Leading Edge
Review

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# Chromatin Connections to Pluripotency and Cellular Reprogramming

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The pluripotent state of embryonic stem cells (ESCs) provides a unique perspective on regulatory programs that govern self-renewal and differentiation and somatic cell reprogramming. Here, we review the highly connected protein and transcriptional networks that maintain pluripotency and how they are intertwined with factors that affect chromatin structure and function. The complex interrelationships between pluripotency and chromatin factors are illustrated by X chromosome inactivation, regulatory control by noncoding RNAs, and environmental influences on cell states. Manipulation of cell state through the process of transdifferentiation suggests that environmental cues may direct transcriptional programs as cells enter a transiently "plastic" state during reprogramming.

## Introduction

Embryonic stem cells (ESCs) have attracted special attention by virtue of their unique properties and extraordinary potential in regenerative medicine. ESCs are distinguished by unlimited self-renewal and the capacity to differentiate into any cell type, the hallmarks of pluripotency. The remarkable ease with which somatic cells are converted to an "ESC-like" state (or induced pluripotent stem cells [iPSCs]) by expression of four transcription factors (Oct4, Sox2, Klf4, and c-Myc) or other combinations (Stadtfeld and Hochedlinger, 2010; Takahashi and Yamanaka, 2006) has focused interest on the regulatory mechanisms by which pluripotency is established and maintained. In this Review, we integrate recent findings regarding the connections of a core ESC transcriptional network, chromatin remodeling and modification, and somatic cell reprogramming.

## Unique Chromatin Structure of Pluripotent Cells

Chromatin—chromosomal DNA as packaged with histones—provides the cellular context for gene expression and cell fate determination. Changes in chromatin structure are mediated through chemical modification of histones (e.g., acetylation, methylation, demethylation, and ubiquitination) and DNA methylation, as well as the action of DNA-binding proteins and chromatin-remodeling enzyme complexes. The chromatin of ESCs is "open" (Gaspar-Maia et al., 2011) (Figure 1). At the histological level, stainable, transcriptionally silent constitutive heterochromatin is dispersed. The exchange of both histone and nonhistone proteins, including heterochromatin protein 1 (HP1), linker histone H1°, and core histones H2B and H3 in chromatin is hyperdynamic. Upon differentiation, heterochromatin appears heterogeneous and clustered in distinct blocks, and hyperdynamic chromatin proteins become immobilized. The open nature of ESCs' chromatin is also reflected in global transcriptional hyperactivity (Efroni et al., 2008). Recent findings demonstrating that cells of the day 3.5 mouse blastocyst exhibit a similar open chromatin conformation are reassuring in relating the chromatin state of ESCs to an in vivo context (Ahmed et al., 2010).

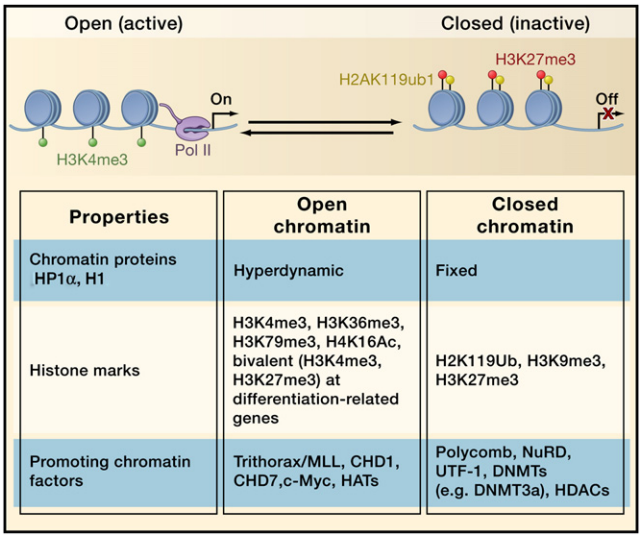
## Networks for Pluripotency

Pluripotency is established through the aegis of transcription factors that operate within highly interconnected protein-protein and protein-DNA networks (Young, 2011). Similarly, these networks are intricately intertwined with chromatin-remodeling and modifying complexes to regulate chromatin organization and gene expression.

### *Core Pluripotency Networks*

The "core" pluripotency factors, such as the transcription factor Oct4, are cell specific in their expression. Sox2 and Nanog constitute additional "elite" factors, though other factors, including Sall4, Rex1, Dax1, and Tcl1, are functionally important. Proteomic studies based on affinity purification of Oct4, Nanog, and Sox2, coupled with purification of associated proteins and microsequencing (Liang et al., 2008; Pardo et al., 2010; Wang et al., 2006), reveal a tight protein-protein interaction network in which core factors are associated with one another in multiprotein complexes and also with many chromatin-associated activities and complexes (see Figure 2). Varying stability and stoichiometry of such complexes provides a means to fine-tune developmental decisions.

Comprehensive chromatin occupancy studies of Oct4, Sox2, and Nanog, taken together with analyses of numerous other transcription factors and chromatin marks, have established




| Properties | Open chromatin | Closed chromatin |
|---|---|---|
| Chromatin proteins HP1α, H1 | Hyperdynamic | Fixed |
| Histone marks | H3K4me3, H3K36me3, H3K79me3, H4K16Ac, bivalent (H3K4me3, H3K27me3) at differentiation-related genes | H2K119Ub, H3K9me3, H3K27me3 |
| Promoting chromatin factors | Trithorax/MLL, CHD1, CHD7,c-Myc, HATs | Polycomb, NuRD, UTF-1, DNMTs (e.g. DNMT3a), HDACs |

**Figure 1. Properties of Open and Closed Chromatin**

At the top, simplified views of open and closed chromatin are depicted. As differentiation proceeds, chromatin becomes closed. Cellular reprogramming reverses the chromatin state. The table summarizes the protein characteristics of open and closed chromatin and the factors that promote each state.

extraordinary complexity in regulatory connections among the core transcription factor network (Boyer et al., 2005; Chen et al., 2008; Kim et al., 2008) (see Figure 2). The pattern of target gene occupancy describes a network with multiple, prominent "hubs" (Kim et al., 2008) defined by highly combinatorial binding of factors, both at promoters and enhancer elements (Chen et al., 2008; Kim et al., 2008). As a class, "common" targets, defined by chromatin occupancy by multiple core factors, tend to be expressed in ESCs and then turned off upon differentiation. These genes are highly enriched for the active chromatin mark H3K4me3 and lose this mark and acquire a repressive mark, such as H3K27me3, upon differentiation.

Among target elements bound by multiple pluripotency factors, the predicted binding motif conforms to an Oct4 or composite Oct4-Sox2 consensus site. This striking finding underscores the centrality of Oct4 and suggests that Oct4 binding recruits other factors to critical regions and promotes assembly of multiprotein factor complexes. Oct4 dependence of chromatin structure surrounding the Nanog locus in ESCs is consistent with this view (Levasseur et al., 2008).

### *Contributions of the Myc Network*

The pervasive transcription factor c-Myc, a member of the initial iPSC reprogramming cocktail (Takahashi and Yamanaka, 2006), is a major regulator of cell proliferation and largely associated with active transcription and open chromatin. Recent findings suggest that Myc factors prevent lineage-specific differentiation, in part through direct repression of GATA6 expression (Smith et al., 2010; Varlakhanova et al., 2010). c-Myc also contributes importantly to the control of proliferation through regulation of miRNAs (Lin et al., 2009a).

c-Myc protein recruits multiple activities implicated in chromatin modification or structure, including histone acetyltransferases (GCN5, p300), chromatin-remodeling complexes, histone deacetylases (HDACs), and histone demethylases (Lin et al., 2009b). Consistent with these interactions, induction of c-Myc expression increases histone acetylation and methylation, including H3K4me3 deposition. In ESCs, c-Myc binds ~3000 promoters (Kim et al., 2008, 2010; Lin et al., 2009a), including some pluripotency factors (e.g., Sox2) and numerous chromatin-associated or modifier gene targets. Distinct protein-protein and transcriptional networks associated with c-Myc are separable from the core networks (Kim et al., 2010) (see Figure 2). During iPSC generation, targets of the c-Myc network are activated prior to expression of the core factors (Stadtfeld and Hochedlinger, 2010). These findings implicate targets of the Myc network in an early phase of reprogramming, possibly through facilitating chromatin accessibility. The c-Myc module is highly represented in ESC-associated signatures that have been widely used in assessing the relatedness of cancer and embryonic cells (Kim et al., 2010).

