CellPress

Cell Stem Cell
Perspective

Polycomb/Trithorax Antagonism: Cellular Memory in Stem Cell Fate and Function

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https://doi.org/10.1016/j.stem.2019.03.005

Stem cells are continuously challenged with the decision to either self-renew or adopt a new fate. Self-renewal is regulated by a system of cellular memory, which must be bypassed for differentiation. Previous studies have identified Polycomb group (PcG) and Trithorax group (TrxG) proteins as key modulators of cellular memory. In this Perspective, we draw from embryonic and adult stem cell studies to discuss the complex roles played by PcG and TrxG in maintaining cell identity while allowing for microenvironment-mediated alterations in cell fate. Finally, we discuss the potential for targeting these proteins as a therapeutic approach in cancer.

Stem cells provide an essential building block for tissue regeneration and repair throughout life. In the developing embryo they mediate organogenesis, whereas in the adult organism they take up residence in specialized niches where they contribute to tissue growth or regeneration. Stem cells possess the unique capacity to self-renew or to differentiate into defined sets of specialized cells. This decision (self-renewal versus differentiation) is tightly regulated to permit efficient tissue repair while ensuring maintenance of the stem cell population. Within the niche, growth factors provide extracellular cues through the activation of specific signaling pathways such as Wnt, Notch, and Hedgehog (reviewed in Fernandez-Alonso et al., 2017). Transduction of these signals to the nucleus activates specific gene expression programs whereby the cells either "remember" their identity as stem cells (self-renew) or partially erase their cellular memory to take on a new identity (differentiate).

Key players of the cellular memory system were initially identified through genetic studies in *Drosophila* that revealed the existence of a functional antagonism between the Polycomb group (PcG) and the Trithorax group (TrxG) protein families. Identified first, members of the PcG family were functionally defined as genes that ensure proper segmentation of the *Drosophila* embryo (Lewis, 1978). Subsequently, TrxG family members were identified through genetic screens for mutations that rescue segmentation defects in PcG-mutated flies (Kennison and Tamkun, 1988). Importantly, PcG and TxG proteins are conserved in mammals, and molecular studies showed that they work as epigenetic regulators of gene expression that modify chromatin structure to facilitate (or prevent) access of the transcriptional machinery to specific genes. Molecular characterization of PcG and TrxG proteins revealed a large diversity of enzymatic functions contributing to each group, yet a clear distinction exists between PcG proteins that act to maintain genes in a transcriptional repressed state through chromatin compaction and TrxG proteins that antagonize repression to maintain a transcriptional permissive chromatin environment (Schuettengruber et al., 2017). Thus, the PcG-TrxG antagonism, initially identified in *Drosophila*, is conserved in mammals as a means of maintaining and transmitting cellular memory. The importance of TrxG-PcG antagonism to the precise control of cell-fate decisions is further underscored by the high prevalence of mutations of these genes in cancer.

In this Perspective, we discuss the complex roles played by mammalian PcG and TrxG proteins in regulating cell-fate decisions in stem cells. Throughout the review, we use a broad definition for these proteins, including all mammalian orthologs of functionally defined *Drosophila* PcG and TrxG as well as all proteins that are part of the mammalian PcG (PRC1 and PRC2) and TrxG (SWI/SNF, Trx, Trr, and Set1) protein complexes. Drawing on studies from embryonic stem cells (ESCs) as well as the hematopoietic and skeletal myogenesis systems, we highlight specific examples that demonstrate contrasting roles for PcG and TrxG proteins in the cell-fate decision process, as well as reveal the importance of the cellular environment in modulating PcG and TrxG functions to decide between self-renewal and differentiation. Finally, we discuss the potential for targeting PcG and TrxG proteins for therapeutic purposes in cancer.

Mechanisms of PcG Protein Function

PcG proteins play a key role in preserving cellular memory of transcriptional repression at developmentally important genes. In essence, their role is to ensure that developmentally regulated genes do not become expressed outside of their spatial and temporal contexts. How does this repression occur? Based on the extensive list of PcG proteins and the diversity of their attributed functions, gene repression appears to occur through multiple processes, which include histone methylation, ubiquitylation, nucleosome remodeling, protein *O*-GlcNAcylation, and chromatin binding (Schuettengruber et al., 2017). Two protein complexes (Polycomb repressive complexes 1 and 2; PRC1 and PRC2) serve as the current paradigm for understanding PcG-mediated transcriptional repression. Here we will review our current understanding of the mechanisms through which PRC1 and PRC2 repress gene expression.

PRC1

PRC1 complexes (see Figure S1) are characterized by the presence of the PcG proteins RING1 and PCGF that are responsible

for ubiquitinating histone H2A at lysine 119 (H2AK119ub). Biochemical studies with recombinant proteins revealed that PRC1 complexes act to establish a compact chromatin structure (Francis et al., 2004). Furthermore, examination of nuclear architecture using chromatin conformation capture techniques showed that chromatin compaction occurs through looping of PRC1-associated loci that assemble into repressive domains spanning up to 140 kb (Kundu et al., 2017). Although PRC1-repressed genes are often inferred by the presence of H2AK119ub, this repression-associated histone mark is not required to mediate chromatin condensation and repression of the Hox locus (Eskeland et al., 2010). Instead, polymerization of PRC1 complexes (Isono et al., 2013) appears to allow for compaction of chromatin by bridging adjacent nucleosomes (Lau et al., 2017). Interestingly, even though PRC1-mediated chromatin compaction is commonly thought to limit DNA accessibility, RNA polymerase II (RNA Pol II) and p300-CBP have been detected at PRC1-repressed genes (Breiling et al., 2001), suggesting that binding of PRC1 does not precede the engagement of RNA Pol II but instead impedes transcriptional elongation by preventing access of additional co-factors (Lehmann et al., 2012). Work is still ongoing to further understand the mechanism through which PRC1 mediates transcriptional repression.

PRC2

PRC2 complexes (see Figure S2) are characterized by the presence of one "Enhancer of Zeste" subunit (either Ezh1 or Ezh2) as well as Suz12 and Eed. The Ezh1 or Ezh2 subunit acts as a methyltransferase to introduce the H3K27me3 repressive mark characteristic of PRC2-repressed chromatin. PRC2 complexes containing the PALI1 and EPOP accessory subunits (PRC2.1) show increased H3K27 methyltransferase activity compared to PRC2 complexes (PRC2.2) that lack these proteins (Beringer et al., 2016; Conway et al., 2018). Although the H3K27me3 mark itself does not induce aggregation, it likely serves as a docking site for additional proteins that facilitate compaction. These additional proteins include (1) Eed, which allows propagation of the repressive H3K27me3 mark across CTCF-delimited topologically associating domains (TADs), and (2) the chromodomain-containing Cbx proteins that are part of the PRC1 complex. From these data, a model has been proposed whereby association of PRC2 with nucleosomes is sufficient to induce a moderate level of chromatin compaction (Margueron et al., 2008), but PRC1-association with the H3K27me3 mark is required to further increase compaction and establish the large condensed aggregates characteristic of heterochromatin. Interestingly, PRC1 and PRC2 do not always co-localize to specific loci (Ku et al., 2008) and PRC2 is able to repress transcription in the absence of PRC1 (Iovino et al., 2013), suggesting different PcG-mediated repression mechanisms at different developmentally regulated genes.

Mechanisms of TrxG Protein Function

TrxG proteins are essential to mediate spatiotemporal activation of developmentally regulated genes. Mechanistically, they work through counteracting the repressive effects of PcG-mediated chromatin compaction. This has been demonstrated first in *Drosophila*, whereby it was shown that proper Hox gene expression can be recapitulated in TrxG mutant flies if they also have a compounding PRC2 mutation (Klymenko and Müller, 2004). Two types of complexes have emerged to mediate this de-repressive activity: (1) the COMPASS-like methyltransferase and demethylase complexes, and (2) the ATP-dependent SWI/SNF chromatin remodeling complexes. The mechanism through which TrxG proteins counteract PcG-mediated repression remains the focus of intense studies, but growing evidence suggests that TrxG proteins work through the eviction of PcG proteins from specific loci.

