

# Chromatin Repressive Complexes in Stem Cells, Development, and Cancer

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The chromatin environment is essential for the correct specification and preservation of cell identity through modulation and maintenance of transcription patterns. Many chromatin regulators are required for development, stem cell maintenance, and differentiation. Here, we review the roles of the polycomb repressive complexes, PRC1 and PRC2, and the HDAC1- and HDAC2-containing complexes, NuRD, Sin3, and CoREST, in stem cells, development, and cancer, as well as the ongoing efforts to develop therapies targeting these complexes in human cancer. Furthermore, we discuss the role of repressive complexes in modulating thresholds for gene activation and their importance for specification and maintenance of cell fate.

## Introduction

The organization of DNA into chromatin is essential for the preservation of genomic integrity in eukaryotic cells and is required for the correct transmission of genetic information over generations. In addition to the physical role of compacting and protecting DNA, the chromatin conformation is closely correlated with the expression state of the genes within its structure. Genes present in a dense chromatin environment are less available to the transcriptional machinery and transcribed to a lesser extent than genes found in looser, and more permissive, chromatin domains. Chromatin is subject to highly dynamic modifications, playing important roles in regulating the availability of DNA and thus gene expression. This regulation includes the exchange of histone variants, nucleosome remodeling by ATP-dependent remodeling complexes, as well as posttranslational modifications of DNA and histones (Kouzarides, 2007).

Protruding N-terminal tails of the core histones (Luger et al., 1997), as well as the linker histone H1, are subject to a vast array of posttranslational modifications, some of which are associated with the transcriptional state of the underlying gene, whereas others appear to play roles in chromatin processes such as cell-cycle regulation or the DNA damage response. Histone modifications have different biochemical functions: One, they serve as docking sites for proteins containing conserved domains interacting with the modified residues, thus recruiting other factors to relevant genomic loci. Two, charged modifications, such as lysine acetylation, neutralize the positive charge of the histones, leading to decreased binding of the negatively charged DNA strand, thus loosening the chromatin structure and promoting transcriptional activity (Kouzarides, 2007).

The various cells of an adult organism display distinct phenotypes, yet they all rely on the same underlying genome. In order to establish cell identity, the correct set of genes must be transcribed, while other genes must be kept in a silent state, and this pattern of gene expression must be maintained in the differentiated cell and propagated through cell generations. Because chromatin regulators ensure stable and heritable cell and tissue

specific gene-expression patterns over subsequent cell generations, they are important for specifying and maintaining cell identity (Orkin and Hochedlinger, 2011).

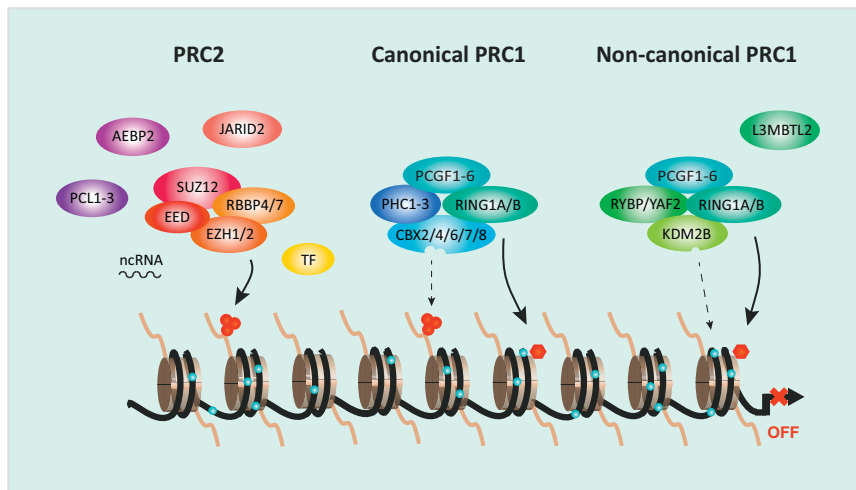
With chromatin modifiers being important for maintaining cell identity, it is not surprising that their deregulation can have deleterious effects on cell fate and functions. Indeed, many chromatin modifiers are essential for normal development and are often found deregulated in human disease, including cancer. One intriguing prospect of this is that whereas genetic mutations are irreversible and thus difficult to target clinically, chromatin modifications are reversible and might thus present promising therapeutic targets. In fact, intense research efforts are currently going into developing inhibitors specifically targeting chromatin-associated proteins, some of which are already in clinical trials and others in clinical use (Helin and Dhanak, 2013).

In this review, we discuss the role of chromatin-mediated transcriptional repression with a particular focus on polycomb repressive complexes, PRC1 and PRC2, and the HDAC1- and HDAC2-containing complexes, SIN3, NuRD, and CoREST. We describe their mechanisms of action in stem cells and development, as well as their deregulation in cancer and emerging strategies for targeting them therapeutically.

## Polycomb Repressive Complexes

The Polycomb group (PcG) proteins were originally identified in *Drosophila*, as transcriptional repressors required for the correct spatiotemporal expression of developmental regulators along the body axis and mutant flies develop abnormally with homeotic transformations. The PcG proteins assemble into large multi-protein complexes, the best-characterized being PRC1 and PRC2 (Figure 1). PRC1 homologs have been identified in metazoan species from flies to mammals, whereas the PRC2 homologs are also found in plants and nematodes (Margueron and Reinberg, 2011).

**PRC1.** *Drosophila* PRC1 consists of Pc (Polycomb, a chromodomain-containing protein with affinity for H3K27me3), dRing (catalyzing H2A ubiquitylation), Psc (Posterior sex combs, involved in chromatin compaction), and Ph (Polyhomeotic).



**Figure 1. Schematic Representation of Polycomb Repressive Complexes**

PRC2 catalyzes methylation of H3K27 (red circles). Several publications have shown that PRC2 recruitment relies on interacting proteins (such as JARID2, AEBP2, and PCL1-3), transient interactions with cell-type-specific transcription factors or noncoding RNAs. Canonical (CBX-containing) PRC1 complexes are recruited (dashed arrow) to H3K27me<sub>3</sub>, while noncanonical (PRC2-independent) PRC1 is recruited (dashed arrow) to CpG islands (blue circles) by KDM2B. Both CBX-containing and PRC2-independent PRC1 complexes catalyze the ubiquitylation of H2AK119 (red hexagons).

*Recruitment of Polycomb Repressive Complexes.* In *Drosophila*, PcG proteins are recruited to DNA stretches termed Polycomb Response Elements

(PREs). A distinct PRE for mammalian cells remains elusive, but mammalian PRCs bind CpG rich promoters of their target genes (Ku et al., 2008), and CpG-rich sequences have been shown to mediate PRC2 recruitment (Mendenhall et al., 2010). Several different recruitment mechanisms for PRC2 have been suggested, including association with near-stoichiometric interaction partners (such as PCL1-3, AEBP2, JARID2), association with transcription factors, and recruitment by ncRNA (Di Croce and Helin, 2013). The WD40 domains of the RBBP4/7 subunit confer general histone-binding activity to PRC2, while those of EED specifically interact with H3K27me<sub>3</sub>, thus providing a potential mechanism for spreading and propagation of the mark. In addition, JARID2 and AEBP2 have both been shown to confer weak CpG-rich DNA-binding activity to the complex, while the Tudor domains of PCL1-3 were recently shown to bind H3K36me<sub>2/3</sub> (Di Croce and Helin, 2013). The involvement of ncRNAs in PRC recruitment has been most extensively studied in the context of X chromosome inactivation. The accumulation of H3K27me<sub>3</sub> on the inactive X chromosome is dependent on XIST expression, and the A repeats of XIST have been shown to bind PRC2 (Zhao et al., 2008). However, XIST lacking the A repeats is capable of recruiting PRC2, indicating the involvement of other domains of XIST in PRC2 recruitment (Kohlmaier et al., 2004). While a number of studies show association of ncRNAs with PRC2 members, the reports differ in the types of RNAs identified, specific binding areas of the RNAs, and the PRC2 component involved in the interaction, and the exact role of ncRNAs in PRC2 recruitment remains unclear (da Rocha et al., 2014; Davidovich et al., 2013; Kaneko et al., 2014; Zhao et al., 2010).