In regard to the potential roles of c-Myc in influencing chromatin structure, the identification of the Tip60-p400 complex (also known as NuA4 HAT) as a c-Myc associated complex (Kim et al., 2010) is notable. Tip60-p400 was also identified as essential for maintaining the ESC state (Fazzio et al., 2008). The multisubunit Tip60-p400 complex has two chromatin regulatory activities. Tip60 serves as a protein acetyltransferase. p400, a member of the Swi2/Snf2 family, functions in exchange of histones H2AZ-H2B within nucleosomes. RNAi inhibition of components of the Tip60-p400 complex leads to differentiation of ESCs (Fazzio et al., 2008). Based on gene expression profiling, it has been suggested that Tip60-p400 and Nanog lie within a common pathway, as Nanog depletion leads to less p400 binding at targets (Fazzio et al., 2008). Alternatively, common gene target analysis places Tip60-p400 targets closer to a c-Myc-regulated network, consistent with proteomic findings (Kim et al., 2010).

Although overexpression of c-Myc has been described as dispensable for somatic cell reprogramming (Nakagawa et al., 2010; Wernig et al., 2008), it is likely that endogenous Myc activity is necessary, given numerous connections to chromatin activities and the core network.

### *Links between Core Pluripotency and Chromatin Factor Complexes*

Multiple mechanisms and levels of control promote globally "open" chromatin in ESCs while simultaneously allowing for repression of differentiation-related genes. This balance reflects interplay between the critical regulators that are essential for pluripotency and chromatin-remodeling and modification complexes. The core pluripotency and Myc networks are highly interconnected to these chromatin complexes through protein-protein and target gene interactions (Figure 2). It is not well understood how interactions between core pluripotency factors and chromatin-associated proteins are mediated and to what extent they are direct or indirect. In principle, the interactions provide a means for recruiting chromatin factors to target genes bound by the transcription factors. Alternatively, pre-bound chromatin complexes may establish a suitable chromatin "milieu" and facilitate the assembly of transcription factors at their sites of action. Here, we discuss some examples of these connections.

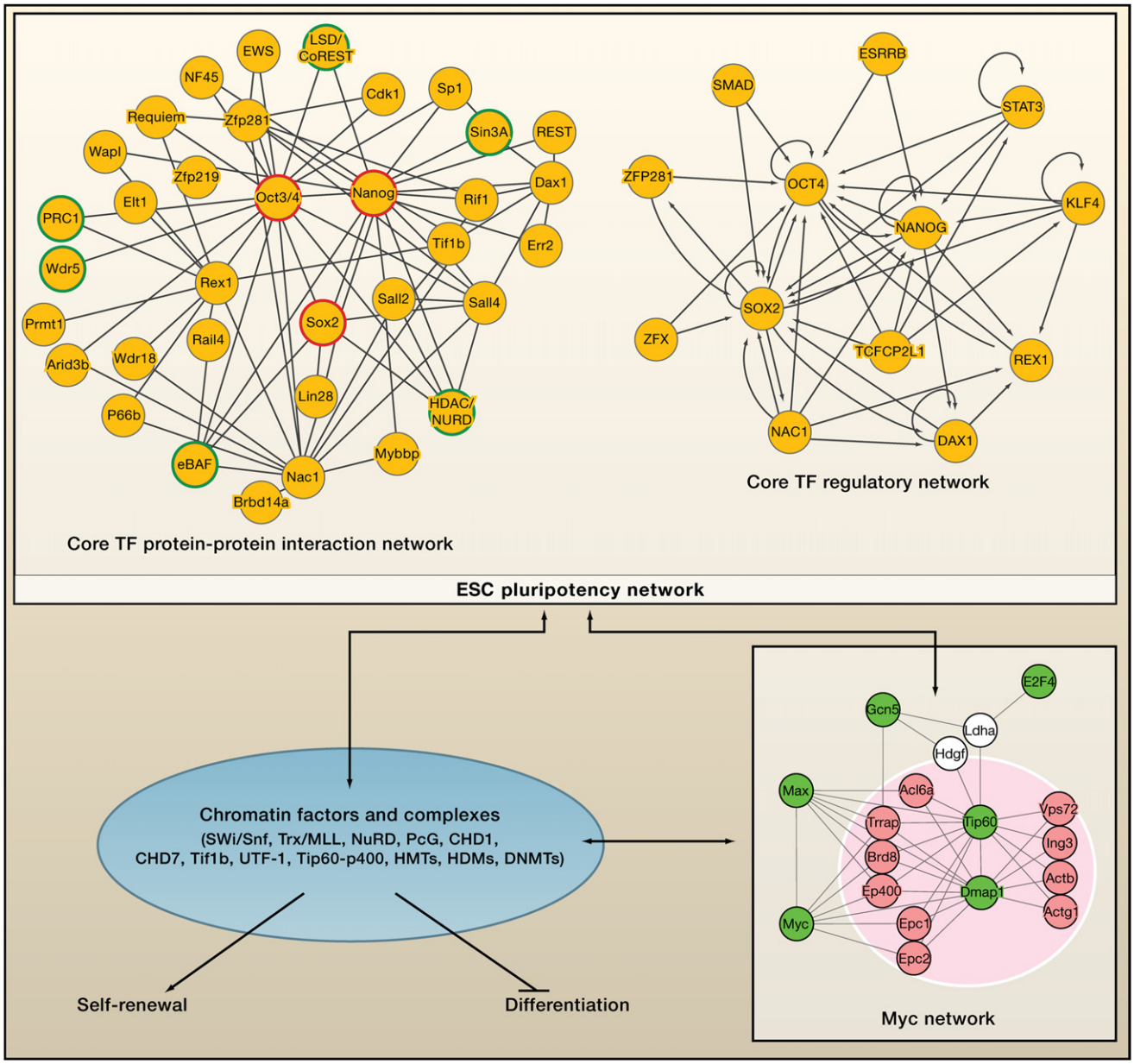



**Figure 2. Networks and Their Interconnections in ESCs**

Protein-protein interactions derived from microsequencing of protein complexes purified from ESCs are shown on the upper left. The network is a consensus view of proteins from Mallanna et al., 2010; Pardo et al., 2010; van den Berg et al., 2010; and Wang et al., 2006. The triad of core pluripotency factors, Oct4, Nanog, and Sox2, are circled in red. Components of chromatin-remodeling or modifying complexes are highlighted in green circles. In the upper right, the transcriptional regulatory network as established through ChIp-ChiP and ChIP-seq studies is summarized (Boyer et al., 2005; Kim et al., 2008; Loh et al., 2006). The Myc network to the lower right refers to the protein-protein and protein-DNA networks reported in Kim et al., 2010. The factors in the core and *c-myc* regulatory networks cross-regulate each other and regulate, and are regulated by, chromatin factor components illustrated in the lower left. The output of these complex regulatory interactions is maintenance of self-renewal and blocking of lineage-specific differentiation.

Oct4-, Nanog-, and Sox2-associated proteins include components of the Swi/Snf (or Brg/Brahma-associated factors [BAF]) complex, a molecular machine that moves nucleosomes. Swi/Snf complexes are found in all cells, but the precise composition varies based on inclusion of alternative subunits (Lessard and Crabtree, 2010). The combinatorial assembly of Swi/Snf complexes underlies developmental stage-specific epigenetic control. Within ESCs, the complex is characterized by the presence of the core subunit Brg1, BAF155, and BAF60A. Overexpression of Swi/Snf components has been reported to enhance reprogramming by Oct4, Sox2, and Klf4 (Singhal et al., 2010).

The NuRD complex associates with Oct4 and Nanog, as well as other critical pluripotency factors, such as Sall4. Moreover, HDACs associate with core factors as part of HDAC/Sin3a or

HDAC/CoREST/LSD1 complexes. Loss of MBD3, a core component of NuRD, undermines pluripotency of ESCs in part through a failure to block trophectoderm differentiation (Kaji et al., 2007; Zhu et al., 2009). Of note, Sall4, which is critical for pluripotency of ESCs and in extra-embryonic endoderm stem cells (Lim et al., 2008), contains a conserved peptide region that mediates interaction with RbAp48, a histone-binding NuRD core subunit shared with HDAC/Sin3a and polycomb complex 2 (PRC2) (Lejon et al., 2011; Kidder et al., 2009; Lauberth and Rauchman, 2006). It is provocative that loss of Lin-53, a RbAp48 homolog in *C. elegans*, removes the barrier to direct reprogramming of germ cells into neurons by the transcription factor Che-1 (Tursun et al., 2011).