COMPASS-like Methyltransferase Complexes

Mammals have three COMPASS-like complexes (see Figure S3) that are defined by the presence of one subunit with a histone H3 lysine 4 (H3K4) methyltransferase activity—Set1 (Set1A or Set1B), Trx (MLL1 or MLL2), or Trr (MLL3 or MLL4)—as well as a core set of common subunits that include Ash2L, WDR5, RBBP5, and DPY30. The main function of these complexes is to maintain gene loci in a transcriptionally permissive state. Notably, COMPASS complexes are all able to methylate H3K4 but with different specificities. On the one hand, Set1- and Trx-COMPASS are responsible for introducing the H3K4me3 mark (Hu et al., 2013b) that serves as a docking site for multiple transcriptional factors at gene promoters, including the TAF3 subunit of the general transcription factor TFIID, the chromatin remodeling factor CHD1, and the core COMPASS subunit WDR5 (Kamps et al., 2015). On the other hand, the Trr-COMPASS complex mediates H3K4 mono-methylation (H3K4me1) at enhancers, which stabilizes binding of the TIP60 acetyltransferase complex (Hu et al., 2013a). Furthermore, Trr-COMPASS uniquely contains a histone demethylase subunit, UTX, that is able to actively remove the repressive H3K27me3 mark, thus directly counteracting PRC2-mediated repressive function. Besides H3K4 methylation and H3K27 demethylation, Trr-COMPASS also contributes to maintaining an open chromatin state through additional mechanisms that include (1) MLL4-mediated recruitment of the acetyltransferases p300-CBP (Dorighi et al., 2017) to mediate H3K27 acetylation (H3K27ac) at enhancers, which in turn antagonizes PRC2-mediated H3K27 methylation, and (2) UTX-mediated recruitment of the SWI/SNF chromatin remodeling complex to open chromatin structure at specific loci (Miller et al., 2010). Interestingly, acetylation events mediated by p300-CBP have been shown to further stabilize SWI/SNF binding to facilitate chromatin opening. Finally, it has been proposed that H3K4 methylation of chromatin prevents DNA methylation (Ooi et al., 2007) to facilitate DNA binding by site-specific DNA-binding transcription factors (TFs). Thus, the COMPASS-like methyltransferase complexes act to maintain open chromatin structure at specific loci to facilitate stable association of transcriptional activators.

SWI/SNF Complex

SWI/SNF complexes are characterized by the presence of either BRM (SMARCA2) or BRG1 (SMARCA4) subunits that possess an ATP-dependent DNA helicase activity. Recent literature has suggested further subdivisions within the SWI/SNF family (Kadosh and Crabtree, 2015), revealing the existence of cell-specific SWI/SNF complexes such as the ESC-specific esBAF complex (see Figure S4). However, these differences in subunit composition between various types of SWI/SNF complexes likely reflect a need for differential recruitment to specific loci rather than differences in their mechanism of action.

Functionally, the BRM and BRG1 subunits of SWI/SNF possess an ATP-dependent DNA helicase activity, allowing them to unwrap DNA from histones by distorting the histone octamer. This unwrapping allows for sliding of nucleosomes across

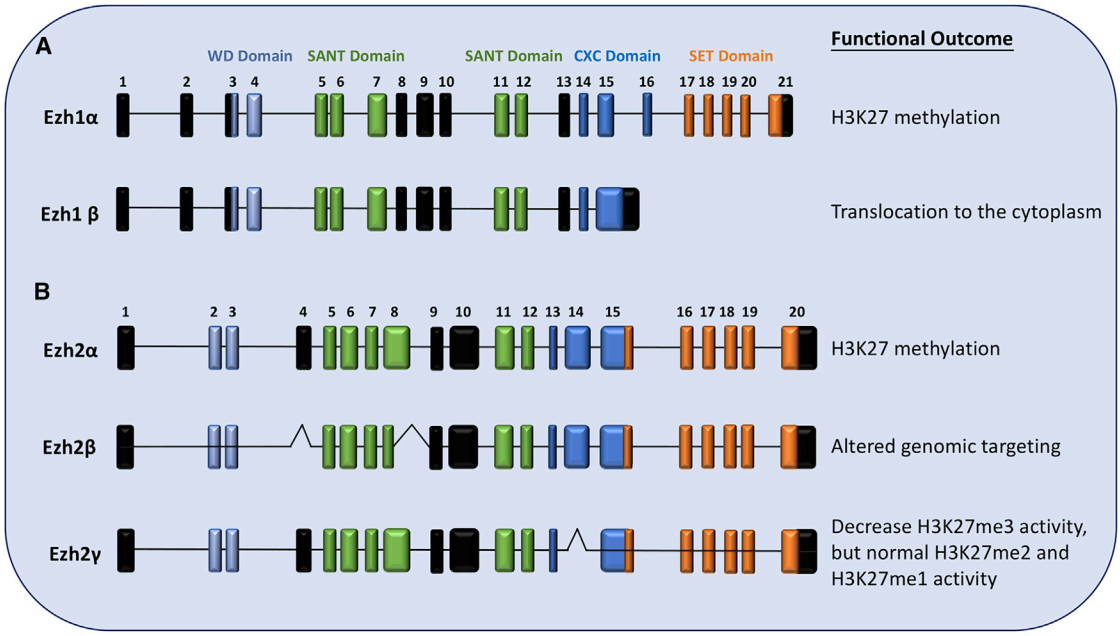

Figure 1. Alternative Splicing of Ezh1 and Ezh2 Alters Their Functional Activity
The exon structure of different splice variants of (A) Ezh1 and (B) Ezh2 are shown. The positioning of the WD (light blue), SANT (green), CXC (dark blue), and SET (orange) domains are indicated.

DNA (Sinha et al., 2017). In the case of BAF complexes, BAF250A and BAF250B subunits also provide a ubiquitin ligase activity that targets histone H2B lysine 120 (H2BK120ub) to prevent chromatin compaction and promote transcriptional elongation (Fierz et al., 2011). Even though a mechanism of action has not yet been established, the SWI/SNF complexes can also act to evict PRC1 and PRC2 from enhancers at target loci (Kia et al., 2008; Stanton et al., 2017). Thus, SWI/SNF helps to establish transcriptional competency by modifying the position of nucleosomes within promoters and enhancers while also excluding PcG proteins from binding at these positions.

PcG Complexes as Activators?

Recent studies have suggested that PRC1 and PRC2 can be found associated with actively transcribed genes (Cohen et al., 2018; Frangini et al., 2013; Gao et al., 2014; Stojic et al., 2011; van den Boom et al., 2016; Zhao et al., 2017). We note that in many of those cases, PRC1 and PRC2 complexes appear to act as rheostats to help control (reduce) transcriptional output from active genes, thus in fact still mechanistically acting as repressors (Cohen et al., 2018). Interestingly, examination of PcG function in the regulation of 3D genome structure has provided important clues into the apparent ''activation'' function of PRC2. Indeed, it was shown that PRC2 promotes spatial proximity between specific promoters and enhancers (Gentile et al., 2018). However, even in those cases, true gene activation may only occur after an eventual loss of PcG binding as previously suggested (Creppe et al., 2014). Finally, non-canonical PRC1 and PRC2 complexes have a diverse set of subunits (see Figures S1 and S2), and can directly contribute to the activation of gene expression through inhibition of PRC1 ubiquitin ligase activity (Gao et al., 2014) or through the recruitment of co-activators such as the acetyltransferase p300 (Zhao et al., 2017). The full extent to which canonical PcG complexes contribute to transcriptional activation remains to be established.

Modulation of PcG and TrxG Function in Stem Cells

Splicing of PcG and TrxG Proteins

Although the functional diversity of PcG and TrxG complexes is increased through the use of paralogs for certain subunits, PRC1 and PRC2 can also be functionally modulated through alternative splicing, which leads to the formation of specific protein isoforms with altered functions. The consequences of alternative splicing for PcG function have been thoroughly explored in the case of PRC2, whereby both Ezh1 and Ezh2 have been shown to exist as multiple isoforms in response to alternate exon usage (see Figure 1). For instance, post-mitotic muscle cells express a cytoplasmic Ezh1β isoform that lacks the enzymatic SET domain and acts to sequester the Eed subunit away from nuclear Ezh1α and Suz12, thereby inhibiting PRC2 enzymatic activity (Bodega et al., 2017; Brand and Dilworth, 2017). In response to oxidative stress, Ezh1β is targeted for degradation through ubiquitylation, allowing Eed to enter the nucleus and associate with Ezh1α and Suz12 to repress muscle-specific genes as the cells enter a survival mode (Bodega et al., 2017). Similarly, Ezh2 undergoes alternative splicing to generate several distinct isoforms. For instance, the Ezh2β isoform is generated through exclusion of exon 4 and partial exclusion of exon 8 (via an alternate splice donor site) to create a version of the protein with altered target gene selection (Grzenda et al., 2013). Yet another isoform, Ezh2γ, is generated by exclusion of exon 14, which disrupts the protein's CXC domain (Mu et al., 2018), leading to the formation of an enzyme that

