewal [7–9]. Indeed, several groups have recently reported that ES cells depleted of several known subunits of BAF complexes have defects in gene regulation, self-renewal, and pluripotency. Homozygous deletion of genes encoding either of two related BAF subunits, *Arid1a* and *Arid1b*, resulted in partial defects in ES cell self-renewal [10,11]. While *Arid1a*$^{-/-}$ ES cells exhibited upregulation of markers of primitive endoderm and failure to differentiate into mesoderm when induced to do so, *Arid1b*$^{-/-}$ ES cells expressed markers of both mesoderm and trophectoderm. These data suggest that differential incorporation of BAF250 isoforms within BAF complexes may alter the spectrum of genes targeted. Similarly, knockdown (KD) of BAF components *Brg1* or *Baf155* in ES cells results in defects in self-renewal and differentiation, underscoring the importance of BAF complexes in ES cells [12,13].

Several BAF complexes appear to be expressed in ES cells, including an ES cell-specific complex called esBAF that consists of a unique subunit composition relative to BAF complexes in other cell types [10,13,14]. The esBAF complex appears to play a direct role in mediating the gene regulatory functions of several core ES cell TFs, since BAF subunits interact directly with TFs Oct4, Sox2, and Nanog and occupy overlapping regions on chromatin [15,16]. In addition, BAF complexes in ES cells bind to many genes encoding core ES cell TFs: *Oct4*, *Sox2*, *Nanog*, *Dppa2*, *Dppa4*, *Sall4*, and *Myc* [16]. For some pluripotency genes, BAF complexes function to tonically silence transcription [16], maintaining their expression at adequate, but not excessive, levels. Conversely, the pluripotency TF Myc functions to silence some BAF subunits expressed in differentiated cells but not ES cells, thereby maintaining the correct composition of ES cell-specific BAF complexes [17].

Chd1 regulates ES cell specific heterochromatin organization

Unlike differentiated cells, ES cells lack significant regions of heterochromatin, the relatively condensed, transcriptionally silent chromatin structure enriched for repressive histone modifications and DNA methylation [1,18]. Recently, the Swi/Snf superfamily ATPase Chd1 was found in an RNAi screen of genes expressed in several types of stem cells to be necessary for ES cell self-renewal and pluripotency [19]. ES cells depleted of Chd1 accumulated large blocks of heterochromatin and exhibited upregulation of genes expressed during neural differentiation. Consistent with these data, Chd1 KD ES cells exhibited a high propensity for neural differentiation, with a concomitant defect in formation of primitive endoderm and cardiac mesoderm.

Chromatin localization of Chd1 mirrors that of RNA Polymerase II and histone H3K4me3, suggesting a role for Chd1 in transcriptional activation [19]. However, transcriptional profiling experiments showed that Chd1 KD ES cells exhibited upregulation of far more genes than downregulation. Interestingly, Chd1 binds the promoter of the *Oct4* gene (*Pou5f1*), and is required for Oct4 expression in ES cells. Conversely, Oct4 binds the regulatory region of *Chd1* gene, suggesting the existence of a regulatory loop.

Chromatin remodelers are required for differentiation

While not required for ES cell self-renewal, two other ATP-dependent nucleosome remodeling factors, Lsh and NURF, appear to be required for proper ES cell differentiation. KD of Lsh, a Swi2/Snf2 superfamily ATPase that functions in retrotransposon silencing, resulted in failure to methylate and silence the promoters of pluripotency TFs during differentiation [20]. NURF is a multisubunit nucleosome remodeling complex involved in gene regulation in metazoans. ES cells lacking NURF subunit Bptf exhibit defects in differentiation of all three germ layers [21].

Two bi-functional chromatin regulatory complexes, the Tip60-p400 and NURD complexes, have also been shown to play important roles in ES cell self-renewal and pluripotency [15,22–26]. The NURD complex exhibits two chromatin regulatory activities: ATP-dependent nucleosome remodeling, catalyzed by the Mi-2 Swi2/Snf2 superfamily ATPase, and histone deacetylase activity, catalyzed by Hdac1 and Hdac2. Homozygous deletion of the non-catalytic NURD subunit Mbd3 allows mutant ES cells to self-renew in the absence of leukemia-inhibitory factor (LIF) [23]. Furthermore, loss of Mbd3 impairs ES cell differentiation in general and alters the spectrum of cell types produced during differentiation [23,25]. Knockout or KD of Mbd3 in ES cells causes misregulation of a number of genes [23,25], including upregulation of genes expressed in trophectoderm (TE) [25]. Consistent with these data, in vitro differentiation of Mbd3−/− ES cells in embryoid bodies (EBs) results in induction of markers of TE [23], a cell type not normally produced during differentiation of ES cells in vitro or in vivo.

In addition, a second form of the NURD complex lacking Mbd3 and Rbbp7, has recently been identified in ES cells [15]. This complex, called NODE (Nanog and Oct4 associated deacetylase), appears to form a high molecular weight complex with both Nanog and Oct4 that functions to repress expression of developmentally regulated genes in ES cells [15]. Similarly, one or both NURD-like complexes has been shown to interact with ES cell TF Sall4 [32]. KD of Mta1, a subunit shared by the NURD and NODE complexes, caused different changes in gene expression than Mbd3 KD or KO, confirming the different functions of these complexes [15]. Unlike Mbd3 loss, which inhibits ES cell differentiation, Mta1 KD resulted in upregulation of differentiation genes of multiple lineages, as well as ES cell differentiation. It remains to be determined how the levels, assembly and activities of the two NURD-like complexes are regulated in ES cells.