Transcription intermediary factor-1b (TIF1b, or TRIM28 and KAP1), a scaffold protein that recruits chromatin complexes, interacts with several proteins within the pluripotency network, including Oct4 and Nanog (Seki et al., 2010; Wang et al., 2006). Previously, TIF1b was linked to silencing and formation of heterochromatin through interaction with HP1, the histone methyltransferase SETDB1, and NuRD. Nonetheless, TIF1b was identified through a genome-wide siRNA screen for factors that are required to sustain Oct4-driven GFP expression (Hu et al., 2009). A phosphorylated form of TIF1b interacts with the ESC-specific form of the Swi/Snf complex, localizes to euchromatin, and modulates iPSC generation (Seki et al., 2010). In part, this may involve recruitment of Oct4 to phosphorylated TIF1b at target genes, such as Nanog.

Evidence also suggests that binding of chromatin factors to gene regulatory elements of the pluripotency factors provides a means for crosstalk. For example, Swi/Snf complexes occupy the Oct4, Sox2, Nanog, Sall4, and c-Myc genes, among many others (Lessard and Crabtree, 2010). Indeed, ~60%–70% of the target genes of Oct4, Nanog, or Sox2 are bound by Brg1. The consequences of Brg1 binding to target genes in ESCs appear to be complex. It has been proposed that ESC-specific genes, such as Nanog and Oct4, are "tonically" repressed by Brg1 in order to maintain expression within optimal limits. In addition, Swi/Snf appears important for repression of pluripotency genes on differentiation, as well as for facilitating chromatin compaction (Schaniel et al., 2009). As Swi/Snf complexes promote or repress gene expression, further work is needed to clarify how their diverse actions contribute to pluripotency and the exit to differentiation.

The pluripotency regulatory network is also directly linked to the control of histone-modifying proteins/complexes, as illustrated by the Jumonji (Jmj) family H3K9 demethylases Jmjd1a/KDM2A and Jmjd2c/KDM4B, which act on H3K9me2 and H3K9me3, respectively (Loh et al., 2007). Both genes lie downstream of Oct4 and are regulated positively through its action. Nonetheless, depletion of either factor in ESCs leads to differentiation, though with differing phenotypes. KDM2A and KDM4B appear to act on the Tcl1 and Nanog genes, respectively (Loh et al., 2007). Thus, Oct4 directly controls epigenetic regulators that act on target genes that are essential for pluripotency. Recently, KDM5B/Jarid1B, a H3K4me3 demethylase that is primarily targeted to intragenic regions and recruited to H3K36me3 via interaction with a chromodomain protein MRG15, has been proposed to activate self-renewal-associated genes by repressing cryptic initiation and maintaining an H3K4me3 gradient to support transcriptional elongation (Xie et al., 2011).

## Regulation of ESC Chromatin Structure by Opposing Systems

The interplay between self-renewal and differentiation in ESCs is reflected by the levels of the active mark H3K4me3 and the repressive mark H3K27me3 at target genes and more globally. The complexity of pathways operating to modulate these histone modifications and global chromatin architecture is only now becoming apparent. Whereas ESCs favor a transcriptionally "permissive" state, potent repressor pathways are critical for repressing expression of differentiation-promoting genes and for sequencing exit from pluripotency.

### *Polycomb as a Repressive System*

As a major repressive system in development, polycomb group (PcG) proteins have received attention as silencers of differentiation pathways in pluripotent cells. PcG proteins act in two different multiprotein complexes known as PRC1 and PRC2 (Margueron and Reinberg, 2011). Four core proteins, EED, Suz12, Ezh2, and RbAp46/48, comprise PRC2. PRC1 is more diverse, as it is composed of core subunits Ring1A and 1B with a variety of other proteins. PRC1 catalyzes monoubiquitination of histone H2A at lysine 119. Through the SET domain of Ezh2 or the related protein Ezh1 (Margueron et al., 2008; Shen et al., 2008), PRC2 catalyzes di- and trimethylation of histone H3 lysine 27. H3K27me3 also binds to EED and stimulates activity of the complex (Xu et al., 2010). This repressive mark has been proposed to serve as a docking site for PRC1, though this may not be the sole mechanism of recruitment. Domains marked by H3K27me3 may be quite large (>100 kb) or on the scale of a few kilobases.

Chromatin occupancy studies reveal that PRC2 and PRC1 components bind numerous differentiation-related genes that are silent but "poised" for expression in ESCs (Boyer et al., 2006; Lee et al., 2006; Young, 2011). These targets display a "bivalent" chromatin mark, defined by the presence of active H3K4me3 and repressive H3K27me3 marks (Bernstein et al., 2006). Upon ESC differentiation, PcG-bound targets are expressed in concert with loss of H3K27me3. In the simplest interpretation, polycomb-mediated repression is essential for maintenance of pluripotency. This conclusion is inconsistent with the capacity of EED null ESCs to give rise to all three germ layers (Chamberlain et al., 2008). Subsequent studies reveal added complexity, particularly with respect to PRC2, and provide a more nuanced view of the role of PcG in pluripotency (Shen et al., 2008, 2009).

An unanticipated finding in proteomic studies was identification of Jarid2 (or Jmj), the founding member of the Jmj family of proteins, as a tightly associated component of PRC2 purified from ESCs and required for proper ESC differentiation (Li et al., 2010a; Pasini et al., 2010; Peng et al., 2009; Shen et al., 2009). The Jmj family is comprised of lysine demethylases, such as aforementioned Oct4-regulated Jmjd1a and Jmjd2c. In mice, Jarid2 is essential for development of the neural tube and the heart, though precise mutant phenotypes are highly sensitive to genetic background. In genome-wide chromatin occupancy

studies in ESCs, Jarid2 binding extensively (>90%) overlaps that of other PRC2 components and H3K27me3. Jarid2 appears to facilitate recruitment of the PRC2 complex to chromatin, possibly through its affinity for GC-rich DNA (Li et al., 2010a). Paradoxically, Jarid2 is enzymatically inactive, as it lacks conserved residues for cofactor binding, and H3K27me3 is not as affected upon its loss as predicted by its role in recruitment to chromatin (Pasini et al., 2010; Peng et al., 2009; Shen et al., 2009). The precise contribution of Jarid2 to the enzymatic activity of the complex is controversial. A possible role of Jarid2 in recruiting PRC1 and poised RNA polymerase II to PcG targets has been suggested (Landeira et al., 2010). Jarid2 is a common target of multiple pluripotency factors and is rapidly downregulated on differentiation. Thus, the subunit composition of PRC2 during differentiation is dynamic.

PcG function is critical to the balance of ESC self-renewal and differentiation, particularly in sequencing transcriptional events that are necessary to exit the pluripotent state and culminate in successful lineage specification (Shen et al., 2009; Shen et al., 2008). Additionally, potential regulatory interactions may exist between Swi/Snf complexes and PcG. Chromatin occupancy of genes encoding various PcG components has been interpreted as consistent with opposition of Swi/Snf and polycomb function (Lessard and Crabtree, 2010). Recent genetic findings also point to an antagonistic relationship between PcG and Swi/Snf function in control of specific genes and in oncogenesis (Wilson et al., 2010).

How the composition and modification of PcG complexes change during differentiation is likely to provide new insights into cell fate transitions (Margueron and Reinberg, 2011). For example, Ezh2 is a substrate for various kinases, including Akt, CDK1, and CDK2. Phosphorylation of Ezh2 has different reported consequences depending on the residue that is modified. Effects on recruitment to chromatin, H3K27 methylation activity, binding to the long noncoding RNA HOTAIR, and differentiation have been described (Kaneko et al., 2010; Tsai et al., 2010).

Although it is often presumed that the histone-modifying activities of PRC2 and PRC1 are synonymous with repressive function, the situation is not so straightforward. PRC1 compacts chromatin structure and represses Hox gene expression independently of histone ubiquitination (Eskeland et al., 2010; Francis et al., 2004). In the absence of the core Ring1B subunit of PRC1, Hox genes are modestly derepressed and chromatin decompaction occurs. Moreover, PRC1 may prevent expression at bivalent genes in part through impaired transcription elongation, perhaps countering the actions of c-Myc in promoting expression. To add to this complexity, PcG complexes may act redundantly in repression, independent of H3K27me3 (Leeb et al., 2010). Moreover, Sox2 overexpression mitigates the defects caused by EED loss by promoting histone acetylation and without restoring histone methylation (Ura et al., 2011). Hence, much remains to be explored regarding the mechanisms by which PcG complexes repress gene expression and influence chromatin structure.