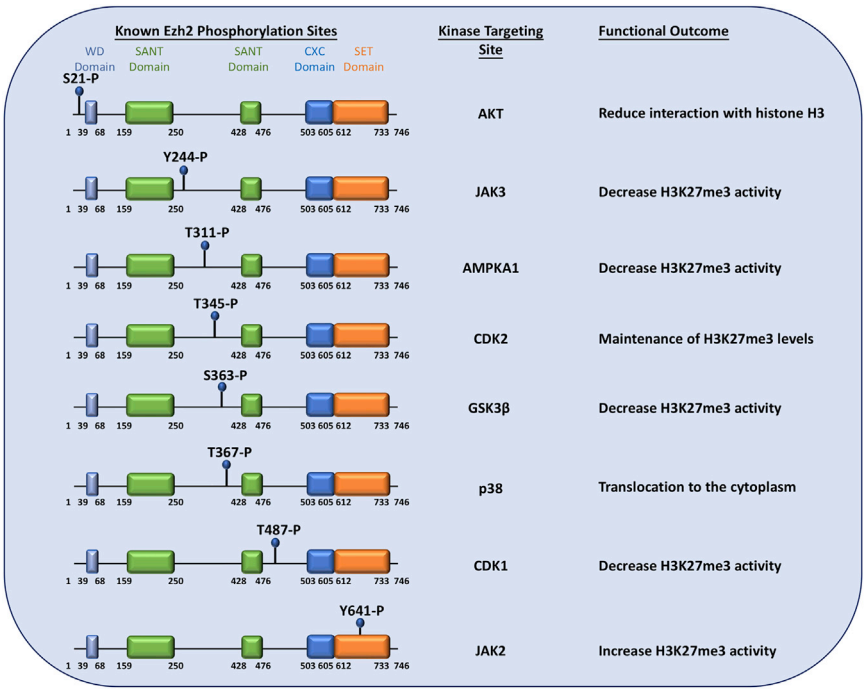

Figure 2. Phosphorylation Sites within the Ezh2 Protein that Alter Its Functional Activity

Amino acid numbers for the start and end of the WD (light blue), SANT (green), CXC (dark blue), and SET (orange) domains are indicated. Blue balloons indicate sites of phosphorylation by the indicated kinase.

efficiently catalyzes H3K27 mono- and di-methylation but cannot generate H3K27me3. Interestingly, this isoform promotes differentiation of ESCs through decreasing H3K27me3 on bivalent genes, which tilts the balance toward a transcriptionally permissive H3K4me3 state on those genes (Mu et al., 2018). Besides Ezh1 and Ezh2, Eed also exists as several isoforms that are generated through alternate promoter usage (Martin et al., 2006). In this case, the distinct isoforms allow for recognition of different histone modifications and may stabilize the complex at different regions of the genome.

Although alternative splicing has not been explored as deeply for TrxG proteins, the TrxG gene Ash2L has been shown to generate two distinct protein isoforms (a long, 80-kDa isoform, Ash2L1, and a short, 60-kDa isoform, Ash2L2) through alternate promoter usage. Interestingly, studies in ESCs showed that even though both proteins contribute to self-renewal, only the loss of the Ash2L1 isoform leads to defects in mesodermal differentiation (Xie et al., 2018), suggesting overlapping and non-overlapping functions. The mechanistic underpinning of these differential functions remains to be established.

Thus, despite a limited number of examples, it is clear that alternative splicing plays an important role in regulating the activity of PcG and TrxG complexes through the generation of alternate protein isoforms with distinct functions.

Post-Translational Modifications of PcG and TrxG Proteins

PcG and TrxG proteins are susceptible to intrinsic and extrinsic signaling cues that modulate their function. For example, studies of post-translational modifications (PTMs) affecting Ezh2 have provided us with an overview of distinct functional outcomes that notably affect the PcG-TrxG antagonism (see Figure 2). First, it has been shown that AKT signaling leads to phosphorylation of Ezh2 at S21, which in turn reduces PRC2 interaction with histone H3, decreasing the levels of H3K27me3 and increasing proliferation of cancer cells (Cha et al., 2005). In contrast, phosphorylation of Ezh2 on a different residue (T487) by the cyclin-dependent kinase CDK1 leads to reduced methyltransferase activity that favors osteogenic differentiation of mesenchymal stem cells (Wei et al., 2011). Phosphorylation of yet another residue (T367) by p38 in breast cancer leads to translocation of the methyltransferase into the cytoplasm, where it is sequestered from its target genes (Anwar et al., 2018). Thus, PTMs can dramatically affect the function of PRC2. An alternate means through which PTMs alter the TrxG-PcG antagonism is via phosphorylation of TFs that recruit the complexes to specific genomic sites. For example, in prostate cells, it has been shown that phosphorylation of ERG at S96 activates transcription through disrupting its interaction with PRC2 and relieving PcG-mediated transcriptional repression (Kedage et al., 2017). In an opposing manner, phosphorylation of TFs can stimulate interactions with PcG and TrxG proteins. For instance, p38-mediated phosphorylation of Mef2D allows for recruitment of Trx-COMPASS complexes to muscle-specific genes (Rampalli et al., 2007). Thus, cellular signaling pathways can influence PcG and/or TrxG function through PTMs of the complexes themselves, or through modification of TFs that target these complexes to their genomic targets.

Targeting PcG and TrxG Proteins to Genomic Loci

Targeting PcG Proteins

Genome-wide analyses of PRC1 and PRC2 binding in ESCs revealed that these proteins cover large (up to 150-kb) domains that are also enriched for H3K27me3 and H2AK119ub, respectively (Boyer et al., 2006; Ku et al., 2008; Kundu et al., 2017). This has led to the suggestion of a 2-step mechanism whereby PcG complexes are first recruited to discrete genomic sites, followed by chromatin spreading to form large repressive chromatin domains (Højfeldt et al., 2018; Margueron et al., 2009). Whereas in *Drosophila*, PcG recruitment is mediated through direct sequence-specific binding of PRC1 and PRC2 complexes to Polycomb response elements (PREs), such a mechanism does not appear to exist in mammals. Instead, PRC1 and PRC2 complexes are recruited through interaction with sequence-specific DNA-binding TFs, such as YY1 in ESCs (Woo et al., 2010) and proliferating muscle progenitors (Caretti et al., 2004), Runx1 in megakaryocytes (Yu et al., 2012), TAL1

in erythroid progenitors (Pinello et al., 2014), and Snail and REST during neuronal differentiation (Arnold et al., 2013).

In addition to sequence-specific TFs, it has been proposed that long non-coding RNAs (lncRNAs) may be involved in recruiting PRC complexes to specific genomic sites. For instance, it has been shown that the lncRNA HOTAIR recruits PRC2 to the HOXD cluster, repressing transcription in *trans* over a large region of 40 kb (Rinn et al., 2007). However, recent studies using artificial tethering of HOTAIR to a transgene showed that repression of the promoter occurs in the absence of PRC2, and that PRC2 is recruited to the gene only after transcription has been turned off (Portoso et al., 2017). Furthermore, PRC2 binding to RNA appears to occur promiscuously (Davidovich et al., 2013) and can antagonize its binding to chromatin (Beltran et al., 2016), suggesting a model whereby PRC2 binding to RNA molecules serves to exclude PRC2 from active genes rather than recruit it to specific genomic locations to mediate transcriptional repression. Thus, the importance of lncRNAs for genomic targeting of PcG proteins remains controversial.

Interestingly, genome-wide analyses in ESCs revealed a strong enrichment for PcG proteins at promoters containing hypo-methylated CpG islands (Boyer et al., 2006). Follow-up studies showed that PRC1 and PRC2 complexes bind to hypomethylated CpG islands at genes that are not transcriptionally active (Mendenhall et al., 2010). Consistent with an important role for non-methylated CpG islands in the recruitment of PcG complexes, introduction of exogenous guanine-cytosine–rich sequences (as short as 220 bp) in the ESC genome is sufficient to mediate binding of PRC1 and PRC2 and to establish large H3K27me3 domains (Jermann et al., 2014). Mechanistically, the binding of PRC1 complexes to these promoters is likely mediated through the non-canonical PRC1 complex subunit KDM2B that recognizes non-methylated CpG islands through its CXXC domain (Wu et al., 2013). In the case of PRC2, recruitment is likely mediated through PCL1 or PCL2 subunits that specifically recognize non-methylated CpG islands (Li et al., 2017). It should be noted that PRC1 and PRC2 are not localized to all hypo-methylated CpG island promoters at non-transcribed genes, suggesting that additional layers of complexity (e.g., sequence-specific DNA-binding TFs) contribute to the targeting of these repressive complexes to specific genes.