Tip60-p400 functions in the same pathway as Nanog to promote self-renewal

Like NURD, Tip60-p400 also exhibits two chromatin regulatory activities. The p400 protein is a Swi2/Snf2 superfamily member that functions in exchange of dimers of histones H2AZ-H2B (or other H2A variants) within nucleosomes [27–30]. Tip60-p400 complex has a second catalytic subunit, Tip60, which functions as a protein acetyltransferase [31]. KD of p400, Tip60, or any other member of the Tip60-p400 complex results in partial differentiation of ES cells, coincident with induction of markers of all three germ layers [26]. In ES cells, p400 was found to bind to the promoters of both highly expressed genes and silent genes, many of which are induced during differentiation. However, upon KD of either Tip60 or p400, many targets of Tip60-p400 that are normally silent in ES cells became upregulated, while expression very few of the highly-expressed targets of the complex were significantly affected [26]. These data were surprising, given the established functions of Tip60 as a histone acetyltransferase and transcriptional co-activator [31].

In addition, Tip60-p400 complex was found to functionally overlap with the core pluripotency TF Nanog. Although Nanog and Tip60-p400 were not found to interact physically, they share a significantly overlapping set of target genes, and have similar affects on gene expression upon KD in ES cells. Epistasis analysis suggests that for some common targets, Nanog and Tip60-p400 complex function in a common pathway to repress differentiation-induced genes in ES cells, although the nature of this pathway remains unclear [26].

One noteworthy feature of ES cell chromatin is that specific regulatory sites, particularly those at lineage specific transcription factor loci, are silenced but remain poised for activation. This specialized chromatin state is promoted by the incorporation of the histone variant, H2AZ, which is incorporated into chromatin by Tip60-p400 [33]. H2AZ is enriched at regions flanking transcriptional start sites, and can promote both transcriptional activation and repression [34]. H2AZ incorporation can influence nucleosome positioning, H1 linker binding, and chromatin remodeling enzyme activity [35]. In ES cells, H2AZ is enriched at silent developmentally regulated promoters, as are repressive TFs and repressive covalent histone modifications [33]. In addition, depletion of H2AZ causes increased expression of H2AZ occupied promoters, indicating that H2AZ contributes to silencing of developmental regulators. In differentiated cells, H2AZ is enriched at active promoters. These data suggest that H2AZ incorporation may be one of several mechanisms that contribute to keeping genes that encode developmental regulators in a silent but poised state in ES cells.

What mechanisms ensure the correct regulation of chromatin remodelers during differentiation?

The studies summarized here show that ATP-dependent nucleosome remodeling enzymes are required for ES cell self-renewal, for pluripotency, and for differentiation into specific lineages. A general theme that emerges from these studies is that the activities of chromatin remodelers are dynamically regulated to maintain pluripotency and that their function can change upon differentiation. In many instances a direct interaction between remodeling complexes and pluripotency TFs in ES cells suggests that the TFs may direct remodelers to their sites of action, which in turn promotes self-renewal. For example, in ES cells, members of the BAF class of Swi/Snf complexes interact with pluripotency TFs to inhibit expression of developmental regulators [15,16]. In contrast, Tip60-p400 lies in the same pathway as Nanog, but does not directly interact with this TF [26]. Upon ES cell differentiation, BAF activity turns off pluripotency specific genes, such as Nanog, and activates expression of developmental regulators. How is BAF function altered from a role in repression of differentiation to that of an activator? BAF subunit composition plays an important role in modulating its function (Figure 1), but the mechanisms that trigger alterations in the make up of BAF complexes have not been defined, nor have the mechanisms by which they alter function. Also, how is the specialized chromatin structure that characterizes distinct ES cell developmental states established, and how does this chromatin structure affect chromatin remodeling at specific loci and on a genome wide basis? Lastly, the mechanisms by which the activities of the different chromatin remodeling enzymes are integrated to regulate chromatin structure in ES cells are not well understood. While some nucleosome remodeling enzymes are likely recruited to specific pluripotency and differentiation genes through interactions with pluripotency TFs, other factors appear to localize more broadly, perhaps via interactions with specific histone modifications. The relative contributions of TF-specific recruitment of nucleosome remodelers to localized regions and widespread binding of nucleosome remodelers to large chromatin domains in ES cells remain unclear. A better understanding of how ATP dependent chromatin remodeling enzymes are targeted and

regulated in ES cells will help clarify the mechanisms by which chromatin is prepared to achieve a developmentally appropriate gene expression program.

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Figure 1.
The composition of the BAF complexes is regulated in a cell type specific fashion. In ES cells, the BAF complex consists of an ES specific combination of subunits and interacts with the pluripotency TFs Oct4 (purple) and Sox2 (green). It remains to be determined whether the BAF complex associates with master regulatory TFs that direct cell type specification in other cell types. BAF subunits that are present in all cell types indicated are coloured gray. Subunits that change between cell types are burgundy, teal and orange.