### *Trithorax as an Agent of Active Gene Expression and Self-Renewal*

Classical studies in *Drosophila* revealed functional antagonism between polycomb and Trithorax (Trx) with respect to Hox gene expression. Whereas polycomb is associated with the repressive H3K27me3 mark, Trx complexes write the active H3K4me3 mark. Mammalian Trx, a homolog of yeast COMPASS, contains a histone methyltransferase (Set1a/b, MLL1–4) and a subunit that recognizes H3K4me3 (Wdr5), as well as other components (Ash2, RBbp5, Dpy-30, etc.). Recently, Trx has been linked to the pluripotency network through study of Dpy-30 (Jiang et al., 2011) and Wdr5 in ESCs (Ang et al., 2011). Protein complexes containing Oct4 interact directly or indirectly with Wdr5 and recruit it to target genes, many of which are also bound by Nanog and Sox2. Furthermore, Wdr5 is required to maintain local and global H3K4me3 and sustain self-renewal. Whereas substantial target gene overlap is seen between Oct4 and Wdr5, an equivalent or higher degree is seen with c-Myc targets. Thus, Wdr5 (and hence, Trx) may link the core and c-Myc regulatory networks. In this context, it is of interest that c-Myc may interact with MLL complexes and therefore either recruit MLL complexes to specific targets or stabilize MLL complexes at their targets.

### *Contribution of CpG-Binding Proteins to Local Chromatin Structure*

CpG islands (CGIs) are prominent in mammalian genomes. Commonly, promoters are embedded within CGIs that lack DNA methylation and are marked by H3K4me3. Recent studies suggest that CGIs influence local chromatin structure through the recruitment of CpG-binding proteins, such as Cfp1 (Thomson et al., 2010). Histone modification is directed by the presence of CGIs in the absence of a promoter and, hence, influenced by the genetic "environment." These findings are consistent with the association of Cfp1 with the Setd1 histone H3K4 methyltransferase/COMPASS complex (Lee and Skalnik, 2005). Cfp1 null ESCs exhibit various defects, including a decrease in global cytosine DNA methylation, reduced levels of heterochromatin, and reduced H3K4me3 mark at CGIs (Tate et al., 2009), and are unable to differentiate in vitro, a phenotype that is reminiscent of loss of the MBD3 subunit of NuRD. CGIs may further sculpt local chromatin structure through recruitment of histone demethylases. For example, CGIs recruit the H3K36-specific demethylases KDM2A (jhdm1a, FbxL11), leading to depletion of H3K36me2 (Blackledge et al., 2010). Recruitment is blocked by CpG DNA methylation. Although H3K36 methylation is enriched on active genes and appears antagonistic to PRC2 (Yuan et al., 2011), experiments have been unsuccessful to date in defining the consequences of KDM2A loss in ESCs (Blackledge et al., 2010). Recent findings indicate that the 5-methylcytosine hydroxylase TET1, which localizes to transcriptional start sites, may oppose aberrant DNA methylation at CpG-rich promoters (Williams et al., 2011a).

### *Global Chromatin Regulators*

Through a focused RNAi screen in ESCs, the chromatin-remodeling enzyme, Chd1, was shown to be essential for pluripotency and sustained Oct4 expression (Gaspar-Maia et al., 2009). Chd1 is a member of the ATPase SNF2-helicase family, recognizes H3K4me2/3 through its chromodomains, localizes to active genes in euchromatin, and is associated with transcriptional activation. The Chd1 locus appears to be a target of the pluripotency network. Depletion of Chd1 in ESCs is associated with an increase in foci of heterochromatin marks, such as H3K9me3 and HP1, as well as reduced exchange of linker histone H1.

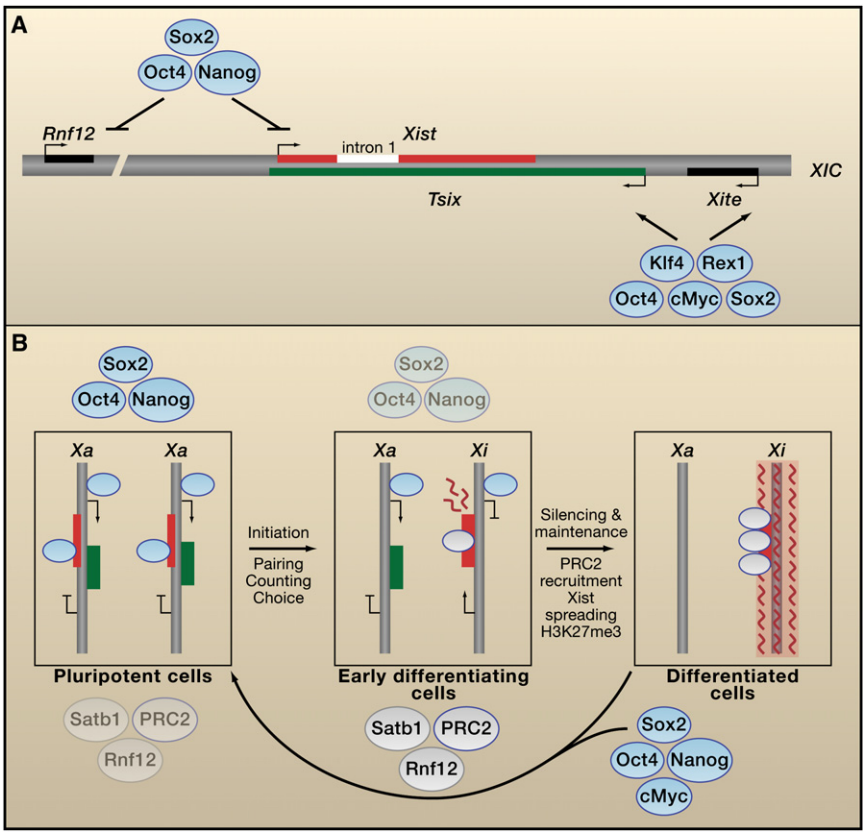



**Figure 3. Pluripotency Factors and X Chromosome Inactivation**

(A) Schematic depiction of X inactivation center (XIC) on the X chromosome with positions of selected noncoding RNAs and the Xist activator Rnf12 as indicated. In undifferentiated female ESCs, *Xist* (intron 1) and possibly Rnf12 are occupied and transcriptionally suppressed by Oct4, Sox2, and Nanog, whereas *Xite* and *Tsix* control regions are bound and transcriptionally activated by Oct4, Sox2, Klf4, Rex1, and cMyc.

(B) In female ESCs, *Xist* is silenced, while *Tsix* is activated by the pluripotency factors shown in (A). Upon differentiation, X chromosome inactivation ensues through a multistep process that involves initiation, silencing, and maintenance of the silenced X. The initiation and onset of silencing are tightly linked with the downregulation of pluripotency factors and the concomitant upregulation of chromatin regulators that mediate XCI, such as Satb1 and PRC2. Introduction of Oct4, Sox2, Klf4, and cMyc into differentiated cells gives rise to induced pluripotent stem cells, which is accompanied by X chromosome reactivation in mouse.

Chd1-depleted ESCs retain features of pluripotent cells but tend to differentiate along the neuronal lineage. Despite the association of Chd1 with ~30% of genes marked by H3K4me3, gene expression changes in its absence are paradoxically limited. Chd1 is presumably one of a larger class of factors that contribute to proper maintenance of open chromatin structure in pluripotent cells.

Recent findings suggest that undifferentiated embryonic cell transcription factor 1 (UTF1) may counterbalance effects of proteins such as Chd1 on overall chromatin structure. UTF-1 is a tightly chromatin-associated protein that occupies >1700 target genes, including many that overlap with pluripotency factors and c-Myc (Kooistra et al., 2010). UTF1-depleted ESCs continue to self-renew but are defective in differentiation. Upon UTF1 depletion, expression of numerous genes is altered, but notably, ~90% are upregulated, a finding that is consistent with the prior assignment of UTF1 as a repressor. UTF1 depletion is also associated with increased release of nucleosomes from chromatin on micrococcal nuclease treatment. These observations implicate UTF1 in preventing chromatin decondensation and possibly limiting transcriptional promiscuity in the setting of the open chromatin state of ESCs.

## X Inactivation as a Model to Study Coupling of Pluripotency Factors and Chromatin Structure

The intricate relationship of pluripotency and epigenetic programs is highlighted by X chromosome inactivation (XCI), a mechanism in placental mammals that ensures proper gene dosage of X-linked genes in females compared with males by randomly inactivating one of the two X chromosomes in female cells (Navarro and Avner, 2009). Here, we review recent insights into the role that pluripotency factors play in regulating long noncoding (lnc)RNAs and heterochromatin formation during XCI.