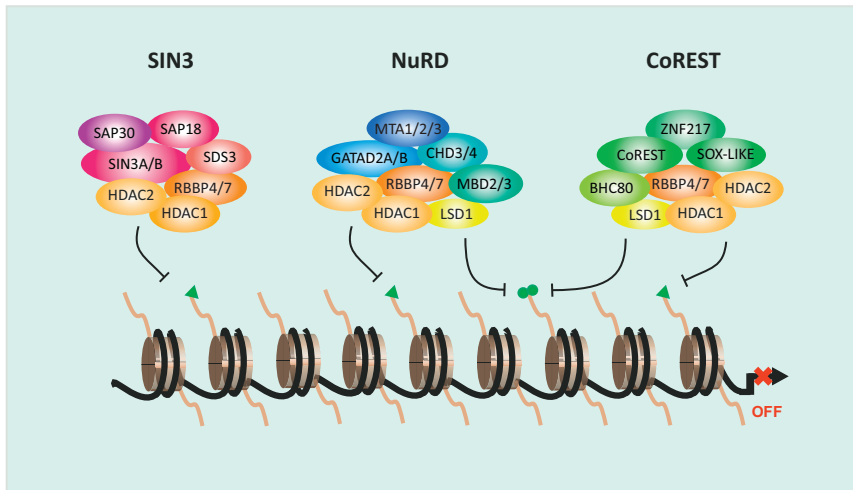
Mammalian genomes encode several homologs of each of the *Drosophila* PRC1 components with five CBX homologs (CBX2/4/6/7/8), two ubiquitin ligases (RING1A/B), six PCGF family members (PCGF1–6, homologous to Psc), and three PHC family members (Phc homologs). In mammalian cells, PRC1 catalyzes H2AK119 ubiquitylation (H2AK119ub1) and promotes chromatin compaction (Di Croce and Helin, 2013).

*PRC2.* Mammalian PRC2 contains the core components EZH2 or its closely related homolog EZH1 (homologs of *Drosophila* E[z]), EED (homolog of Esc), and SUZ12 (homolog of Su[z]12), all three of which are required for catalytic activity in vitro, whereas association with the histone chaperone RBBP4/7 seems to be required for catalytic activity in vivo. The EZH component contains a SET domain, which catalyzes the methylation of lysine 27 of histone H3 (Margueron and Reinberg, 2011).

*Transcriptional Repression by PRCs.* Whereas H3K27 is the essential physiological substrate for PRC2 (Pengelly et al., 2013), the precise functional importance of PRC-mediated histone marks remains unclear. The functional role of H3K27me<sub>3</sub> has primarily been studied as a recruitment mechanism for CBX-containing PRC1 complexes, and, in *Drosophila*, the catalytic activity of E(z) is required for target gene repression (Müller et al., 2002), whereas H2AK119ub1 is believed to promote chromatin compaction and transcriptional repression. In vitro data show that PRCs promote condensation of nucleosomal arrays (Francis et al., 2004), and PRC binding in *Drosophila* mediates chromatin compaction and organization into functional domains, called PcG bodies, as well as long-range interactions important for higher-order chromatin organization (Bantignies et al., 2011; Sexton et al., 2012). Recently, the E3 ligase activity of the Ring1 component of PRC1 was shown to be dispensable for recruitment to and compaction of chromatin at the *Hox* loci in mESCs (Endoh et al., 2012). However, the catalytic activity was indispensable for target gene repression, indicating that H2A ubiquitylation and chromatin condensation represent two separate mechanisms of PRC1-mediated repression (Endoh et al., 2012). Alternative roles of H2AK119ub1 in PRC-mediated repression include prevention of H3K4me<sub>3</sub> deposition, inhibition of RNA polymerase II activity, and prevention of H2A-H2B dimer eviction from transcribed regions (Di Croce and Helin, 2013).

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Several lines of evidence obtained in *Drosophila* and mammalian cells have shown that PRC1 recruitment to target sites is dependent on PRC2 and H3K27me<sub>3</sub>. However, recent studies in PRC2 knockout mESCs have shown only a minor decrease in H2AK119ub1 levels despite a global loss of H3K27me<sub>3</sub> (Leeb et al., 2010). An explanation for this observation has been provided by the characterization of PRC2-independent RING1-containing complexes without any CBX component, which rely on their complex partners RYBP/YAF2 and L3MBTL2, as well as the association with DNA binding proteins



**Figure 2. Schematic Representation of HDAC1- and HDAC2-Containing Complexes**

HDAC1 and HDAC2 of SIN3, NuRD, and CoREST catalyze the removal of acetyl groups from histone tails (green triangles). The NuRD subunits CHD3/4 are ATP-dependent chromatin remodelers and LSD1 present in CoREST (and possibly also NuRD) catalyzes demethylation of H3K4me1/2 (green circles). Recruitment of HDAC1- and HDAC2-containing complexes is thought to rely on chromatin-binding domains within each complex or additional interaction partners (not depicted).

CHD3/4 helicase subunit and histone deacetylation catalyzed by HDAC1 and HDAC2. Additional components include the scaffolding proteins GATAD2A/B, conferring histone-binding properties to the complex, while the MBD2/3 and

such as KDM2B for their recruitment to CpG-rich promoters (Farcas et al., 2012; Gao et al., 2012; Qin et al., 2012; Tavares et al., 2012; Wu et al., 2013). Similar to what has been shown in *Drosophila* (Lagarou et al., 2008), the mammalian KDM2B-RING1B complex appears to have higher catalytic activity toward H2AK119ub1 than PRC1.

Accompanying the changes in transcriptional programs during differentiation, PRC binding changes dynamically (Bracken et al., 2006; Mohn et al., 2008). Whether the patterns of PRC binding in various cell types depend on differential expression of interaction partners or ncRNAs or whether PRC binding differs simply as a consequence of differential gene-expression patterns and recruitment to untranscribed genes is still unclear. Elucidating the mechanisms regulating PRC binding to target genes is essential for our understanding of the nature of PRC-mediated transcriptional repression.

#### HDAC1- and HDAC2-Containing Complexes

HDAC1 and HDAC2 are highly homologous class I histone deacetylases found in large multimeric complexes, the most extensively studied being Sin3, NuRD, and CoREST (Figure 2), which are found in species from yeast to human. In addition to the HDAC1 and HDAC2 catalytic core and RBBP4/7 that are shared among the complexes, they incorporate different subunits, thus providing target specificity or additional catalytic activities. Importantly, many of the subunits have several homologs, allowing for combinatorial assembly of specific complexes with context-dependent functions (Kelly and Cowley, 2013).

**SIN3.** Mammalian genomes encode two homologs of SIN3 (SIN3A/B), which associate individually with HDAC1 and HDAC2, RBBP4 and RBBP7, SDS3, and the SIN3-associated proteins SAP18 and SAP30 to form the core SIN3 complex. Different studies have identified additional interaction partners including MeCP2 (methyl-CpG-binding protein), RBP1 (RB-binding protein), BRMS1 (breast cancer metastasis suppressor), ING1/2 (inhibitor of growth), SAP25, SAP130, and SAP180, as well as the histone demethylase RBP2/KDM5A (Hayakawa and Nakayama, 2011; Kadamb et al., 2013).