Lastly, it has long been proposed that PRC1 is recruited to chromatin through the PRC2-dependent histone mark H3K27me3, which serves as a docking site for the CBX subunits of PRC1 (Di Croce and Helin, 2013). Likewise, the JARID2 subunit of PRC2 recognizes H2AK119ub (Kalb et al., 2014), the histone mark put in place by PRC1, which allows for cooperative binding of the two PcG complexes (Schwartz and Pirrotta, 2014). However, several recent studies suggest that recognition of specific histone marks serves to stabilize binding rather than mediate the recruitment of PRC1-PRC2 complexes to specific sites. For example, loss of H2AK119ub in ESCs only partially disrupts PRC2 association with target loci (Endoh et al., 2012). Furthermore, deletion of both ubiquitin ligase subunits of PRC1 (RING1A and RING1B) in epithelial progenitors leads to a global decrease of PRC2 binding but does not alter PRC2 genomic localization (Cohen et al., 2018), strongly suggesting that H2AK119ub is not involved in targeting PRC2 to specific gene loci. Similarly, even though PRC2 can bind to the H3K27me3 modification that it catalyzes, accurate targeting of PRC2 to its genomic targets can be achieved in the absence of any pre-existing H3K27me3 marks (Højfeldt et al., 2018). Taken together, these studies point to a crucial role for PRC1-PRC2-mediated histone marks (H2AK119ub and H3K27me3) in stabilizing cooperative binding of the PcG complexes rather than mediating their site-specific targeting. Another important role of these histone modifications may be to allow self-propagation through spreading away from targeting sites to generate large repressive domains (Margueron et al., 2009).

Targeting TrxG Proteins

Large-scale changes in the transcriptional program of cells undergoing self-renewal or differentiation require coordinated recruitment of TrxG proteins at specific developmentally regulated genes. TrxG genomic binding is mediated through interactions with multiple sequence-specific TFs. For instance, in ESCs, the TF Oct4 targets COMPASS (Ang et al., 2011) and SWI/SNF (King and Klose, 2017) complexes to specific genes to maintain the pluripotency program. In hematopoiesis, the Trx-COMPASS complex is targeted to erythroid genes through interaction with the TF NFE2 to mediate H3K4 methylation at the β-globin promoters (Demers et al., 2007), whereas in leukemic T cells, Trr-COMPASS is recruited to the genome through interaction with the TF TAL1 to mediate removal of H3K27me3 marks (Benyoucef et al., 2016). Besides ESCs and hematopoietic cells, the mechanism leading to TrxG complex recruitment has been thoroughly explored in muscle differentiation (Figure 3). Upon the decision to differentiate in muscle progenitors, the Six4 homeobox protein recruits Trr-COMPASS to specific genes involved in muscle development whereby the demethylase subunit UTX removes the repressive H3K27me3 mark to activate transcription (Chakroun et al., 2015; Seenundun et al., 2010). At the same time, Trx-COMPASS is targeted to muscle-specific genes through its interaction with the ubiquitously expressed TF Mef2D (Rampalli et al., 2007), whereas the SWI/SNF complex is recruited through a direct interaction between its BAF60C subunit and the muscle-specific transcriptional activator MyoD (Forcales et al., 2012). Interestingly, binding sites for Six4, Mef2, and MyoD are all found within the myogenin gene-proximal promoter (Aziz et al., 2010; Yee and Rigby, 1993) that fully recapitulates myogenin expression during development when placed upstream of a reporter gene (Yee and Rigby, 1993). This suggests that regulation through these sites is sufficient to mediate the development-specific myogenin expression. Although the specificity of TF binding provides a means to coordinate the activation of multiple genes, cell signaling helps control the timing of activation. Indeed, activation of the p38-MAPK signaling pathway allows for phosphorylation of Mef2D that is necessary for high-affinity interaction with Trx-COMPASS (Rampalli et al., 2007) and phosphorylation of BAF60C that is required for its incorporation into the muscle-specific SWI/SNF complex (Forcales et al., 2012). Thus, multiple TFs work in a coordinated manner to establish a transcriptionally permissive state through the recruitment of TrxG proteins at muscle developmental genes.

Upon initial recruitment, additional mechanisms are likely to play an important role in stabilizing the interaction of TrxG proteins with their target loci. Indeed, several TrxG proteins contain "reader" domains that are capable of recognizing DNA or histone modifications. For instance, KMT2A and KMT2B subunits

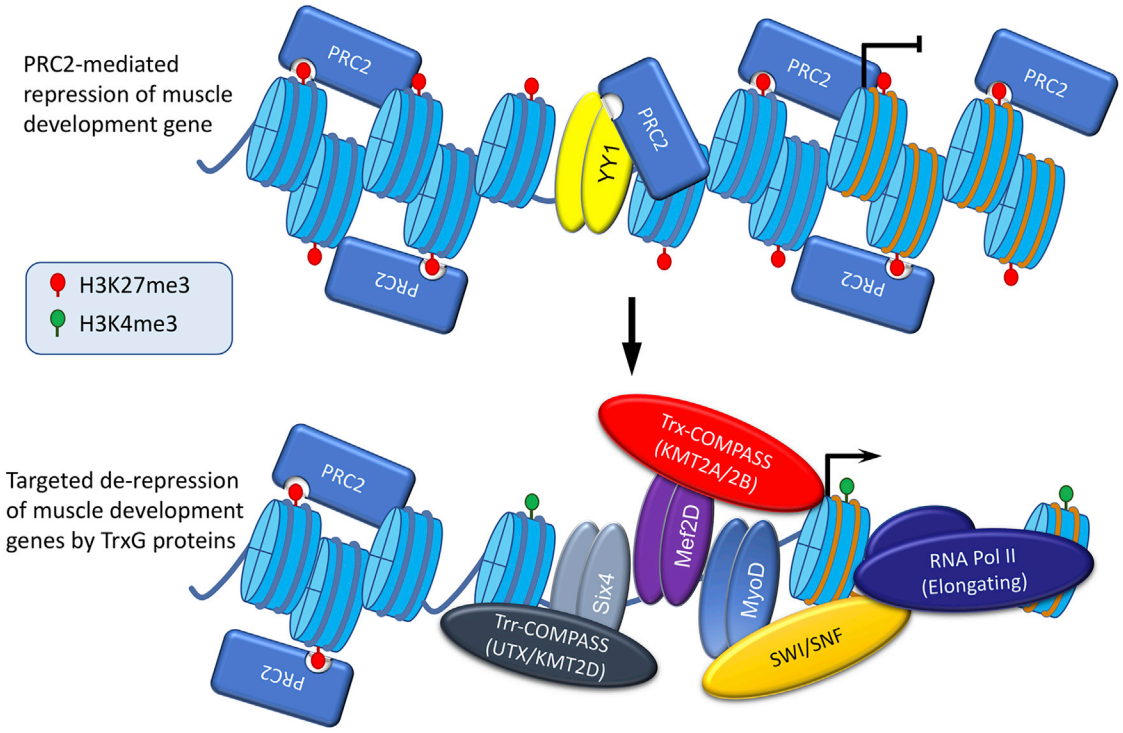

Figure 3. TF-Mediated Targeting of TrxG and PcG Proteins to Muscle Developmental Genes
Top: PRC2-mediated repression of muscle developmental genes. In proliferating MuSCs, the gene encoding the muscle-specific TF myogenin is repressed through the recruitment of the PRC2 complex, which establishes a chromatin environment marked by H3K27me3. The targeting of the PRC2 complex to the myogenin locus is mediated by the DNA-bound TF YY1. Bottom: targeted de-repression of muscle developmental genes by TrxG proteins. Upon environmental cues to undergo differentiation, an exchange of TFs takes place at the myogenin promoter, where YY1 is replaced by the TFs MyoD, Six4, and Mef2D. The binding of MyoD allows for the recruitment of the SWI/SNF nucleosome remodeling complex to the myogenin promoter. Six4 binding allows the recruitment of Trr-COMPASS, which is responsible for removing H3K27me3 marks at the myogenin gene. Finally, Mef2D binding allows for the recruitment of the Trx-COMPASS complex that puts the transcriptionally permissive H3K4me3 mark in place. Thus, the recruitment of different TrxG protein complexes to the myogenin promoter can be explained by targeting through DNA-binding TFs.

of the Trx-COMPASS complex both possess a CXXC domain that binds to non-methylated CpG islands, whereas the Cfp1 subunit of Set1-COMPASS serves the same purpose (Lee et al., 2007). Other examples include WDR5, a protein present in all COMPASS complexes, that recognizes the H3R2me2s (Guarnaccia and Tansey, 2018), as well as the BRG1 (or BRM) and BAF180 subunits of SWI/SNF that all possess bromodomains, allowing stabilization of binding to acetylated histone H3 (Charlop-Powers et al., 2010; Shen et al., 2007).