### *Pluripotency Factors and XCI Reversal*

Though much has been learned regarding the earliest steps of XCI during female ESC differentiation (Figure 3), the mechanism by which the paternally silenced X becomes reactivated specifically in the pluripotent inner cell mass (ICM) remains elusive. Recent evidence documents a direct role for several pluripotency factors during the reversion of XCI in the ICM. Oct4, Sox2, and Nanog bind to intron 1 of the lncRNA critical for XCI, *Xist*, in undifferentiated female ESCs to suppress its expression (Navarro et al., 2008), whereas Oct4, Sox2, Rex1, c-Myc, and Klf4 associate with the control region of the antagonizing lncRNA *Tsix* to stimulate expression (Donohoe et al., 2009; Navarro et al., 2008) (Figure 3A). Consistent with this finding, reactivation of XCI in the ICM strictly correlates with Nanog expression, and *Nanog*-deficient blastocysts fail to undergo XCI reprogramming (Silva et al., 2009). Interestingly, transcriptional reactivation of the silenced (paternal) X in the ICM was suggested to occur prior to loss of *Xist* coating and the ensuing H3K27 trimethylation of the Xi, indicating that another *Xist*-independent mechanism may operate during XCI reprogramming (Williams et al., 2011b).

Recently, Gribnau and colleagues made the unexpected observation that deletion of intron 1 of *Xist*, encompassing all known pluripotency binding sites, is insufficient to activate Xist expression in ESCs (Barakat et al., 2011). This suggests that other targets of Oct4, Nanog, and Sox2 may exist that control

*Xist* transcription. A candidate factor is the X-linked ubiquitin ligase Rnf12 (Jonkers et al., 2009), which functions as a dose-dependent activator of *Xist* transcription. Indeed, the *Rnf12* gene promoter, like *Xist* intron 1, is occupied and suppressed by pluripotency factors in undifferentiated ESCs (Navarro et al., 2011), and *Rnf12* deletion in female ESCs abrogates XCI, although different views exist regarding Rnf12's precise role in this context (Barakat et al., 2011; Shin et al., 2010).

Pluripotency factors may play additional roles during XCI by influencing the processes of X chromosome "pairing" and "counting" (Donohoe et al., 2009). "Pairing" denotes the physical association of both Xs to establish asymmetries between the future Xa and Xi and provides the basis for "counting" to ensure that only cells with two Xs undergo XCI. Specifically, Oct4 protein was shown to associate with Ctcf, a chromatin insulator protein that is involved in X chromosome pairing. Accordingly, pairing and counting were abrogated in *Oct4*-deficient cells, resulting in two Xi.

Given that exit from pluripotency correlates with the onset of XCI, it is conceivable that transcriptional repressors of pluripotency genes may also directly influence XCI. Indeed, the chromatin organizer and transcription factor Satb1, which physically associates with and inhibits the *Nanog* and *Klf4* promoters in differentiating ESCs (Savarese et al., 2009), functions as a "competence factor" for XCI during early embryonic differentiation (Agrelo et al., 2009), possibly by reorganizing chromatin structure during XCI initiation or by regulating *Xist*/*Tsix* expression. Together, these results demonstrate a direct role for pluripotent transcription factors and their repressors at multiple steps of XCI.

### *Cellular Reprogramming and X Chromosome Reactivation*

The reactivation of the somatically silenced X upon overexpression of Oct4, Sox2, c-Myc, and Klf4 in generation of iPSC from female fibroblasts lends support for direct involvement of pluripotency factors in XCI reversal and chromatin remodeling (Maherali et al., 2007) (Figure 3B). Differentiation of these iPSCs results in random XCI, indicating that introduction of reprogramming factors is sufficient to trigger a process that eventually erases the epigenetic imprint of the previously inactive X chromosome. Of note, pluripotent epiblast stem cells (EpiSCs), which are derived from postimplantation embryos (Brons et al., 2007; Tesar et al., 2007), express Oct4 and Sox2 at comparable levels as ESCs but nevertheless exhibit XCI (Guo et al., 2009), suggesting that these factors are insufficient to reprogram the silenced X. In contrast, overexpression of Klf4 or Nanog, which are downregulated in EpiSCs relative to ESCs, facilitates the conversion of EpiSCs into ESC-like cells as well as XCI reactivation (Guo et al., 2009; Silva et al., 2009). It should be interesting to test whether Rex1, which is also overexpressed in ESCs compared with EpiSCs, plays a similar role in XCI reactivation.

It remains unclear whether the same coupling of XCI and pluripotency factors applies to human ESCs. Human ESCs resemble mouse EpiSCs more than mouse ESCs and invariably exhibit signs of XCI (Hoffman et al., 2005; Silva et al., 2008b). Consistent with this, human iPSCs seem to retain the inactive X chromosome of their somatic donor cell (Tchieu et al., 2010). However, forced expression of OCT4, KLF2, and KLF4 endows human ESCs with a mouse-like state that displays two Xa, providing evidence that the function of pluripotency factors in XCI reprogramming may be conserved (Hanna et al., 2010).

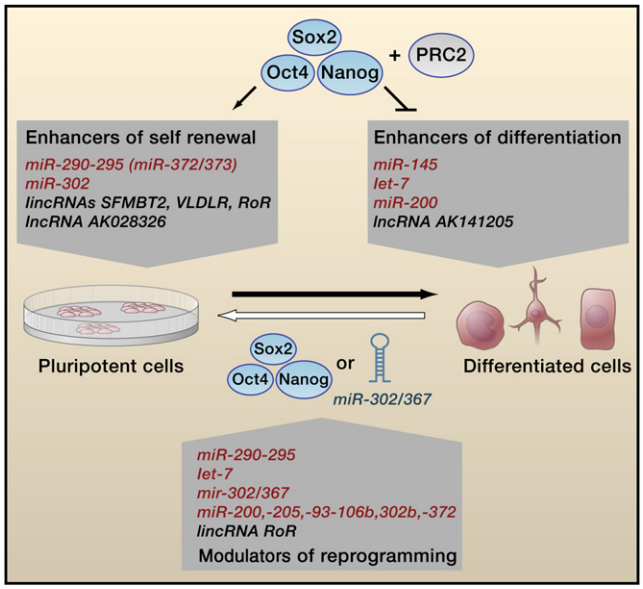



**Figure 4. Noncoding RNAs Modulate ESC Self-Renewal, Differentiation, and Cellular Reprogramming**

Shown are examples of microRNAs (in red) and lncRNAs (in black) that are occupied and either activated by Oct4, Sox2, and Nanog or silenced by the same factors in combination with PRC2 in pluripotent cells, as well as their roles in self-renewal and differentiation. Manipulation of several noncoding RNAs in the context of iPSC formation has been shown to enhance cellular reprogramming. Note that some miRNAs, such as members of the miR-200 family, may directly target PRC1 and PRC2 components, such as Bmi-1 and Suz12, respectively. Expression of the miR-302/367 cluster has been suggested to be sufficient for iPSC formation.

## Role of Noncoding RNAs in Regulating Chromatin State and Pluripotency

Accumulating evidence suggests that the principles of RNA-mediated gene control during XCI apply to several other loci and cellular processes. Though there are several species of noncoding (nc) with different functions in mammalian cells (Pauli et al., 2011), we focus here only on miRNAs and lncRNAs because they are implicated in pluripotency, chromatin structure, and reprogramming (Figure 4).

### *Role of miRNAs in Pluripotency and Reprogramming*

miRNAs are short ncRNAs that inhibit gene expression mostly by destabilizing and repressing target RNAs. Their biogenesis depends on the RNA-processing enzymes Dicer, Drosha, and its essential cofactor Dgcr8. Deletion of either enzyme in mouse ESCs results in severe growth and differentiation defects, indicating roles in self-renewal and pluripotency (Pauli et al., 2011). In an elegant complementation approach, Blelloch and colleagues found that the ESC-specific miR-290-295 microRNA family members rescue the proliferative defect of *Dgcr8* knockout ESCs by inhibiting suppressors of G1-S progression, such as Lats2, p21, and Rbl2 (Wang et al., 2008). Genome-wide binding studies suggest that pluripotency factors, including

Oct4, Sox2, Nanog, and Tcf3, occupy the promoters of these as well as other miRNA genes in ESCs (Marson et al., 2008). For example, many differentiation-associated miRNA gene loci, such as let-7 and miR-145, are bound by the same pluripotency factors in combination with components of the PRC2 complex in ESCs, resulting in their transcriptional suppression. The dual role that pluripotency factors play in binding to active and repressed miRNA genes in ESCs is akin to that seen for protein-coding genes that are involved in self-renewal and differentiation (Boyer et al., 2006; Lee et al., 2006). Intriguingly, let-7 targets the pluripotency factors Lin28 and Sall4, whereas miR-145 targets OCT4, SOX2, and KLF4 transcripts for degradation, establishing negative feedback loops that are typical of many miRNAs and ensure rapid suppression of the self-renewal program upon initiation of differentiation (Melton et al., 2010; Xu et al., 2009b).