**NuRD.** The NuRD (nucleosome remodeling deacetylase) complex couples two important chromatin-modifying activities, namely nucleosome remodeling through the ATP-dependent

MTA1/2/3 subunits mediate binding to DNA and transcription factors, respectively (Hayakawa and Nakayama, 2011; Lai and Wade, 2011). Some results have suggested that NuRD interacts with the histone demethylase LSD1/KDM1A, potentially adding yet another catalytic activity to its repertoire (Wang et al., 2009b). However, this association is not observed in other purifications, possibly reflecting context-specific interactions.

**CoREST.** Originally described as corepressor of REST (RE1-silencing transcription factor), CoREST was subsequently found in complex with HDAC1 and HDAC2 and RBBP4/7 (although not retrieved in some purifications) with additional subunits including Sox-like protein, ZNF217, BHC80, and the histone demethylase LSD1 (Hayakawa and Nakayama, 2011). LSD1 has catalytic activity toward H3K9me1/2 and H3K4me1/2 (Metzger et al., 2005; Shi et al., 2004). However, in the context of CoREST, LSD1 seems to preferentially target H3K4me1/2, while primarily exerting its function as a H3K9 demethylase when associated with nuclear receptors (Kooistra and Helin, 2012).

**Transcriptional Regulation by HDAC1- and HDAC2-Containing Complexes.** SIN3, NuRD, and CoREST are all large, multimeric complexes that serve as scaffolds for assembling different catalytic activities at relevant genomic loci. For NuRD, the CHD3/4 helicase activity has been shown to promote deacetylase activity, possibly by promoting the accessibility of the nucleosome substrate through ATP-dependent nucleosome sliding (Xue et al., 1998). It is noteworthy that all three complexes combine their core deacetylase activity with demethylase interaction partners. For CoREST, the two catalytic activities appear to be interdependent with deacetylation promoting demethylation (Lee et al., 2006a), pointing to a functional interplay extending beyond mere colocalization.

In accordance with histone acetylation being associated with transcriptional activation, HDAC-containing complexes reversing this modification are traditionally described as corepressors promoting transcriptional repression of their target genes. Importantly, however, it has been shown that dynamic acetylation and deacetylation is required for active transcription to occur, thus pointing to important roles of HDAC-containing complexes in activating transcription in addition to their function as corepressors (Clayton et al., 2006; Kelly and Cowley, 2013). Indeed,

chromatin immunoprecipitation (ChIP)-sequencing studies show that HDACs also colocalize with acetyltransferases at transcriptionally active loci, presumably acting to reset acetylation levels after gene activation (Wang et al., 2009c). Thus, the transcriptional regulation exerted by HDAC1- and HDAC2-containing complexes might be highly context dependent.

**Recruitment of HDAC1- and HDAC2-Containing Complexes.** Recruitment of the HDAC1- and HDAC2-containing complexes seems to rely on cell-type-specific transcription factor binding and chromatin-binding domains within certain subunits (Hayakawa and Nakayama, 2011). Each of the complexes contain at least two subunits with histone-binding properties such as PHD-fingers, chromodomains and Tudor domains, as well as the WD40 repeats of RBBP4/7 (Kelly and Cowley, 2013).

The existence of several homologs for most of the components indicates that specific complex composition might confer distinct binding patterns and influence their biological function in different cell types (Kelly and Cowley, 2013). For instance, NuRD complex containing MBD2 is functionally distinct from MBD3-NuRD (Le Guezennec et al., 2006). MBD2 recruits NuRD to methylated CpGs, whereas MBD3 is unable to bind methyl-cytosine due to amino acid substitutions in the methyl-binding domain. However, NuRD is recruited to some target genes independently of their methylation status, and the MBD component is thus only partly responsible for NuRD recruitment (Baubec et al., 2013). Similarly, the MTA proteins are incorporated into distinct NuRD complexes with differential transcription factor binding and recruitment to specific genomic loci (Lai and Wade, 2011).

Thus, for PRCs and HDAC1- and HDAC2-containing complexes, it seems that specific subunit composition and association with cell-type-specific interaction partners is important for regulating their recruitment and biological function.

### Chromatin Repressive Complexes in Pluripotent Stem Cells

ESCs display an open and permissive chromatin structure with low levels of DNA methylation and a greater abundance of activating histone modifications, such as H3K4me3 and histone acetylation. In addition, structural proteins such as heterochromatin protein 1 (HP1), the linker histone H1, and the core histones display highly dynamic kinetics in their association with chromatin in ESCs, further opening the chromatin structure (Azuara et al., 2006; Meshorer et al., 2006). The hyperdynamic nature of ESC chromatin correlates with a very high level of transcriptional activity and a high abundance of general transcription factors and chromatin modifiers (Efroni et al., 2008), central to maintaining transcriptional patterns in the open chromatin structure. Upon differentiation, the overall chromatin structure shifts toward a tighter, more restrictive configuration with decreased transcriptional activity and concomitant accumulation of H3K27me3 (Zhu et al., 2013), as well as induction of large H3K9me3-positive heterochromatic foci (Meshorer et al., 2006).

Chromatin regulators along with tightly regulated transcription factor circuits play important roles in balancing self-renewal and pluripotency in ESCs, and the open chromatin environment appears to be important for the maintenance of pluripotency. Conversely, the open, permissive chromatin environment necessitates the action of chromatin repressive complexes in order to protect against inappropriate transcription of differentiation factors, as well as for the orchestration of differentiation through the

timely repression of pluripotency-associated genes (Orkin and Hochedlinger, 2011).

**Polycomb Repressive Complexes in Embryonic Stem Cells.** PRCs are highly expressed in ESCs and have been shown to bind CpG-rich promoters of genes for transcription factors and signaling molecules controlling development (Boyer et al., 2006; Bracken et al., 2006; Lee et al., 2006b). In addition, PcG proteins and their marks are found at some repetitive elements and involved in imprinting and X chromosome inactivation (Casa and Gabellini, 2012).

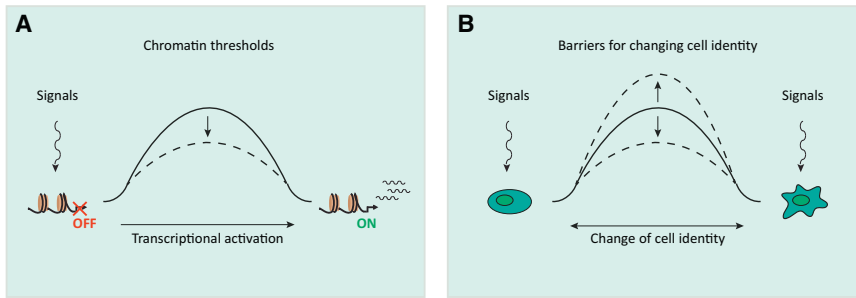
While the PRC2 components are essential for mouse development, mESCs lacking *Eed*, *Suz12*, or *Ezh2* can be derived from knockout embryos, yielding similar phenotypes with retention of self-renewal capacity, loss of H3K27me2/3 and in vitro differentiation defects. Consistent with the in vitro defects, chimeric embryo complementation studies show that knockout mESCs initiate differentiation but display abnormal long-term repression of pluripotency factors and lack robust induction of differentiation factors (Chamberlain et al., 2008; Montgomery et al., 2005; Pasini et al., 2007; Shen et al., 2008). Notably, the passage number of *Eed*<sup>-/-</sup> cells influences the phenotype: high-passage *Eed*<sup>-/-</sup> cells display more pronounced derepression of target genes and a global loss H3K27me1, whereas the phenotype of low-passage *Eed*<sup>-/-</sup> cells appears identical to those of *Ezh2*<sup>-/-</sup> and *Suz12*<sup>-/-</sup> cells (Chamberlain et al., 2008).