Finally, it is important to note that TrxG proteins can also facilitate binding of additional TrxG proteins in an enzyme-independent manner. For example, it has been shown that in T cells, the UTX subunit of Trr-COMPASS facilitates binding of SWI/SNF to specific genes in a demethylase-independent manner (Miller et al., 2010). Similarly, the MLL4 subunit of Trr-COMPASS facilitates binding of the TrxG-associated acetyltransferase p300 to enhancers in a methyltransferase-independent manner (Dorighi et al., 2017; Lai et al., 2017).

In summary, multiple interactions help establish stable binding of TrxG proteins to lineage-specific genes where they antagonize PcG-mediated repression. Nevertheless, the initial targeting to specific genes appears to rely on interactions with sequence-specific DNA-bound TFs, similar to what has been observed for PcG proteins.

PcG Proteins in Stem Cells

In both embryonic and adult stem cells, three main mechanisms have been identified through which PcG proteins regulate cell-fate decisions (quiescence versus self-renewal versus differentiation). First, they prevent cell-cycle exit by maintaining cell-cycle inhibitors in a repressed state. Second, they prevent differentiation by maintaining repression at developmentally regulated genes. Third, they suppress alternate cell lineages when cells commit to differentiation.

Promoting Self-Renewal

Maintenance of cell-cycle progression is a key function of PcG proteins in adult stem cells. For instance, canonical PRC1 complexes containing the PCGF4 (Bmi1) (Lessard and Sauvageau, 2003; Park et al., 2003) and Cbx7 (Klauke et al., 2013) subunits were shown to be necessary for self-renewal of hematopoietic stem cells (HSCs). Similarly, the Ezh1-containing PRC2 complex promotes survival of slow-dividing long-term HSCs (Hidalgo et al., 2012). In fact, it is likely that the Ezh2-containing forms of PRC2 complexes also promote cell survival, because exogenous expression of Ezh2 in HSCs allows for continuous serial transplantation of bone marrow in mice (Kamminga et al., 2006). Mechanistically, PRC1 and PRC2 promote self-renewal through repression of the cell-cycle inhibitor p16Ink4a, thereby preventing cell-cycle arrest and p53-mediated cell death.

Similarly, PcG proteins have been shown to promote expansion of muscle satellite cells (MuSCs). Indeed, loss of the PRC1 complex subunit PCGF4 (Bmi1) leads to decreased proliferation of MuSCs, resulting in a reduced capacity to regenerate muscle after injury (Robson et al., 2011). Conversely, exogenous expression of PCGF4 (Bmi1) and telomerase (TERT) in MuSCs derived from a Duchenne muscular dystrophy patient led to increased proliferation and immortalization (Cudré-Mauroux et al., 2003). Interestingly, the persistent expression of PCGF4 (Bmi1) in these cells also blocked their capacity to differentiate, suggesting an important role for PRC1 in inhibiting differentiation (as further described in the next paragraph). Finally, it has been demonstrated that age-related senescence of MuSCs is associated with increased expression of p16Ink4a due to loss of PRC1-mediated gene silencing at the CDKN2A locus (Sousa-Victor et al., 2014). Thus, PcG plays a key role in promoting self-renewal of various adult stem cell populations by preventing cell-cycle exit.

It is interesting to note that PcG complexes do not appear to be required for self-renewal of ESCs. Indeed, mutant ESC populations have been successfully derived that lack the PRC2 subunits Ezh2 (Shen et al., 2008), Eed (Chamberlain et al., 2008), and Suz12 (Pasini et al., 2007), or the PRC1 subunit RING1B (Leeb and Wutz, 2007). As such, it appears that maintenance of the pluripotency gene regulatory network is sufficient to ensure that ESCs do not exit the cell cycle.

Blocking Differentiation

In addition to their function in maintaining cell-cycle progression, PRC1 and PRC2 appear to play a key role in preventing cell differentiation. Indeed, it has been shown that loss of PRC2 in primed ESCs is sufficient to induce spontaneous differentiation toward the mesendodermal lineage (Collinson et al., 2016; Shan et al., 2017). A similar outcome was observed upon deletion of the PRC1 subunit RING1B, which predisposes ESCs to undergo aberrant differentiation through the activation of lineage-specific TFs (Leeb and Wutz, 2007). Mechanistically, genome-wide analyses of PRC1 and PRC2 binding confirmed that these proteins are enriched at genes encoding lineage-specific TFs in ESCs (Boyer et al., 2006; Lee et al., 2006). Furthermore, the enrichment of bivalent H3K27me3 and H3K4me3 marks at the promoters of these developmentally regulated genes suggests that PRC2 is particularly important for maintaining a poised transcriptional state (Bernstein et al., 2006). Interestingly, in naive ESCs, loss of PRC2 also results in an upregulation of developmentally regulated genes but does not lead to loss of self-renewal, suggesting that pluripotency genes dominate over lineage-determining genes in this particular context (Chamberlain et al., 2008; Shen et al., 2008).

In adult stem cells, PRC1 and PRC2 are also known to block differentiation through the suppression of lineage-specific gene expression. Maintenance of the HSC identity is ensured by canonical PRC1 complexes containing Cbx7 (but not Cbx2, Cbx4, or Cbx8) that act to repress genes of the hematopoietic progenitor cell lineage, and thus prevent differentiation (Klauke et al., 2013). A similar role is played by PRC2 complexes in muscle, where loss of Ezh2 in MuSCs leads to premature differentiation toward the myotube cell fate (Juan et al., 2011). Thus, PcG proteins function to maintain the stem cell state by preventing the expression of pro-differentiation genes.

Suppressing Alternate Cell Fates during Differentiation

In addition to their function in preventing stem cell differentiation, PcG proteins play a major role in regulating lineage-specific differentiation through the repression of alternate cell fates. Given the large number of cell types in higher eukaryotes and the great complexity of cell-fate decisions, the suppression of alternate cell fates is probably the most complex role of PcG proteins. It is tempting to speculate that this complexity is reflected at least in part by the remarkable diversity of PRC1 and PRC2 subunit composition, whereby specific subunits mediate cell-specific functions. We highlight some of these cell-specific functions in the remaining paragraph.

PRC2 complexes play an important role in ensuring the efficient specification of the ectodermal lineage, where it has been shown that Ezh2 represses genes of the mesendoderm fate during embryoid body formation (Collinson et al., 2016; Shan et al., 2017). In contrast, canonical PRC1 complexes have been shown to promote the efficient differentiation of ESCs toward the mesodermal lineage by reinforcing repression of both pluripotency genes and ectodermal genes (Morey et al., 2015). Similarly, the non-canonical PRC1 complex containing PCGF1 facilitates efficient ectodermal differentiation through suppression of mesodermal genes (Gao et al., 2012), whereas the non-canonical PRC1 complex containing PCGF5 ensures suppression of all non-blood mesodermal lineages (Chagraoui et al., 2018). As another means of influencing alternate cell fates, PRC1 complexes can act to suppress the expression of specific signaling pathways. For instance, the non-canonical PRC1 complex containing PCGF6 acts to facilitate neural differentiation of ESCs by reinforcing the repression of the SMAD2-TGFβ signaling pathway that would otherwise favor mesoderm and endoderm fates (Yao et al., 2018). In addition, this same non-canonical PRC1 complex plays a key role in silencing germ cell-specific genes in ESCs (Endoh et al., 2017). Adding to the complexity of PcG-mediated repression of lineage-specific genes, canonical PRC1 complexes containing distinct CBX family members prevent expression of different cell-specific gene expression programs. Indeed, the Cbx2-containing PRC1 complexes repress trophoblast, mesodermal, and endodermal lineage genes, whereas Cbx4-containing PRC1 complexes do not repress trophoblast genes but do repress mesodermal and endodermal lineage genes (Morey et al., 2012).

Although the role of PcG complexes in suppressing alternate cell fates is less well characterized in adult stem cells, evidence suggests that they also play an important role in this process. For instance, it has been shown that deletion of RING1A causes T cell progenitors to differentiate toward the B cell lineage, implying that PRC1-mediated repression of the B cell program is essential for T cell development (Ikawa et al., 2016).

Taken together, these findings indicate that PRC1 contributes to cell-fate decisions by creating a compact repressive chromatin environment at specific gene loci (ensuring cell-fate memory by preventing spurious differentiation), conspicuously "avoiding" genes from certain developmental programs that escape repression, thereby providing stem cells with an opportunity to partially erase their memory and take on a new fate.

TrxG Proteins in Stem Cells

In both embryonic and adult stem cells, TrxG proteins contribute to cell-fate decisions through two main mechanisms, both of which entail counteracting PcG-mediated gene repression. First, TrxG proteins are involved in preserving stem cells' "memory of self" through maintaining pluripotency (or multipotency) genes in an active state. Second, TrxG proteins are necessary to establish a new "cell memory" through the activation of lineage-specific gene expression upon differentiation.