Consistent with their role in regulating ESC self-renewal, modulation of miRNAs affects the reprogramming of somatic cells into iPSCs. For example, ectopic expression of ESC-specific miRNAs from the miR-290-295 cluster or its human ortholog, hsa-miR-372/373, in fibroblasts enhances the formation of iPSCs in a c-Myc-dependent fashion (Judson et al., 2009; Subramanyam et al., 2011). In agreement with let-7's inhibitory effect on ESC self-renewal, its suppression also promotes the derivation of iPSCs (Melton et al., 2010). The finding that ectopic expression of LIN28, which is critical for the biogenesis of let-7, is sufficient to reprogram somatic cells in combination with OCT4, SOX2, and NANOG (Yu et al., 2007) is in further accordance with this result. Overexpression of another group of miRNAs, which has previously been shown to promote a mesenchymal-to-epithelial transition, also enhances the formation of iPSCs from mouse fibroblasts (Li et al., 2010b; Samavarchi-Tehrani et al., 2010) (Figure 4). Together, these studies document that different microRNAs influence diverse cellular processes to influence somatic cell reprogramming into iPSCs.

Two recent provocative studies report that enforced expression of the *miR-302/367* gene cluster alone is sufficient to induce pluripotency in fibroblasts, possibly by targeting the epigenetic regulators AOF1, AOF2 (LSD1, KDM1A), MECP1-p66, and MECP2 (Anokye-Danso et al., 2011; Lin et al., 2011). These surprising observations imply that the derepression of one or several (chromatin) suppressors of pluripotency genes can be as potent as enforced expression of the respective pluripotency genes. These results may further explain how lentiviral infection of human fibroblasts alone gives rise to pluripotent cells at extremely low efficiency (Kane et al., 2010). The authors of that study found that insertional mutagenesis near the *DICER* locus resulted in the dysregulation of hundreds of miRNAs. It will now be important to identify the critical targets of *mir-302/367* in order to test whether their downregulation also suffices to induce pluripotency.

### *Role of lncRNAs in Chromatin Structure, Pluripotency, and Reprogramming*

In addition to the association of lncRNAs with XCI, Hox gene regulation, and genomic imprinting (Nagano and Fraser, 2011), recent studies in mouse and human ESCs have identified more than 900 so-called long intergenic nc (linc)RNAs, which are implicated in the control of ESC self-renewal and pluripotency (Guttman et al., 2009, 2010; Khalil et al., 2009). Of these, one-third appear to be bound by Oct4 and Nanog in their promoter regions, directly linking their transcription with the core pluripotency network.

Though the roles of lncRNAs in maintaining pluripotency are still poorly understood (see below), increasing evidence suggests that lncRNAs that are involved in XCI, genomic imprinting, and Hox gene regulation associate with components of activating or repressive histone-modifying complexes, such as PRC2, G9a, LSD1, CoREST, SMCX, and WDR5/MLL, leading to transcriptional activation (Ørom et al., 2010; Wang et al., 2011) or silencing of target genes in *cis* or *trans* (Khalil and Rinn, 2011; Nagano and Fraser, 2011).

Gain- and loss-of-function experiments with a few ESC-specific lincRNAs suggest involvement in cellular differentiation, proliferation, and reprogramming. For example, Rinn, Daley, and colleagues have recently identified 10 lincRNAs that are upregulated in human iPSCs compared with ESCs, indicating a possible role in cellular reprogramming (Loewer et al., 2010). Knockdown and overexpression of one of these lincRNAs, lincRNA-RoR, reduced and slightly enhanced, respectively, the formation of iPSCs from human fibroblasts, providing the first evidence for the involvement of a lincRNA in cellular reprogramming. Another study reported two lncRNAs whose regulatory sequences are bound by Oct4 and Nanog, respectively, and that appear to control the self-renewal and differentiation potentials of mouse ESCs (Sheik Mohamed et al., 2010).

The lncRNA *Gtl2*, which is part of the ~1 Mb long *Dlk1-Dio3* imprinted locus, is aberrantly silenced by DNA hypermethylation and histone hypoacetylation during cellular reprogramming into iPSCs (Liu et al., 2010; Stadtfeld et al., 2010). The silenced status of this cluster in iPSCs tightly correlates with the developmental failure of these iPSCs to contribute efficiently to tissues in mice and entire animals. *Gtl2* is a maternally expressed gene that is thought to negatively regulate the paternally expressed *Dlk1* gene, involved in fetal growth, within the same gene cluster. Although the precise mechanism underlying aberrant silencing remains unclear, the observation that binding sites for Oct4 and Nanog have been identified in the upstream region of the *Gtl2* locus suggests that ectopically expressed pluripotency factors may recruit chromatin factors to the cluster that mediate epigenetic silencing (Navarro et al., 2010).

It should be noted that other ncRNAs have recently been identified in ESCs based on their interaction with PRC2 (Kanhere et al., 2010; Zhao et al., 2010). However, their role in regulating chromatin structure and gene expression in pluripotent cells remains unclear.

## Environmental Influences on Chromatin Structure and Cellular State

Here, we discuss recent findings interrogating the effects of environmental factors on the chromatin and developmental states of pluripotent cells (Figure 5). In brief, mouse ESCs can be maintained in a self-renewing pluripotent state in the presence of Lif/Stat3 and Bmp/Smad/Id signaling (Wray et al., 2010) or, alternatively, in the presence of two chemical inhibitors, dubbed "2i," of the MAP kinases Erk1 and Erk2 and glycogen synthase kinase 3 (Gsk3) (Ying et al., 2008). Genomic targets of the core pluripotency triad, Oct4, Sox2, and Nanog, are frequently co-occupied by the downstream effectors of Lif and Bmp signaling, Stat3 and Smad1, as well as by the histone acetyltransferase p300 and by

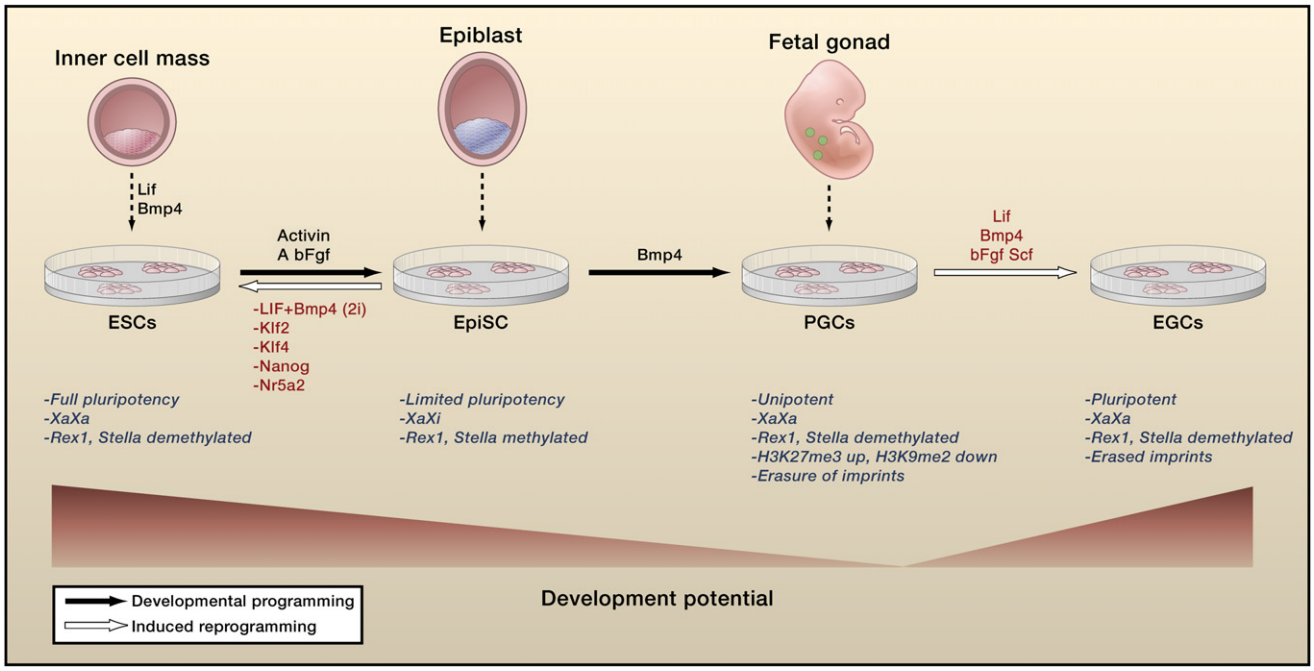



**Figure 5. Examples of Culture-Induced Epigenetic Programming and Reprogramming**

ESCs, derived from the inner cell mass of blastocysts, are maintained in an undifferentiated state in the presence of Lif and Bmp4. Exchange of Lif and Bmp4 with bFgf and activin A induces their differentiation into EpiSCs, which are normally derived from the epiblast of postimplantation embryos and have limited differentiation potential. The ESC-to-EpiSC transition is accompanied by characteristic epigenetic changes, such as X inactivation and methylation silencing of *Rex1* and *Stella* genes, which can be reversed by replating of cells in Lif/Bmp4 or 2i or upon overexpression of Klf2, Klf4, Nanog, or Nr5a2. When exposed to Bmp4 EpiSCs continuously give rise to unipotent PGCs that undergo genome-wide epigenetic remodeling, X reactivation, and erasure of genomic imprinting. In the presence of Lif, Bmp4, and bFgf, these PGCs undergo dedifferentiation into pluripotent EGCs. Note that ESCs, EpiSCs, and EGCs have unlimited self-renewal potentials, and PGCs represent a transient cell population that cannot be maintained in culture. JAKi, JAK inhibitor.