Ring1b-deficient mESCs have reduced levels of H2AK119ub1, a slight deregulation of some target genes and a loss of differentiation potential (Leeb and Wutz, 2007), whereas *Ring1a/Ring1b* double knockout mESCs lose the ability to self-renew after a few passages and show defects in cell-cycle regulation, pointing to PRC2-independent roles of Ring1a/Ring1b (Endoh et al., 2008). Knockdown studies show that the noncanonical PRC1 components Rybp or Kdm2/Fbxl10 are dispensable for self-renewal, while loss of either factor diminishes H2AK119ub1 levels and compromises the in vitro differentiation potential (Gao et al., 2012; Wu et al., 2013). Recently, different Cbx subunits of PRC1 were shown to have specific roles with Cbx7 being required for maintaining the pluripotent state of mESCs, with a shift in composition to Cbx2/4 being important during differentiation (Morey et al., 2012).

**HDAC1- and HDAC2-Containing Complexes in Embryonic Stem Cells.** *Hdac1* and *Hdac2* are both dispensable for mESC self-renewal, but whereas *Hdac2*<sup>-/-</sup> cells retain their in vitro differentiation potential, *Hdac1* knockout disrupts normal differentiation (Dovey et al., 2010). Several NuRD subunits have been shown to interact with core pluripotency factors, including Oct4 and Nanog, forming the NODE complex (Nanog- and Oct4-associated deacetylase) (Liang et al., 2008), which might be functionally distinct from Mbd3-containing NuRD. While the relative contributions of different *Hdac1*- and *Hdac2*-containing complexes remain unclear, several studies show important roles of subunits of each of these complexes in mESCs.

In vitro culture of *Sin3a*<sup>-/-</sup> blastocysts yield smaller colonies and insufficient outgrowth of the ICM, showing important roles of Sin3a in the establishment of mESCs (Cowley et al., 2005), consistent with the peri-implantation lethality observed for *Sin3a*<sup>-/-</sup> mice. Mbd3-deficient mESCs can be derived and propagated in culture but display defects during differentiation (Kaji et al., 2006; Rais et al., 2013). Knocking out *Lsd1* in mESCs leads





**Figure 3. Chromatin Sets Thresholds for Gene Activation Controlling Cell Identity**

(A) The chromatin environment sets thresholds for gene activation, ensuring that persistent exposure to appropriate signals (e.g., increasing transcription factor levels) is required for transcriptional activation. Loss of chromatin repressive complexes (dashed line) lowers the threshold and increases transcriptional noise.

(B) Changes in chromatin thresholds in turn alter the barriers against changes in cell identity mediated by extrinsic (e.g., growth factors or hormones) and intrinsic (e.g., transcription factor levels or somatic mutations) signals. The biological outcome (whether the barriers are increased

or decreased) depends on which genes become aberrantly activated by the loss of chromatin repressive complexes (dashed lines). For instance, if a repressive complex acts to limit the expression of an oncogene, loss of the complex would promote oncogenesis. Conversely, if the complex binds a tumor suppressor, its loss would increase the barrier for oncogenic transformation. Thus, loss of a chromatin repressive complex can influence cell identity in different directions, depending on the genes they regulate and the integration of the combined signals the affected cell receives.

to reduced CoREST levels, a slight deregulation of gene expression and defects during embryoid body formation with incomplete silencing of pluripotency-associated genes (Foster et al., 2010; Wang et al., 2009a). Lsd1 has also been shown to colocalize with NuRD at the enhancers of pluripotency-associated genes, where it is required for the downregulation of H3K4me1-levels during differentiation (Whyte et al., 2012).

**Chromatin Repressive Complexes and Pluripotency.** Many repressive complexes do not seem to be essential for the self-renewal of ESCs, while both in vivo and in vitro data demonstrate their requirement for pluripotency. It has been suggested that one of the key functions of chromatin regulation is in noise reduction, meaning that the presence of nucleosomes and other chromatin-bound factors act to limit the propensity of promiscuous transcriptional activity such that several cues need to act in concert in order for transcription to take place (Figure 3). Studies from yeast support this theory: By competing with transcription factors and the transcriptional machinery for access to promoters, chromatin acts to increase the threshold for gene activation and limit transcriptional noise (Lam et al., 2008; see also, e.g., Chi and Bernstein, 2009). This view might help explain the phenotype of ESCs lacking chromatin repressive complexes: As long as cells are grown in defined media, loss of a complex does not lead to widespread gene activation or changes in cell identity. However, it might lower the threshold for gene activation giving rise to transcriptional noise. Thus, during differentiation in an environment with multiple signals and different types of cells, the more relaxed chromatin makes cells lacking repressive complexes more susceptible to aberrant activation of gene expression, which can result in differentiation and developmental failures. The normal role of the repressive chromatin complexes is therefore to ensure that sustained and strong signals are required for changing the transcription program and the specification of differentiation. The importance of defined media in this context is illustrated by the early observations that knockout of e.g., *Ezh2* or *Mbd3* was incompatible with the establishment of pluripotency (Kaji et al., 2007; O'Carroll et al., 2001). Both observations have since been refuted by the establishment of knockout mESCs lacking either factor, most likely through the refinement of experimental procedures or the introduction of optimized culture conditions such as 2i/LIF (Rais et al., 2013; Shen et al., 2008).

In the context of pluripotent cells, a much-debated feature is the observation of bivalent domains in the promoters of developmental genes, defined by the presence of H3K4me3 alongside H3K27me3 (Azuaa et al., 2006; Bernstein et al., 2006). This co-occurrence observed by ChIP-sequencing approaches might represent the two marks existing simultaneously on the same histone tail, on opposite H3 tails of the same nucleosome or on neighboring nucleosomes. In addition, it has been argued that bivalent promoters might simply represent an artifact of heterogeneous cell populations. Although additional observations of bivalent domains in the early embryo and differentiated cell types and the application of sequential ChIP and mass spectrometry approaches have shown the existence of truly bivalent promoters, their functional relevance remains unclear. With all CpG-rich promoters being H3K4me3-positive in mESCs (Mikkelsen et al., 2007) and PRC2 being recruited to CpG-rich stretches, the co-occurrence of H3K4me3 and H3K27me3 is to be expected. Bivalent genes are found to be transcriptionally inactive but are generally thought to be poised for activation upon differentiation, thus providing plasticity to the chromatin structure (Voigt et al., 2013). However, recent studies show that loss of H3K4me3 from bivalent promoters does not disrupt the large-scale responsiveness of gene activation upon *all trans*-retinoic-acid-induced differentiation of mESCs, thus questioning the prevailing view of the functional relevance of bivalency (Denissov et al., 2014; Hu et al., 2013).

ChIP analyses show binding of the Chd4 component of NuRD at bivalent polycomb target gene promoters and reveal potential coregulation of the two complexes with common target genes gaining H3K27ac and losing PRC2 binding, as well as H3K27me3 in *Mbd3*<sup>-/-</sup> cells (Reynolds et al., 2012b). This indicates that NuRD and PRC2 might be functionally linked through occupation of some of the same genomic loci, where NuRD might facilitate PRC2 recruitment and methylation through deacetylation of H3K27. This potential coregulation is reminiscent of observations in *Drosophila*, where HDAC1/RPD3 collaborates with PcGs in repressing a subset of PcG target genes (Tie et al., 2001).

Interestingly, Hdac1 and Mbd3 have been found to associate with the promoters of many actively transcribed genes, including core pluripotency factors. Importantly, however, comparative analysis of mESCs showed that target gene expression was primarily upregulated upon *Hdac1* knockout, indicating that NuRD

acts as a transcriptional repressor even at actively transcribed genes (Kidder and Palmer, 2012). In a recent study, Reynolds et al. investigated the role of NuRD in regulating pluripotency and lineage commitment of mESCs. The authors found that rather than silencing pluripotency-associated genes, NuRD is required to restrict transcript levels below a threshold, thereby sensitizing cells to differentiation cues and facilitating lineage commitment in response to the relevant stimuli (Reynolds et al., 2012a). Thus, NuRD and other repressive complexes might not function as traditional silencers but rather by fine-tuning expression levels of their target genes (Hu and Wade, 2012; Reynolds et al., 2013).