Maintaining Pluripotency or Multipotency

Self-renewal of ESCs requires the activity of multiple TrxG proteins that work together to induce and maintain expression of genes from the pluripotency transcriptional network. For example, ESCs express a cell-specific SWI/SNF complex (esBAF) composed of a unique combination of subunits (see Figure S4) that allow targeting of the complex to key genes in the pluripotency network (Ho et al., 2009). Once targeted to pluripotency genes, SWI/SNF promotes transcription by reducing nucleosome occupancy at promoters (Lei et al., 2015). The Set1-COMPASS complex is also essential for pluripotency through ensuring the continued expression of Oct4 (Bledau et al., 2014). Interestingly, Set1-COMPASS supports self-renewal of ESCs in an H3K4 methyltransferase-independent manner (Sze et al., 2017). Similarly, the H3K4 methyltransferase activities of Trx-COMPASS (Ernst et al., 2004; Lubitz et al., 2007) and Trr-COMPASS (Wang et al., 2012, 2016) are dispensable for ESC self-renewal.

Besides ESCs, TrxG proteins also contribute to the expansion of adult stem cell pools. Again, different adult stem cells possess distinct SWI/SNF complexes with variable subunit composition (see Figure S4). For instance, in MuSCs, it has been shown that expansion of the stem cell pool is dependent on the activity of BRG1-containing SWI/SNF complexes, which act to maintain expression of Pax7 (Padilla-Benavides et al., 2015). In HSCs, SWI/SNF complexes containing the BAF45A (Krasteva et al., 2017) and BAF180 (Lee et al., 2016) subunits are required for expansion of the stem cell pool. Interestingly, Set1A-COMPASS is not required for HSC maintenance in homeostasis, even though it plays an important role in preventing attrition of the long-term HSC population in conditions of stress hematopoiesis (Arndt et al., 2018). Seemingly more important for HSC self-renewal, the Trx-COMPASS complex is essential for maintaining the stem cell population as well as for bone marrow to repopulate the niche after transplantation (McMahon et al., 2007). Taken together, the current literature suggests that stem cell self-renewal is mediated via specific TrxG complexes that differ depending on the cell type and cell environment.

Establishing New Cellular Memory at Lineage-Specific Genes

During differentiation, TrxG proteins are required to ensure activation of lineage-specific gene expression programs. Multiple COMPASS complexes have been well studied whereby they seem to have co-evolved to create a memory of active gene expression at developmentally regulated genes. First, the Set1A-COMPASS complex functions in proliferating ESCs to prepare their eventual differentiation. It does this by ensuring that developmentally regulated genes are tri-methylated at H3K4 to create bivalently (H3K27me3/H3K4me3) marked promoters that are poised for activation upon differentiation (Sze et al., 2017). Upon differentiation, TF-dependent targeting of Trr-COMPASS complexes to enhancers of lineage-specific genes allows the activation of cell-specific gene expression programs (Wang et al., 2016). Although the activation of developmental genes entails the promoters transitioning from a bivalent (H3K27me3/H3K4me3) to a monovalent (H3K4me3) state, it is surprising that the H3K27 demethylase activity of UTX (the core component of Trr-COMPASS) is not required for the removal of H3K27me3 marks at these sites during differentiation (Wang et al., 2012). Nevertheless, UTX contributes to mesodermal differentiation of ESCs, where it plays a demethylase-independent role in activating key genes such as Brachyury and Wnt3 (Wang et al., 2012). Finally, the Trx-COMPASS complex is dispensable for ESC differentiation toward the three-germ layers, but is required to activate the primordial germ cell gene expression program (Hu et al., 2017).

The SWI/SNF complexes also play an essential role in mediating ESC differentiation. For instance, BAF250A-containing SWI/SNF complexes are required for ESC differentiation toward mesodermal and endodermal lineages (Gao et al., 2008). Furthermore, inclusion of the SMARCC2 subunit favors ectodermal differentiation at the expense of endoderm formation (Wade et al., 2015). It is likely that lineage preferences of SWI/SNF complexes containing distinct subunits reflect a differential ability to interact with lineage-specific TFs.

In adult stem cells, TrxG proteins also play an essential role in activating differentiation programs. In muscle, for example, it was shown that the exchange of BRG1 and BAF60A-containing SWI/SNF complexes for BRM and BAF60C-containing SWI/SNF complexes permits MuSCs to exit the cell cycle and activate a muscle-specific gene expression program (Albini et al., 2015). Furthermore, all three COMPASS complexes—Trx- (Rampalli et al., 2007), Trr- (Faralli et al., 2016), and Set1- (Vethantham et al., 2012)—contribute to the activation of muscle gene expression. In hematopoiesis, COMPASS complexes also play an essential role in differentiation. For instance, it was shown that deletion of the core subunit DPY30 in HSCs leads to HSC accumulation and blockage in their blood multilineage reconstitution capacity due to failure in the activation of lineage-specific genes (Yang et al., 2016). Finally, examination of SWI/SNF function in hematopoiesis showed that deletion of BRG1 at various stages of T cell development resulted in a stage-specific block in cell-fate transitions (Chi et al., 2003). In summary, TrxG proteins play essential roles in establishing cell-specific gene expression patterns during stem cell-fate transitions toward multiple lineages.

Model of Cell-Fate Decision in the Context of TrxG-PcG-Mediated Cell Memory

Studies described above have clearly established that cellular memory is maintained through PcG-TrxG antagonism, whereby PcG proteins repress gene expression via chromatin compaction whereas TrxG proteins antagonize this repression via chromatin opening at specific loci where they have been recruited through interaction with lineage-determining TFs. Although the maintenance of self-renewal and cell identity in stem cells is critically dependent on a stable PcG-TrxG antagonism, disruption of this antagonism must occur to permit differentiation, which by definition requires partial loss of cell memory to allow cells to

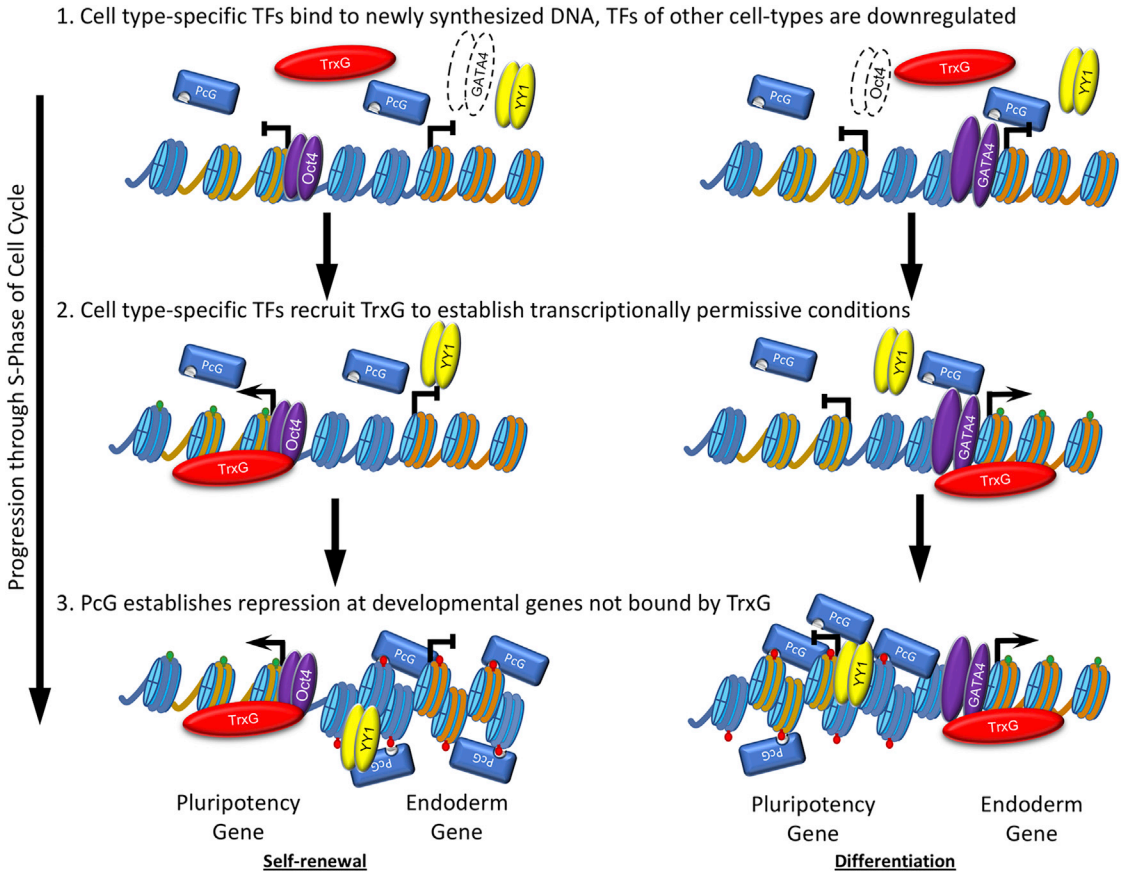

Figure 4. Model for TF-Mediated Recruitment of TrxG Proteins as a Cellular Memory in Stem Cells