Wdr5, a core component of the Trithorax complex depositing H3K4 methylation (Ang et al., 2011), thus establishing links between growth factor signaling, the core pluripotency network, and chromatin regulation (Chen et al., 2008).

### *Reprogramming of Germ Cells into Pluripotent Cells*

Two typical examples for culture-induced changes of epigenetic and developmental state are the conversion of primordial germ cells (PGCs) and derivative spermatogonial stem cells (SSCs) into pluripotent stem cells (Hochedlinger and Jaenisch, 2006). When explanted in culture, PGCs give rise to embryonic germ cells (EGCs) in the presence of bFgf, Lif, and Scf (Matsui et al., 1992; Resnick et al., 1992) or, alternatively, 2i and Lif (Leitch et al., 2010) (Figure 5). Importantly, PGCs are unipotent and hence can only produce sperm or oocytes in vivo, whereas derivative EGCs are pluripotent and contribute to all tissues in mice, including germ cells.

During early stages of PGC reprogramming in bFgf/Lif/Scf, the germ cell specification factor Blimp1/Prdm1 becomes downregulated, whereas its repressed targets c-Myc and Klf4 are upregulated (Durcova-Hills et al., 2008). Given that Oct4 and Sox2 are already expressed in PGCs, this result suggests that PGCs have an inherent potential to become pluripotent, which is normally blocked by the transcriptional repressor protein Blimp1. Of note, Blimp1 cooperates with the arginine methyltransferase Prmt5 during PGC specification (Ancelin et al., 2006). Recent data suggest that translocation of Prtm5 from the nucleus to the cytosol during PGC-to-EGC conversion, as well as during ESC derivation from ICM cells, mediates histone H2A methylation, which in turn leads to the suppression of differentiation-associated genes (Tee et al., 2010). Forced Prmt5 expression, in combination with Oct4 and Klf4, is also sufficient to induce pluripotency from murine fibroblasts (Nagamatsu et al., 2011). Furthermore, Prmt5 physically interacts with Stat3, which is critical for EpiSC-to-ESC conversion and cellular reprogramming into iPSCs (Yang et al., 2010), thus providing an interesting connection between the Lif/Stat3 signaling pathway, chromatin structure, and the establishment of a pluripotent state.

Similar to PGCs, SSCs give rise at extremely low frequency (0.01%) to pluripotent ESC-like germline stem cells (called mGSCs or gPSCs) when grown in the presence of Lif and serum (Hochedlinger and Jaenisch, 2006). However, relatively little is known about the mechanisms by which cells spontaneously revert to pluripotency except for the observations that changes in cell density (Ko et al., 2009) and loss of p53 (Kanatsu-Shinohara et al., 2004) enhance the derivation of these cells. In summary, PGCs and SSCs have established potent epigenetic mechanisms to efficiently suppress the full pluripotency program in vivo, whereas explantation in culture can remove these constraints and facilitate spontaneous conversion into pluripotent cells. Examination of these epigenetic barriers should be informative for further understanding germ cell development and for enhancing cellular reprogramming.

### *Effects of Fgf/Erk, Jak/Stat, and Bmp Signaling on the Epigenetic and Differentiation State of Cells*

The interconversion of ESCs and EpiSCs provides another example of an environment-induced change of epigenetic and developmental states (Figure 5). The differentiation of ESCs into EpiSCs, which mimics the normal progression of preimplantation ICM cells into postimplantation epiblast, is achieved by the replacement of Lif and Bmp4 in established ESCs with bFgf and activin A (Guo et al., 2009). Resultant EpiSCs resemble EpiSCs that are derived directly from embryos in their epigenetic profile (e.g., XCI, methylation of *Stella* and *Rex1* promoter regions) and limited differentiation potential (e.g., capacity to form teratomas but inability to contribute to chimeras).

Replating of EpiSCs in Bmp/Lif or 2i/Lif gives rise, at low frequencies, to reverted ESC-like cells that show reactivation of the silenced X chromosome and demethylation of *Stella* and *Rex1* promoters (Bao et al., 2009). A recent report has linked this reversion to the inhibition of Fgf/Erk signaling by 2i, which appears to relieve Fgf/Erk's suppressive effect on *Klf2* expression (Greber et al., 2010). In a related study, Smith and coworkers identified a limiting role for Jak/Stat3 signaling in EpiSC-to-ESC conversion (Yang et al., 2010). Notably, activation of Jak signaling as well as overexpression of *Nanog* (Silva et al., 2009) or *Klf2* or *Klf4* (Guo et al., 2009; Hall et al., 2009), all of which facilitate an EpiSC-into-ESC conversion, also promoted the progression of partially reprogrammed iPSCs into fully reprogrammed iPSCs, indicating commonalities among these different types of reprogramming. Lastly, exposure of EpiSCs to Bmp4 promotes the delineation of PGCs and subsequently the derivation of EGCs in culture, which showed epigenetic changes that are typical for germ cell maturation, including reactivation of XCI and erasure of imprinted gene methylation (Hayashi and Surani, 2009). Taken together, these results show that Fgf/Erk, Jak/Stat, and Bmp signaling dynamically regulate the interconversion of ESC, EpiSCs, and PGCs/EGCs, hence linking major signaling pathways to changes in the epigenetic configuration and differentiation state of pluripotent cells.

Lif-dependent ESC self-renewal depends on Jak/Stat3 signaling. Of note, activation of Jak2 also contributes to the self-renewal of ESCs in a Lif-independent fashion by interfering with the binding of the heterochromatin factor HP1$\alpha$ at key pluripotency genes (Griffiths et al., 2011). Specifically, constitutive active Jak signaling in ESCs results in the phosphorylation of histone H3 tyrosine 41 (H3Y41), thereby displacing HP1$\alpha$ from many targets that are involved in the self-renewal of ESCs, including Nanog and Sox2. Importantly, these ESCs grow in the absence of 2i or Lif and do not activate Stat3. This result uncovers a previously unrecognized role for Jak signaling in directly communicating with the pluripotency network by controlling chromatin accessibility at crucial self-renewal genes.

The derivation of human ESCs in low oxygen also illustrates the influence of environmental factors on the epigenetic state of pluripotent cells (Lengner et al., 2010). Physiological oxygen levels preserve ESCs in a pre-X-inactivation state that is reminiscent of mouse ESCs, which carry two Xa. Interestingly, low oxygen levels also prevent the spontaneous differentiation of human ESCs (Ezashi et al., 2005; Lengner et al., 2010) and enhance the derivation of iPSCs from fibroblasts (Utikal et al., 2009; Yoshida et al., 2009), suggesting that hypoxic culture conditions in general are beneficial for the establishment and maintenance of very primitive pluripotent cells. Though the mechanisms underlying these observations remain unclear, it is possible that elevated levels of hypoxia-induced Hif-2$\alpha$, which positively regulates *Oct4* at the transcriptional level, contribute to these effects (Covello et al., 2006).

## Pluripotency Factors and Alternative Cellular States

The observation that changes in environmental cues and/or forced expression of transcription factors generate alternate pluripotent cell states (EpiSCs, ESCs, and EGCs) (Figure 5) raises the intriguing possibility that non-iPSC fates might be produced directly from somatic cells upon overexpression of Oct4, Sox2, Klf4, and c-Myc when exposed to appropriate culture conditions, an idea sometimes referred to as transdifferentiation. In the following section, we review recent examples of pluripotent factor-induced transdifferentiation and speculate on the underlying mechanisms.