In the acquisition of pluripotency through reprogramming of somatic cells, the chromatin environment undergoes major reorganization toward an open chromatin structure along with erasure of DNA methylation and redistribution of histone modifications, and many chromatin modifiers appear to influence this process. Cell fusion experiments with mESCs with knockout of PRC1 or PRC2 components show that functional PRCs are required for reprogramming of human B cells (Pereira et al., 2010). Similarly, small hairpin RNA-mediated knockdown of PRC1 or PRC2 components impaired the conversion of human fibroblasts to induced pluripotent stem cells (iPSCs) (Onder et al., 2012), while ectopic *Ezh2* or *Bmi1* expression increases the efficiency of iPSC generation (Buganim et al., 2012; Moon et al., 2011). In this context, it is important to keep in mind the differences between mouse and human pluripotent cells. Indeed, *Ezh2* knockout does not impair iPSC formation from MEFs (Fragola et al., 2013), indicating that *Ezh2* is not required for reprogramming of mouse cells. However, the authors note that despite a global loss of H3K27me3 upon *Ezh2* knockout, this mark is retained on a subset of important developmental regulators, most likely deposited by *Ezh1*-PRC2. Indeed, knockdown of *Eed* in the *Ezh2*-deficient cells diminishes the remaining H3K27me3 and prohibits reprogramming (Fragola et al., 2013). Another important aspect to consider is the potentially distinct requirements of repressive complexes during early and late stages of reprogramming (Ho et al., 2013), as well as effects on proliferation, which are not directly linked to the acquisition of pluripotency, yet would still influence reprogramming efficiency.

Whereas the PRCs are observed to positively influence reprogramming, the opposite situation has been reported for other repressive chromatin regulators. Recently, depletion of the core NuRD component *Mbd3* was shown to increase the efficiency of iPSC generation (Luo et al., 2013; Rais et al., 2013). One explanation for the seemingly discrepant roles of these repressor complexes might be that PRCs are primarily involved in repression of differentiation-associated genes, while NuRD also plays important roles in the regulation of pluripotency-associated genes. In contrast, however, a separate study shows that *Mbd3* is required for the establishment of iPSCs from mouse neural stem cells, as well as the more primed epiblast stem cells and preiPSCs, while ectopic expression of *Mbd3* with *Nanog* promotes reprogramming (dos Santos et al., 2014). These discrepancies might stem from differences in the experimental approaches and culture conditions applied, underlining the context-dependent nature of such studies.

Collectively, a plethora of studies demonstrate important roles of chromatin repressive complexes in governing cell identity and guarding the pluripotent state of ESCs. Tables 1 and 2 summa-

rize the observed phenotypes of loss-of-function studies concerning repressive complexes components in pluripotent cells.

### Chromatin Repressive Complexes in Tissue Stem Cells and Development

During embryonic development, the chromatin environment is modulated to facilitate specification and maintenance of the various cell types. Accordingly, many components of chromatin repressive complexes are required for normal development. The exact phenotypes vary according to the specific component investigated, but general features include defects in early lineage specification upon knockout of *Ring1b* or core PRC2 members (Faust et al., 1995; O'Carroll et al., 2001; Pasini et al., 2004; Voncken et al., 2003) and several members of HDAC1 and HDAC2-containing complexes (Cowley et al., 2005; David et al., 2003; Hendrich et al., 2001; Lagger et al., 2002; Wang et al., 2007a), as well as later developmental problems with defects in cell type specification and tissue development as observed for several components. In the context of PRC2, it is noteworthy that *Jarid2* knockout leads to defects in neural tube formation with embryonic lethality around E15.5 (Takeuchi et al., 1995), whereas *Pcl2* knockout mice display an incompletely penetrant phenotype of skeletal transformation (Wang et al., 2007b), providing evidence that neither interacting protein is solely responsible for PRC2 recruitment.

Interestingly, the existence of multiple homologs of certain components appears to provide some functional redundancy during development. For instance, mice lacking the PRC1 component *Pcgf2/Mel18* or the closely related gene *Pcgf4/Bmi1* are viable with homeotic transformations (Akasaka et al., 1996; van der Lugt et al., 1994), while concomitant deletion of both genes leads to embryonic lethality around E9.5 (Akasaka et al., 2001). In the context of multiple homologs, important factors to consider include spatiotemporal expression patterns, as well as potential distinctive functions of the homologous proteins, which can give rise to distinct phenotypes of loss of single homologs. This is exemplified by the fact that knockout of *Ezh2*, *Ring1b*, *Hdac1*, or *Sin3a* leads to early embryonic lethality, whereas the loss of their closely related structural homologs have less severe consequences on development. The observed phenotypes from knockout studies of components of Polycomb group proteins and HDAC1 and HDAC2-containing complexes are summarized in Tables 1 and 2.

In addition to their roles during early embryonic development, chromatin repressive complexes play important roles in maintaining gene-expression patterns and cell identity of many different tissues. The roles of PRCs and HDAC1- and HDAC2-containing complexes in tissue stem cells and development are discussed below and summarized in Tables S1 and S2 available online.

*Polycomb Repressive Complexes in Tissue Stem Cells and Development.* The PRCs have been most extensively studied in mESCs, but a growing number of studies demonstrate important roles of PRCs in tissue-specific stem and progenitor cells, and conditional knockout studies show that the PRCs are required during many aspects of mammalian development.

In the hematopoietic system, *Bmi1* is required for self-renewal of hematopoietic stem cells (HSCs) through a mechanism involving the repression of the *Ink4a-Arf* locus (Park et al., 2003), and *Bmi1* knockout promotes premature and deficient

**Table 1. Loss-of-Function Phenotypes of Polycomb Repressive Complexes**

	Development	mESCs	Reprogramming	References
<b>PRC1</b>				
<i>Ring1a</i>	Viable with homeotic transformation.		Knockdown impairs reprogramming.	(del Mar Lorente et al., 2000; Onder et al., 2012)
<i>Ring1b</i>	Lethal around E10.5. Gastrulation defects and cell-cycle inhibition.	Global loss of H2AK119ub1. Slight deregulation of target genes. Differentiation defects.		(de Napoles et al., 2004; Leeb and Wutz, 2007; Voncken et al., 2003)
<i>Ring1a/Ring1b double KO</i>		Loss H2AK119ub1 (also on Xi), derepression of target genes, loss of self-renewal and differentiation defects.	Knockout impairs reprogramming.	(de Napoles et al., 2004; Endoh et al., 2008; Pereira et al., 2010)
<i>Pcgf2 (Mel18)</i>	Homeotic transformation, postnatal lethality.			(Akasaka et al., 1996)
<i>Pcgf4 (Bmi1)</i>	Homeotic transformation, neurological and immune defects with peri- or postnatal lethality.		Knockdown impairs and overexpression enhances efficiency.	(Moon et al., 2011; Onder et al., 2012; van der Lugt et al., 1994)
<i>Pcgf2/Pcgf4 double KO</i>	Lethal around E9.5.			(Akasaka et al., 2001)
<i>Cbx2 (M33)</i>	Homeotic transformation and severe immune defects. 50% die perinatally. Remaining pups die postnatally.			(Coré et al., 1997)
<i>Cbx4</i>	Perinatal lethality with severe immune defects.			(Liu et al., 2013)
<i>Cbx7</i>	Increased susceptibility to tumors of liver and lung.			(Forzati et al., 2012)
		Knockdown yields differentiation defects.		(Morey et al., 2012)
<i>Phc1 (Rae28)</i>	Perinatal lethality and homeotic transformation.			(Takahara et al., 1997)
<i>Phc2</i>	Viable with homeotic transformation.			(Isono et al., 2005)
<i>Phc1/2 double KO</i>	Lethal before E11.5.			(Isono et al., 2005)
<i>Rybp</i>	Early postimplantation lethality around E6.5.	Knockdown yields reduction of H2AK119ub1 and differentiation defects.		(Gao et al., 2012; Pirity et al., 2005)
<i>L3mbtl2</i>	Lethal around E9.5. Gastrulation defects.	Decreased proliferation. Differentiation defects.		(Qin et al., 2012)
<i>Kdm2b (Fbx110)</i>	Incompletely penetrant peri-/postnatal lethality with defects in neural tube closure.	Knockdown yields reduction of H2AK119ub1 and differentiation defects.	Knockdown impairs and overexpression enhances efficiency.	(Fukuda et al., 2011; Liang et al., 2012; Wu et al., 2013)