In the absence of TrxG protein recruitment, PcG-mediated repression is the default state in developmentally regulated genes. Because the association of PcG proteins with developmentally regulated genes is delayed after DNA replication in S phase of the cell cycle, TF binding at specific genes allows an opportunity for TrxG recruitment prior to the establishment of PcG-mediated repression. Using endodermal differentiation of ESCs as an example, the replicating ESC must make a cell-fate decision to self-renew (left) or differentiate (right). (1) To mediate *self-renewal*, the ESC-specific TF Oct4 (same as a parental cell) is stabilized and becomes associated with its target loci that mediate self-renewal, whereas the endoderm-specific TF GATA4 is degraded. To mediate *differentiation*, the endoderm-specific TF GATA4 is stabilized and becomes associated with endoderm developmental genes, whereas the ESC-specific TF is degraded. (2) The cell-specific TF Oct4 (self-renewal) or GATA4 (differentiation) then recruits TrxG proteins to specific genomic loci to create a cellular memory of the genes that are to be transcribed in the daughter cells—pluripotency genes in Oct4-expressing ESCs, and endodermal genes in GATA4-expressing endodermal cells. (3) All developmentally regulated (mesodermal and ectodermal) genes that are not bound by TrxG proteins are then subjected to PcG-mediated repression as a default. Through such a mechanism, cells would have a long-term memory of the genes it should be expressing (TrxG bound) and those it should be repressing (PcG bound).

establish a new (differentiated) identity. However, it is currently unknown how the PcG-TrxG antagonism is established in the first place, and how it is disrupted to permit differentiation. Specifically, two outstanding questions remain: (1) what is the mechanism through which PcG and TrxG functions are disrupted in multipotent stem cells to permit a change in cell identity upon differentiation, and (2) how is the PcG-TrxG antagonism re-established after the cells have acquired their new identity?

Interesting clues in answering these questions have been provided by studies analyzing PRC1 and PRC2 binding throughout the cell cycle. Indeed, it was shown that PRC1 and PRC2 complexes transiently dissociate from chromatin upon DNA replication in S phase. Interestingly, the delay between PRC1-PRC2 dissociation and re-association after DNA replication provided enough time to permit binding of lineage-specific TFs to their target loci prior to re-establishment of a compacted chromatin structure (Alabert et al., 2014; Petruk et al., 2017). Based on these findings, it is tempting to speculate that replication-mediated PRC1-PRC2 displacement during S phase provides a critical window of opportunity for lineage-specific TFs to recruit TrxG proteins to developmentally regulated loci (see Figure 4). This first step of TrxG recruitment would then be followed by a second step of binding and subsequent spreading of PcG proteins to mediate gene repression over large domains, except at sites that have been previously targeted by TrxG proteins and are thus protected from PcG-mediated repression. At these sites, PRC1-PRC2 binding or repressive function would be antagonized by TrxG proteins, thus re-establishing the PcG-TrxG antagonism. Although such a 2-step model is speculative, it is consistent with previous findings in erythroid cells that DNA replication during S phase is required for differentiation, for activation of the erythroid TF GATA-1, and for a switch to an active chromatin conformation at the β-globin locus (Pop et al., 2010). Furthermore, MuSCs also require a passage through S phase

after differentiation induction to activate a muscle gene expression program (Andrés and Walsh, 1996), further supporting a crucial role for DNA replication in the establishment of a new cell identity. The second step of our model (i.e., PcG binding and subsequent spreading over large chromatin domains) is supported by experiments showing that accurate PcG binding and H3K27me3 methylation can be re-established entirely *de novo* in the absence of prior H3K27 methylation (Højfeldt et al., 2018).

An implication of our proposed model is that the set of lineage-determining TFs that are present during the short window of opportunity after DNA replication is expected to be absolutely central to the establishment of specific cell fates (e.g., self-renewal versus differentiation). Thus, one would expect for such factors to be highly regulated during cell-cycle progression (i.e., repressed to promote self-renewal or expressed to promote differentiation). Interestingly, this is exactly what has been observed for the master regulator of muscle differentiation, MyoD. Indeed, whereas MyoD undergoes S phase-specific degradation through phosphorylation in proliferating MuSCs (Kitzmann et al., 1998) (presumably to prevent differentiation), a point mutant in the MyoD phosphorylation site, which prevents its degradation in S phase, greatly increases MyoD-directed lineage conversion of fibroblasts to myotubes (Kitzmann et al., 1999), thus promoting differentiation.

Interestingly, it has been suggested that, in some cases, cell differentiation can occur in the absence of cell division (Grinenko et al., 2018; Roch et al., 2015). Chromatin immunoprecipitation of PRC1-PRC2 proteins would be particularly informative in such cases to determine whether non-dividing, differentiating cells lose PRC1-PRC2 genomic binding or whether they use another mechanism (e.g., upregulation of pioneer TFs) to overcome PcG-TrxG antagonism and partially "forget" their identity on their way to a new fate.

The Complexity of PcG and TrxG Functions Challenges the Use of "Epi-drugs" as Therapeutic Approaches in Cancer

Given their crucial role in maintaining cell-fate memory, it may not be surprising that PcG and TrxG proteins are often disrupted in cancer (Zehir et al., 2017). Such disruptions include mutations that can be gain of function (GOF) (McCabe et al., 2012; Sneeringer et al., 2010) or loss of function (LOF) (Ernst et al., 2010), as well as can cause protein degradation (Kim et al., 2015) or overexpression (Peng et al., 2017; Varambally et al., 2002). Importantly, transgenic studies in mice have shown that disrupting PcG and/or TrxG proteins can trigger oncogenic transformation *in vivo* (Andricovich et al., 2018; Simon et al., 2012), providing proof of principle that mutations that perturb the abundance or corrupt the function of PcG and TrxG proteins can, at least in some cases, represent "driver mutations" in cancer. Even when PcG-TrxG proteins are not mutated, they can play critical roles as co-factors for oncogenic TFs, reinforcing oncogenic expression programs (Benyoucef et al., 2016; Xie et al., 2017).

The realization that PcG and TrxG proteins play critical roles in cancer prompted the development of small molecules that inhibit their functions (Helin and Dhanak, 2013; Müller et al., 2018). Such "epigenetic drugs" or "epi-drugs" that reversibly modulate enzymatic activities or protein interactions of specific PcG and TrxG family members are generally considered more amenable to clinical applications than therapeutic strategies based on genome alterations. Although a number of epi-drugs have been successfully developed, their therapeutic utilization as cancer treatments remains a considerable challenge, most likely due to the highly complex and often context-specific functions of PcG and TrxG enzymes. Notably, the functional antagonism between the repressive PcG and the activating TrxG proteins that was discovered in stem cells remains at the heart of new strategies to modulate the functions of these proteins in cancer. In the following paragraphs, we use specific examples to highlight some of the main challenges that remain in understanding the role of PcG and TrxG proteins in cancer and in designing methods to manipulate their antagonist activities for therapeutic purposes. We suggest that further drawing from our mechanistic knowledge of these enzymes in stem cells will lead to a better understanding of their aberrant function in cancer and help design highly specific therapeutic strategies for precision medicine.

Understanding the Defect(s) in PcG and TrxG Proteins that Underlie Cancer Development and Maintenance

Although PcG and TrxG proteins are often disrupted in cancer, precisely defining their defects can be difficult. For instance, a recurrent point mutation at the tyrosine residue 641 (Y641) of Ezh2 found in the germinal center B (GCB) subtype of diffuse large B cell lymphoma (DLBCL) and in follicular lymphoma (FL) was initially defined as LOF based on decreased capacity of the Ezh2 mutant to tri-methylate H3K27 *in vitro* (Morin et al., 2010). However, subsequent studies revealed that the Y641 Ezh2 mutant protein is in fact not deficient in its capacity to introduce a methyl mark, but instead presents an altered binding affinity for its substrates with increased affinity for H3K27me1/me2 (and decreased affinity for non-methylated H3K27) compared to wild-type Ezh2 (Sneeringer et al., 2012; Yap et al., 2011). Thus, the Y641 mutation does not inhibit but instead alters the function of Ezh2, such that the enzyme becomes more efficient at converting H3K27me2 into H3K27me3 but less efficient at recognizing non-methylated H3K27 as a substrate. Overall, this GOF mutation (which is always heterozygous) results in an aberrant increase (not decrease) in H3K27me3 global levels in cancer, a conclusion that is the exact opposite of what was expected initially from the predicted model. This example illustrates the critical importance of functional characterization of each mutation in cancer.