### *Pluripotency Genes in Transdifferentiation*

Initially, Schöler and colleagues showed that fibroblasts expressing the four Yamanaka factors and cultivated in the presence of bFgf and activin A give rise directly to EpiSCs rather than iPSCs, suggesting that growth conditions may dictate the fate of the resultant cell type (Han et al., 2011). Remarkably, when the same reprogramming factors were expressed in fibroblast cultures for a brief time insufficient to produce iPSCs and followed by a change of culture conditions conducive for cardiomyocyte growth, cardiomyocyte-like cells that activated a cardiac reporter, exhibited action potentials, and spontaneously twitched were produced (Efe et al., 2011). Similarly, brief exposure of fibroblast cultures expressing Oct4, Sox2, Klf4, and c-Myc to neural progenitor conditions gave rise to astrocytes and neurons exhibiting typical marker expression and action potentials (Kim et al., 2011). These reports do not exclude the unlikely possibility that rare iPSCs were generated during pluripotency factor expression, which then redifferentiated into the observed cell types.

Such transdifferentiation experiments suggest that, early during the reprogramming process, cell chromatin may become sufficiently "plastic" to assume different cellular states, which are selected for by the extracellular signals provided (Figure 6). Whether these putative intermediates are equivalent with previously reported "partially reprogrammed" iPSCs (Mikkelsen et al., 2008; Sridharan et al., 2009) or "pre-iPSCs" (Silva et al., 2008a) remains to be explored; partial iPSCs and pre-iPSCs have extinguished somatic gene expression patterns and have activated other lineage factors, however, without acquiring pluripotency. It should be informative to test whether their exposure to lineage-specific growth conditions converts them into alternative cell fates.

Consistent with the concept of pluripotency factor-induced transdifferentiation, expression of OCT4 was reported as sufficient to convert human dermal fibroblasts into CD45+ hematopoietic progenitor-like cells in vitro (Szabo et al., 2010). Upon exposure of fibroblast-derived CD45+ progenitors to different hematopoietic cytokines, cells with myeloid, erythroid, and megakaryocytic phenotypes were observed that could, to

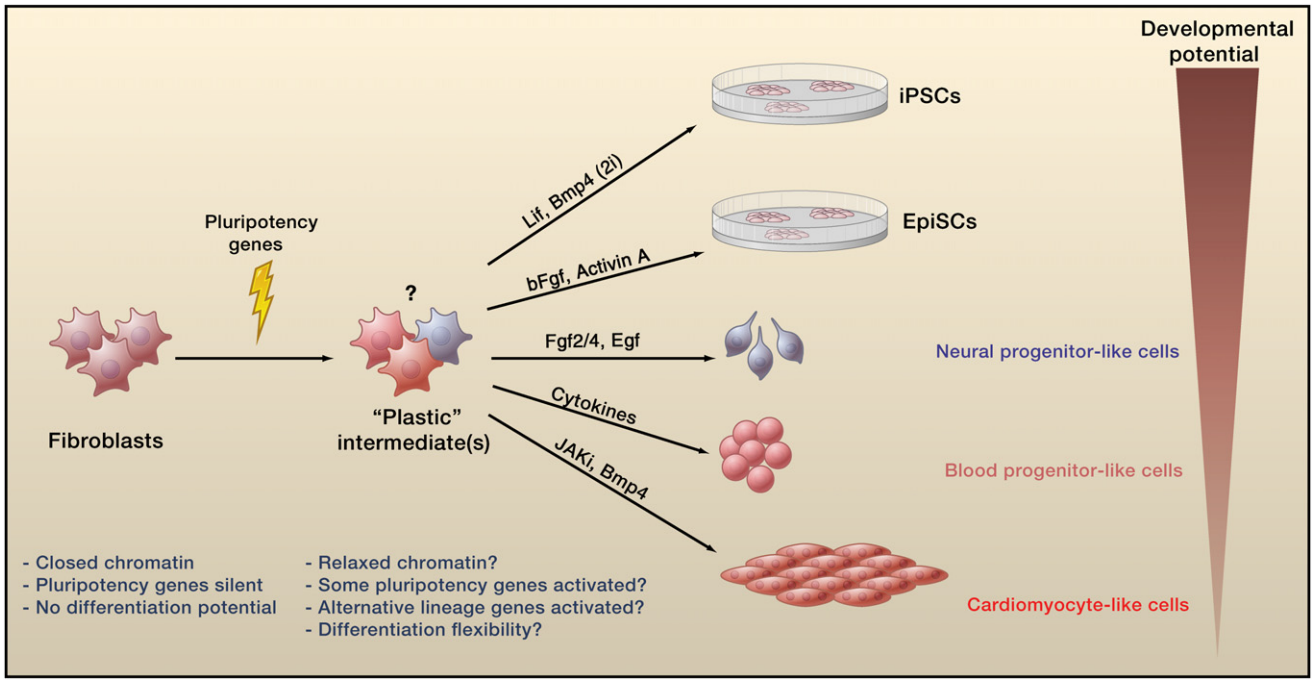



**Figure 6. Proposed Synergism between Pluripotency Gene Expression and Growth Factors in Changing Cellular Identity**
Introduction of individual or combinations of pluripotency genes into fibroblasts may generate putative "plastic" intermediates that are amenable to further reprogramming into iPSCs or EpiSCs when exposed to Lif/Bmp4 (2i) or bFgf/activin A, respectively. Alternatively, such intermediate cells may be converted directly into neural or blood progenitors with limited self-renewal potentials or cardiomyocytes when exposed to appropriate growth factors. Note that the developmental potency of resultant cells appears to depend on the provided growth conditions.

a limited extent, engraft in irradiated mice. An alternative explanation for these results is that rare pre-existing CD45+ progenitors present in the heterogeneous fibroblast population were expanded rather than generated de novo by ectopic OCT4 expression. Consistent with this interpretation, ectopic Oct4 expression in mice expands adult progenitor cells rather than induces dedifferentiation of mature cells (Hochedlinger et al., 2005).

### *Possible Mechanisms Underlying Transdifferentiation*

Nonphysiological binding to lineage-specific target genes is one mechanism by which forced pluripotency factor expression might induce alternative differentiated cell fates. Support for this hypothesis derives from the observation that partially reprogrammed iPSCs exhibit aberrant expression and pluripotency factor binding to differentiation-specific genes (Mikkelsen et al., 2008; Sridharan et al., 2009), and fibroblasts overexpressing OCT4 show abnormal binding to hematopoietic targets that are normally occupied by OCT1 and OCT2 in the differentiated state (Szabo et al., 2010).

Another interpretation is that pluripotency factors themselves are normally involved in early lineage commitment in embryonic cells, and their forced expression in fibroblasts may mimic this effect, resulting in transdifferentiation. The notion that pluripotency factors may serve as lineage specifiers relates to the observation of "pioneer factors," which establish transcriptional competence at some repressed target genes in embryonic cells in order to facilitate their subsequent activation by differentiation-specific family members (Ram and Meshorer, 2009). Indeed, several pre-B cell-specific enhancers, which are occupied and silenced by Sox2 and FoxD3 in undifferentiated ESCs, become activated in lymphoid cells through exchange of Sox2/FoxD3 with Sox4 (Liber et al., 2010). Similarly, FoxD3 binding establishes a DNA methylation-free mark at the *Albumin1* enhancer in ESCs that is critical for subsequent gene activation by FoxA1 in endoderm cells (Xu et al., 2009a). These observations are further reminiscent of the recently reported pattern of poised enhancer elements identified in mouse and human ESCs based on depletion for histone H3 lysine 27 acetylation (H3K27ac) (Creyghton et al., 2010; Rada-Iglesias et al., 2011).

Pluripotency factors have also been suggested to directly activate transcription of lineage targets during early development, and this mechanism may contribute to transdifferentiation. For instance, Nanog has been shown to bind to and activate the Eomes gene specifying definitive endoderm formation (Teo et al., 2011), whereas Tbx3 activates the extra-embryonic endoderm regulator Gata6 by counteracting PRC2-mediated H3K27 methylation (Lu et al., 2011). Likewise, Oct4 function has been suggested to be critical for mesoderm and subsequent cardiac and hematopoietic (Zeineddine et al., 2006; Kong et al., 2009) differentiation from embryonic cells.

Whatever the mechanism underlying transdifferentiation, given the recognition that pluripotency factors may also serve as active lineage determinants, it may now be possible to predict—based on available transcription factor binding data—which cell lineages can be generated by forced expression of

defined pluripotency factors. In addition, a better understanding of how diverse growth factor pathways signal to chromatin will yield critical insights into mechanisms of normal development and provide a framework for attempts to change the identity of one cell type into that of any other cell type by manipulating defined proteins.

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