(Continued on next page)

**Table 1. Continued**

	Development	mESCs	Reprogramming	References
PRC2				
<i>Ezh2</i>	Lethal around E7.5-8.5. Gastrulation defects.	Global loss of H3K27me2/3, differentiation defects.	Knockout/knockdown impairs reprogramming of human cells. Overexpression enhances, yet knockout does not impair mouse iPSC formation.	(Buganim et al., 2012; Fragola et al., 2013; O'Carroll et al., 2001; Onder et al., 2012; Pereira et al., 2010; Shen et al., 2008)
<i>Ezh1</i>			Not required.	(Onder et al., 2012)
<i>Eed</i>	Lethal around E7.5-8.5. Gastrulation defects.	Global loss of H3K27me2/3, slight derepression of target genes and differentiation defects. Late-passage <i>Eed</i> <sup>-/-</sup> cells: Global loss of H3K27me1 and further derepression.	Knockout impairs reprogramming.	(Chamberlain et al., 2008; Faust et al., 1998; Faust et al., 1995; Montgomery et al., 2005; Pereira et al., 2010; Schumacher et al., 1996)
<i>Suz12</i>	Lethal around E7.5-8.5. Gastrulation defects.	Global loss of H3K27me2/3, differentiation defects.	Knockout impairs reprogramming.	(Pasini et al., 2007; Pasini et al., 2004; Pereira et al., 2010)
<i>Jarid2</i>	Lethal at E11.5-15.5 with developmental defects depending on strain.	Differentiation defects.	Not required.	(Lee et al., 2000; Motoyama et al., 1997; Pereira et al., 2010; Shen et al., 2009; Takeuchi et al., 1999; Takeuchi et al., 1995)
<i>Pcl2</i>	Viable with incompletely penetrant defects including homeotic transformations.	Knockdown yields enhanced self-renewal and differentiation defects.		(Walker et al., 2010; Wang et al., 2007b)
<i>Pcl3</i>		Knockdown yields differentiation defects.		(Brien et al., 2012)
<i>Yy1</i>	Peri-implantation lethality.		Knockdown enhances efficiency.	(Donohoe et al., 1999; Onder et al., 2012)



**Table 2. Loss-of-Function Phenotypes of Hdac1- and Hdac2-Containing Complexes**

	Development	mESCs	Reprogramming	References
<b>Hdac1 and Hdac2</b>				
<i>Hdac1</i>	Lethal E9.5-10.5.	Decreased proliferation and differentiation defects.		(Dovey et al., 2010; Lagger et al., 2002)
<i>Hdac2</i>	Perinatal lethality with cardiac malformations.	Not effect on self-renewal or pluripotency.		(Dovey et al., 2010; Montgomery et al., 2007)
<i>Hdac1</i> and <i>Hdac2</i>			Valproic acid increases efficiency.	(Huangfu et al., 2008)
<b>Sin3</b>				
<i>Sin3a</i>	Peri-implantation lethality.	Insufficient outgrowth of ICM during mESC establishment.		(Cowley et al., 2005; Dannenberg et al., 2005)
<i>Sin3b</i>	Perinatal lethality. Pups born in submendelian ratios.			(David et al., 2008)
<i>Sds3</i>	Peri-implantation lethality. Defects in chromosome segregation and early lineage specification.			(David et al., 2003)
<b>NuRD</b>				
<i>Mbd2</i>	Mice are viable. Abnormal maternal behavior.			(Hendrich et al., 2001)
<i>Mbd3</i>	Early postimplantation lethality.	Differentiation defects.	Conflicting data: Knockout/knockdown enhances efficiency. Knockout impairs and ectopic expression enhances efficiency.	(Hendrich et al., 2001; Kaji et al., 2006; Luo et al., 2013; Rais et al., 2013) (dos Santos et al., 2014)
<i>Gatad2a</i>	Postimplantation lethality, morphological defects.			(Marino and Nusse, 2007)
<b>CoREST</b>				
<i>CoREST</i>	Late embryonic lethality due to severe anemia.			(Yao et al., 2014)
<i>Lsd1</i>	Early embryonic lethality around E5.5 with defects in gastrulation and trophoblast specification.	Reduced CoREST levels, slight deregulation of gene expression and differentiation defects.		(Foster et al., 2010; Wang et al., 2009a; Wang et al., 2007a; Zhu et al., 2014)

lymphocytic specification (Oguro et al., 2010). Interestingly, the specific composition of PRC1 with regards to the Cbx component seems to be important for the transition from self-renewal to differentiation during hematopoiesis. As in mESCs, Cbx7 is required for HSC self-renewal, whereas Cbx2/4/8-containing PRC1 seems to be important during differentiation (Klauke et al., 2013). In addition, overexpression of Cbx7 or Kdm2b promotes HSC self-renewal and the number of colony-forming cells during serial transplantations (Klauke et al., 2013; Konuma et al., 2011). Studies of PRC2 in the hematopoietic system show that Ezh2 is required for normal lymphopoiesis (Su et al., 2003) and PRC2 is involved in HSC self-renewal with Ezh2 being important for HSC self-renewal during fetal liver hematopoiesis, while Ezh1 maintains the HSC compartment in the adult bone marrow (Hidalgo et al., 2012; Mochizuki-Kashio et al., 2011), once again highlighting the importance of context-specific incorporation of different homologs. Given the many recent reports of increased expression levels and loss-of-function mutations of PRC2 members, as well as hyperactive oncogenic EZH2 mutants in hematopoietic cancers, it is highly

relevant to further study the role of PRCs in normal and malignant hematopoiesis.

PRCs also contribute the self-renewal capacity of neural stem cells by maintaining the *Ink4a-Arf* locus in a repressed state (Molofsky et al., 2003), and they are involved in the timely repression of neurogenic factors, promoting the neurogenic-to-astrogenic switch (Hirabayashi et al., 2009; Román-Trufero et al., 2009). Important roles of the PRCs have also been described in epidermal stem cells, skeletal and cardiac muscle, hepatic stem cells, and in the skeletal system (Table S1).

While many studies have shown the requirement of PRCs for maintaining the differentiation capacity of both mESCs and tissue-specific stem cells, PRC components appear to be specifically required for self-renewal of a wide range of tissue-specific stem cells. The basis for this differential requirement is not entirely clear, but the consideration of several factors could provide some explanation: The use of defined media and culture conditions might influence the outcome of loss-of-function studies, as illustrated by the fact that some of the phenotypes observed in mESCs grown in serum/LIF have been refuted by

the introduction of 2i/LIF-based medium yielding more homogeneous cell populations influenced by fewer environmental cues. Furthermore, lineage-committed tissue-specific stem cells residing in more complex and heterogeneous environments or grown in less well-defined media outside their niche might be more sensitive to the loss of chromatin factors. During differentiation, the chromatin environment changes to a more restrictive conformation with accumulation of repressive chromatin marks such as H3K27me3 (Zhu et al., 2013). In the context of lineage-committed cells, loss of PRC2 would influence this organization, leading to failures in differentiation and/or altering developmental potential, as illustrated by the enhanced plasticity observed in *Ezh2*-deficient T cells (Tumes et al., 2013).