In the absence of mutations in PcG and TrxG coding or regulatory DNA sequences, these proteins can still be modified through post-transcriptional mechanisms such as splicing. For instance, Ezh2 is aberrantly spliced in cancer with different, sometimes opposite, effects. Indeed, in renal cell carcinomas, increased expression of the splicing factor SF3B3 leads to the inclusion of exon 14 in Ezh2 that is required for maximal H3K27me3 activity, and enforces the proliferation of cancerous cells (Chen et al., 2017). In contrast, in patients with myelodysplastic syndromes (MDSs), mutations of the SRSF2 splicing factor leads to aberrant introduction of a premature termination codon in Ezh2 mRNA followed by non-sense-mediated decay, which in turn impairs HSC self-renewal, promoting MDSs (Kim et al., 2015; Shiozawa et al., 2018; Shirahata-Adachi et al., 2017). These examples illustrate the fact that even in the

absence of mutations, PcG and TrxG proteins can be disrupted in cancer.

Finally, unaltered PcG and TrxG proteins can also serve as oncogenes through interactions with oncogenic TFs—TAL1 in T-ALL (Benyoucef et al., 2016), estrogen receptor in breast cancer (Xie et al., 2017), and RUNX1 in MDS (Sashida et al., 2014)—that recruit them to aberrant genomic locations where they disrupt cellular memory and establish or maintain an oncogenic gene expression program. Again, these findings highlight the many different mechanisms through which TrxG and PcG functions can be altered in cancer even in the absence of mutations.

Defining the Proper Cellular Context for Effective Therapeutic Strategies

One of the most puzzling observations regarding PcG and TrxG proteins' function in cancer is that these proteins can play either a tumor suppressor or an oncogenic role depending on the cellular environment. This dichotomy may be best understood in hematopoiesis whereby cell-fate branching across multiple lineages has been well characterized. An interesting example of this duality is the PcG H3K27 methyltransferase Ezh2 that is part of the PRC2 complex. On the one hand, LOF mutations in Ezh2 (i.e., tumor suppressor function) are found in malignancies of the myeloid lineage (e.g., MDSs, myeloproliferative neoplasms [MPNs], and acute myeloid leukemia [AML]; Ernst et al., 2010; Nikoloski et al., 2010) and the lymphoid T cell lineage (e.g., T-ALL and ETP-ALL; Ntziachristos et al., 2012; Simon et al., 2012). Consistent with a tumor suppressor role in these lineages, the knockout (KO) of Ezh2 in murine HSCs leads to heterogeneous malignancies of myeloid and lymphoid T origins (including MDS, AML, and T-ALL) (Mochizuki-Kashio et al., 2015; Simon et al., 2012).

In contrast, in malignancies of the B cell lineage (e.g., GCB-type DLBCLs, GC-derived non-Hodgkin lymphomas [NHLs], and FL; Béguelin et al., 2013; Morin et al., 2010; Okosun et al., 2014; Sneeringer et al., 2010), Ezh2 is subject to recurrent GOF mutations or otherwise increased levels/corrupted activity, suggesting an oncogenic role. Consistent with this, expression of Ezh2 GOF mutants contributes to the development of B cell malignancies in transgenic mouse models (Béguelin et al., 2013; Berg et al., 2014; Sneeringer et al., 2010), and inhibition of Ezh2 enzymatic activity is a potent therapeutic in B cell malignancies (Konze et al., 2013).

Although this functional duality (tumor suppressor in myeloid and T cells versus oncogene in B cells) may seem surprising, it reflects, at least in part, Ezh2 functions in hematopoietic stem-progenitor cells. Indeed, PRC2(Ezh2) is required for long-term maintenance of HSCs, promoting self-renewal through inhibition of differentiation and limiting cell proliferation (Kamminga et al., 2006; Majewski et al., 2010; Xie et al., 2013). Thus, the phenotypic diversity of malignancies with Ezh2 LOF (myeloid and lymphoid T) is likely explained through increased proliferation and uncontrolled (stochastic) differentiation toward multiple lineages occurring in HSCs upon Ezh2 LOF. In contrast, Ezh2 LOF is unlikely to cause B cell malignancies, given that Ezh2 is necessary for the formation of the B cell germinal center (GC), presumably through increased proliferation by inhibiting cell-cycle inhibitors in those cells (Béguelin et al., 2013, 2017; Caganova et al., 2013). What is more, this same function of inhibiting cell-cycle arrest in B cell GCs may largely be responsible for the oncogenic role of Ezh2 in the B cell lineage, whereby increased (corrupted) Ezh2 activity may lead to uncontrolled proliferation, maybe accompanied by a block in B cell differentiation (through aberrant H3K27 methylation at B cell differentiation genes). Taken together, these studies highlight how perturbation of PcG and TrxG activities can disrupt cell memory and cell-fate decisions in specific cellular environments, in turn leading to highly context-specific oncogenic transformation. These findings also emphasize the need to develop therapies that are cell-type specific.

Making the matter even more complex, studies on the H3K27 demethylase UTX have shown that even within the same cell type, TrxG proteins can function as either oncogenes or tumor suppressors. First, based on the fact that UTX demethylates H3K27me3, a mark that is introduced by the T-ALL tumor suppressor Ezh2 (as described above), one would expect UTX to be an oncogene in the T cell lineage. Indeed, UTX was shown to play a major oncogenic role in T-ALL as a co-factor for the TF TAL1, an important driver of T cell leukemogenesis (Benyoucef et al., 2016). Mechanistically, it was shown that TAL1 interacts with UTX and recruits it to genomic sites that should normally be silenced. Aberrant maintenance of an open chromatin configuration at these sites through active removal of H3K27me3 permits the expression of a TAL1-mediated leukemic gene expression program. As such, UTX inhibition is efficient at eliminating leukemic blasts *in vivo* in T-ALL patient-derived leukemia models (PDX) that express TAL1. Although these findings all point toward an oncogenic role for UTX in T-ALL, it is interesting to note that UTX inhibition does not kill leukemia cells from patients that do not express TAL1, pointing toward a subtype-specific oncogenic role whereby UTX is only oncogenic in the presence of TAL1 (Benyoucef et al., 2016). Consistent with this, UTX-inactivating mutations have been found in T-ALL patients who express the oncogenic TF TLX3 but not in TAL1-positive T-ALL patients (De Keersmaecker et al., 2013; Van der Meulen et al., 2015). Furthermore, UTX has been proposed to be a tumor suppressor in a Notch-mediated murine model of T-ALL (with no expression of TAL1), although the precise mechanism has not been determined (Ntziachristos et al., 2014). Taken together, these findings revealed that even within the same lineage, TrxG proteins can be tumor suppressors or oncogenes depending on specific protein-protein interactions, further highlighting the need for deep mechanistic understanding prior to the clinical use of epi-drugs.

Paradoxically, the highly context-specific functions of TrxG and PcG proteins that render their targeting so difficult in cancer may also represent the Achilles heel of cancer cells, providing us with a unique opportunity to develop highly specific therapeutic approaches that preserve fully quiescent healthy stem cells (that presumably are not dependent on PcG-TrxG for survival) while eliminating TrxG/PcG-dependent cancer cells.

Concluding Thoughts

There remains little doubt that the antagonism between PcG and TrxG plays a central role in maintaining stem cell fate. Furthermore, the ability of these proteins to control cell fate suggests that they may represent useful therapeutic targets through providing a means of modifying cell fate with potential beneficial effects in cancer, disease, and aging. Although approaches

geared toward enzymatic inhibition of PcG and TrxG protein function may represent useful therapeutic strategies, in some cases such approaches are unlikely to preserve the functions of healthy cells from other tissues that in many cases are also dependent upon TrxG and PcG enzymatic activities. Instead, given the contribution of lineage-specific TFs to influencing PcG and TrxG function in the establishment of cell-specific gene expression programs, one could envisage the development of small molecules or peptides directed at disrupting the highly cell-specific interactions between TFs and the PcG-TrxG proteins. Future studies should be directed at elucidating intricate mechanisms through which TFs transduce the environmental cues to direct the fate for all stem cell populations.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.stem.2019.03.005.

ACKNOWLEDGMENTS

Work in the Brand and Dilworth labs is supported by grants from the CIHR (201503MOP, 201709PJT, and 201708IGH to M.B. and FDN-143330 to F.J.D.) and NIH (R01DK098449 to M.B.). J.Z. is the recipient of a doctoral scholarship from the China Scholarship Council.

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