***Hdac1- and Hdac2-Containing Complexes in Tissue Stem Cells and Development.*** While Hdac1 and Hdac2 are considered to act redundantly in most cell types, important exceptions to this view include distinct roles during early embryogenesis, where Hdac1 is essential and required for proliferation through repression of cell-cycle inhibitors (Lagger et al., 2002). In addition, conditional knockout studies with combinatorial ablation of *Hdac1* and *Hdac2* demonstrate distinct roles during epidermal development, where loss of a single allele of *Hdac2* in an *Hdac1* knockout background leads to developmental defects (Winter et al., 2013), and the opposite situation in neuronal development, where *Hdac1* haplo-insufficiency is observed in *Hdac2* knockouts (Hagelkruys et al., 2014).

Studies in knockout mice and tissue-specific stem cells show important roles of Hdac1 and Hdac2-containing complexes in many different tissues, including roles of NuRD, Sin3, and CoREST in the hematopoietic system (Cowley et al., 2005; David et al., 2008; Kerényi et al., 2013; Williams et al., 2004; Yao et al., 2014; Yoshida et al., 2008; Zhang et al., 2012b), roles of NuRD in epidermal stem cells (Kashiwagi et al., 2007), and roles of REST/CoREST and Lsd1 in neural stem cells and during neural development (Qureshi et al., 2010; Sun et al., 2010; Wang et al., 2007a) (Table S2).

Taken together, chromatin repressive complexes are essential for establishing and maintaining cell identity during tissue development and homeostasis, in part through their ability to restrict the expression of important cell-cycle regulators and key developmental genes. One emerging picture is that subunit composition and association with specific interactors provide important means for regulating the function of the complexes in different cell types and developmental stages. Further elucidation of the molecular basis of tissue-specific functions of repressor complexes will be crucial for understanding the consequence of their deregulation in cancer.

### **Chromatin Repressive Complexes in Cancer**

Many cancers display a dedifferentiated stem cell-like phenotype, and several of the factors required for establishing or maintaining stem cell states are also involved in oncogenesis. Thus, bearing in mind that chromatin repressors are crucial for establishing and preserving cellular identity, it is to be expected that chromatin repressors would often be found deregulated in human cancers. Intense research is going into elucidating the mechanism by which chromatin modifiers and modifications promote cancer development or progression. One of the early recurring questions in cancer epigenetics was that of “cause or consequence,” that is, whether the chromatin environment

is deregulated as a consequence of the cancer or if the chromatin regulators play a direct role in driving oncogenesis. However, recent discoveries of copious numbers of recurrent somatic mutations in genes encoding chromatin-associated proteins argue that a deregulated chromatin environment can play a causal role in the disease (You and Jones, 2012). In the following, we will discuss reports of deregulated chromatin repressors and their emerging roles as targets for anticancer therapeutics.

***Polycomb Repressive Complexes and Cancer.*** Increased levels of EZH2 have been correlated with poor outcome in metastatic prostate cancer and poor prognosis in tumors of other tissues (Bracken et al., 2003; Kleer et al., 2003; Takawa et al., 2011; Varambally et al., 2002; Wagener et al., 2010). Recently, recurrent point mutations in the SET domain of EZH2 have been described in diffuse large B cell lymphoma and follicular lymphoma, conferring hyperactivity of EZH2 yielding increased levels of H3K27me3 (Béguelin et al., 2013; Lohr et al., 2012; McCabe et al., 2012a; Morin et al., 2010; Pasqualucci et al., 2011; Ryan et al., 2011). Further evidence for direct roles of H3K27 methylation in cancer includes loss-of-function mutations of the demethylase UTX (Dalgliesh et al., 2010; van Haafden et al., 2009) and the recent discoveries of somatic mutations of lysine 27 in H3.3 in pediatric glioblastoma (Schwartzentruber et al., 2012; Wu et al., 2012). However, this mutation has been shown to inhibit PRC2 activity, leading to lower H3K27me3 levels. Loss-of-function mutations of EZH2, as well as SUZ12, EED, and JARID2, have been identified in myeloid cancers (Ernst et al., 2010; Nikoloski et al., 2010; Puda et al., 2012; Ueda et al., 2012) and T-ALL (Ntziachristos et al., 2012; Simon et al., 2012; Zhang et al., 2012a), as well as cancers of other tissues. Thus, the role of PRC2 in cancer is highly context dependent with EZH2 exerting functions as an oncogene as well as a tumor suppressor.

Indications of PRC1 involvement in human cancer include increased expression levels of BMI1 and correlation with poor prognosis in a range of solid tumors and hematological cancers (He et al., 2009; Mohty et al., 2007; Nowak et al., 2006; Shafaroudi et al., 2008) and reports of oncogenic functions of CBX7 in the hematopoietic system (Klaue et al., 2013; Scott et al., 2007) and some solid tumors (Shinjo et al., 2013; Zhang et al., 2010), as well as tumor suppressor roles in others (Forzati et al., 2012).

***HDAC1- and HDAC2-Containing Complexes and Cancer.*** While somatic mutations in HDACs are rare, there are many reports of HDAC1 and HDAC2 being overexpressed in human cancers, often correlating with poor patient outcome. In contrast, there is also data on cancer-associated loss-of-function mutations of HDAC1 and HDAC2, and knockout mouse models show that these proteins can also exert tumor suppressive roles (West and Johnstone, 2014). In addition, HDACs are aberrantly recruited to target genes in many cancers, in part due to an altered expression level of specific subunits of the HDAC-containing complexes. The SIN3-associated protein BRMS1 is often lost in invasive stages of human cancers (Hurst, 2012), and ING1 and ING2 are often mutated or downregulated in human cancers, pointing to tumor suppressive roles of SIN3 (Guérillon et al., 2014). The MTA subunits are the most studied components of NuRD with a role in cancer. As the name implies, MTA1 (metastasis associated gene 1) was originally identified

by its preferential expression in a metastatic tumor model, and increased expression in a wide range of tumors correlates with tumor grade and poor prognosis (Nicolson et al., 2003). Interestingly, there is an inverse correlation between MTA1 and MTA3 expression during cancer progression, and MTA3 seems to play mainly tumor suppressive functions, thus pointing to the importance of specific subunit composition in regulating complex function (Lai and Wade, 2011). ZNF217 is overexpressed in cancers and was found to recruit CoREST to the *INK4B* locus (Thillainadesan et al., 2012), thus promoting proliferation, and LSD1 expression is elevated in many human cancers (Helin and Dhanak, 2013) but is also reported to be downregulated and involved in the suppression of metastasis in breast cancers (Wang et al., 2009b). Whether LSD1 exerts its functions in cancer mainly as a subunit of NuRD, CoREST, or along with additional factors remains to be elucidated.

**Molecular Mechanisms of Chromatin Repressive Complexes in Cancer.** The mechanisms by which repressive-complex proteins contribute to oncogenesis include their roles in repressing genes activated by stress signals and involved in proliferation. PcG proteins bind the *INK4A-ARF-INK4B* locus, and overexpression of PcG proteins prevents expression of p14 (ARF), p15 (INK4B), and p16 (INK4A) in response to stress signals, including oncogenes (Bracken et al., 2007). Although cancer cells generally display global DNA hypomethylation, CpG islands of tumor-suppressor genes are often aberrantly methylated in cancer. Interestingly, PcG binding has been suggested to predispose promoters for DNA hypermethylation (Ohm et al., 2007; Schlesinger et al., 2007; Widschwendter et al., 2007). Oncogenic fusion proteins have been implicated in aberrant targeting of repressive complexes to chromatin, including PLZF-RAR $\alpha$ -mediated recruitment of PRC1 and PML-RAR $\alpha$ -mediated recruitment of PRC2, DNMTs, and NuRD in leukemia (Lai and Wade, 2011; Richly et al., 2011). NuRD, LSD1, and PcG proteins have been shown to promote the epithelial-to-mesenchymal transition (EMT) through TWIST- or SNAIL1-mediated downregulation of E-cadherin (Tam and Weinberg, 2013), and EZH2 overexpression seems to promote tumor angiogenesis (Lu et al., 2010). Another important aspect is the role of repressive complexes in governing cell identity: indeed, aberrant expression of PcG proteins helps sustain a dedifferentiated phenotype as seen for instance in rhabdomyosarcoma, where knockdown studies and application of specific inhibitors targeting EZH2 are able to partially reinstate muscle cell identity to the tumor cells (Marchesi et al., 2012).

Although the involvement of chromatin repressive complexes in cancer is indisputable, their functional role in oncogenesis is still incompletely understood as they promote oncogenesis in one setting, while protecting against malignant transformation in another. This duality is probably related to the role of chromatin modifiers in modulating transcriptional output of target genes with opposing functions. Rather than directly deciding the transcription programs, alterations in the level of chromatin regulators changes the threshold for transcriptional activation or repression and this altered chromatin balance sensitizes the cell to stimuli promoting oncogenic transformation. Deciphering the role of repressive complexes in specific cancer types will be important for furthering our understanding and guiding new therapies.

### Targeting Chromatin Repressive Complexes in Cancer

While genetic lesions are difficult to target therapeutically, targeting the deregulated chromatin environment is tempting due to the reversibility of the system. Several drugs targeting chromatin modifiers are already being used in the clinic. Most famously, DNA methyltransferase inhibitors are used to treat patients with MDS, where they prolong lifespan and prevent the progression to leukemia (Helin and Dhanak, 2013).

**HDAC Inhibitors.** Another class of molecules already being used in the clinic is HDAC inhibitors, which are currently used in the treatment of T cell lymphoma. The FDA-approved Vorinostat and Romidepsin target class I HDACs and are able to inhibit the function of HDAC1 and HDAC2 in the context of SIN3, NuRD, and CoREST (Khan and La Thangue, 2012; West and Johnstone, 2014). The molecular mechanisms of these drugs are still incompletely understood, but treatment outcomes include cell-cycle arrest via induction of p21, increased apoptosis, antiangiogenic effects via HIF1 inhibition, and sensitization of cancer cells to DNA-damaging agents (Khan and La Thangue, 2012). Despite these encouraging results, there is no clear correlation between acetylation levels and clinical outcome upon HDAC inhibition. In addition, it has been difficult to establish robust biomarkers to predict efficacy, and, thus far, these drugs are limited to treatment of specific hematological cancers (Helin and Dhanak, 2013).

**LSD1 Inhibitors.** In addition to HDAC inhibitors, LSD1 inhibition represents a route of targeting complexes such as NuRD and CoREST. Two recent studies have provided evidence for an important role of LSD1 in acute myeloid leukemia (Harris et al., 2012; Schenk et al., 2012). Neither study observes any global effect on histone methylation, but both report localized increases in H3K4me2 at specific promoters, including the differentiation marker CD11b (Schenk et al., 2012), and certain MLL-AF9 targets (Harris et al., 2012). These studies raise several questions regarding the function of LSD1 in leukemia: First, LSD1 binds throughout the genome as part of several different complexes, yet the effects on H3K4me2 are very localized. Second, although LSD1 inhibition leads to an increase in CD11b expression (Schenk et al., 2012), the increased H3K4me2 at MLL-AF9 target genes is actually accompanied by lower expression (Harris et al., 2012), which is surprising considering that H3K4me2 is usually associated with gene expression. Thus, although these studies are encouraging, the mechanisms underlying the differentiation and apoptosis-inducing properties of LSD1 inhibitors remain to be elucidated.

**EZH2 Inhibitors.** With EZH2 being overexpressed in many cancers and the recent reports on hyperactive EZH2 mutants in follicular lymphoma and diffuse large B cell lymphoma, specific EZH2 inhibitors are attracting interest as potential anticancer drugs. Several highly selective compounds show promising results in reducing H3K27me3 levels, decreasing proliferation, and increasing apoptosis in lymphoma cell lines carrying SET domain mutations and markedly reducing tumor burden and increasing survival in mouse xenograft models (Knutson et al., 2014; Knutson et al., 2012; McCabe et al., 2012b; Qi et al., 2012), and two EZH2 inhibitors have entered clinical trials ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)). Interestingly, EZH2 inhibition has also been shown to inhibit the growth of rhabdoid tumors and lowering intratumor levels of H3K27me3, potentially expanding

the therapeutic range beyond hematopoietic malignancies (Knutson et al., 2013). These pediatric tumors arise from a loss of the SNF5 component of the SWI/SNF remodeling complex (Versteeg et al., 1998). They display elevated EZH2 levels, and conditional knockout of *Ezh2* has been shown to reduce tumor growth (Wilson et al., 2010). Interestingly, this type of tumor only carries few somatic mutations (Lee et al., 2012), potentially making them more dependent on the chromatin environment and one might speculate that other tumor types with few somatic mutations could show similar vulnerability to PRC2 inhibitors. Importantly, despite clear effects on prohibiting cancer growth, it has not been possible to identify consistent transcriptional profiles being reverted upon treatment with EZH2 inhibitors (McCabe et al., 2012b). This lack of consistency indicates that EZH2 targets different pathways even within the same types of tumors, in agreement with a role of chromatin factors in threshold modulation as opposed to directly deciding the transcriptional outcome. With EZH2 exhibiting characteristics of an oncogene as well as a tumor suppressor even within hematological cancers, it will be important to develop tools and biomarkers to predict efficacy of targeting EZH2 therapeutically and to stratify patients accordingly.

**Alternative Ways of Targeting Polycomb Repressive Complexes.** In the context of targeting PcG proteins in cancer, several new drugs are currently being tested. One approach, targeting the EED-EZH2 interface by treatment with a stabilized  $\alpha$ -helix of EZH2, shows disruption of PRC2 complex formation, lower levels of H3K27me<sub>3</sub>, growth arrest, and differentiation of MLL-AF9 driven leukemic cells (Kim et al., 2013). In the context of targeting PRC1, application of small-molecule BMI1 inhibitors reduced global H2AK119ub1 in colorectal cancer cells and decreased tumor load in transplanted mice through a depletion of cancer-initiation cells (Kreso et al., 2014). Another potential approach to targeting PRC1 is by chromodomain inhibitors targeting the CBX-component. Recently, Simhadri et al. reported on the development of a chromodomain antagonist with 10- to 400-fold selectivity for CBX7 over other CBX family members (Simhadri et al., 2014). In addition, studies of BET (bromodomain and extra-cellular) domain inhibitors targeting BRD4 indicate that targeting domains recognizing histone modifications is therapeutically feasible (Di Croce and Helin, 2013). With its oncogenic roles in the hematopoietic system and CBX7 being preferentially involved in undifferentiated cell types, it will be interesting to explore CBX7 inhibition as a strategy for targeting PRC1 in cancer.

### Concluding Remarks

The chromatin environment is an important factor in the establishment and maintenance of cell identity. Accordingly, the protein complexes modulating chromatin are important for many aspects of mammalian development and stem cell function and are often deregulated in cancers. Although the introduction of DNA demethylating agents and HDAC inhibitors in the clinic provides proof-of-concept of the feasibility of targeting the chromatin environment in cancer, and the ongoing development of drugs targeting chromatin modifiers show promising results in preclinical trials, the mechanisms underlying their efficacy are not understood. Thus, further elucidation of the role of chromatin repressive complexes in cancer and the development of robust predictive biomarkers will be paramount in the implementation of personalized therapies to improve patient outcome.

### SUPPLEMENTAL INFORMATION

Supplemental Information includes two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.stem.2014.05.006>.

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