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Polycomb and trithorax opposition in development and disease

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Abstract

Early discoveries in chromatin biology and epigenetics heralded new insights into organismal development. From these studies, two mediators of cellular differentiation were discovered: the Polycomb group (PcG) of transcriptional repressors, and the trithorax group (trxG) of transcriptional activators. These protein families, while opposed in function, work together to coordinate the appropriate cellular developmental programs that allow for both embryonic stem cell self-renewal and differentiation. Recently, both the PcG and trxG chromatin modulators have been observed to be deregulated in a wide spectrum diseases including developmental disorders and cancer. To understand the impact of these findings we outline the past, present, and future.

INTRODUCTION

During embryogenesis, embryonic stem cells (ESCs) activate unique cellular differentiation programs to form all germ layers of a developing organism. These events are meticulously orchestrated at the transcriptional level in both a spatial and temporal context to ensure proper anatomic patterning and function. Transcriptional control mechanisms guiding this process make up a complex regulatory network involving hierarchical chromatin structure, chromatin dynamics, chromatin modulators, epigenetic modifications, and a set of core transcription factors. Remarkably, this regulatory network functions in concert to maintain a permissive chromatin environment in ESCs that allows for pluripotency and self-renewal while remaining poised for differentiation into a myriad of cell types. Two protein families, the Polycomb group (PcG) and trithorax group (trxG), have emerged as key controllers of the biological processes involved in cellular fate determination.

The Polycomb and trithorax groups have a rich history that encompasses nearly a century of research in developmental biology. A renewed interest in these two groups has recently emerged after numerous next-generation sequencing efforts have identified high mutation frequencies within these gene sets in mammalian disease. Many follow-up studies have since demonstrated both loss and gain of function events in PcG and trxG members as crucial mediators of pathogenesis in several of these disease types including developmental

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disorders and human cancer. It is important to note that the early works conducted on Polycomb and trithorax in *Drosophila* were purely genetic in nature with much of the flagship molecular and biochemical analysis largely absent until many years after most of the *PcG* and *trxG* genes were already identified. While this has made the works of the many researchers that pioneered this field all the more impressive, it also presents a unique challenge for those attempting to obtain a complete perspective in this domain in the context of mammalian disease. In this review, we summarize the history and evolution of the Polycomb and trithorax groups in an inclusive manner, as well as discuss contemporary interests in the context of development and disease. In addition, there are many excellent reviews that supplement this overview.^{1–3}

DISCOVERY AND EVOLUTION

Drosophila Melanogaster: A Model Organism for Development

In 1910, Thomas Hunt Morgan isolated the sex-linked *white* mutation in *Drosophila melanogaster* arguably bringing about the advent of modern genetics. Over the following decades, Morgan and his colleagues continued to produce multiple seminal works on the basis of heredity, and perhaps unknowingly, they would ultimately set the genetic foundation for landmark discoveries in developmental biology throughout the remainder of the twentieth century. Much, if not most, of what we currently understand about organismal development originated in these early genetic screens, and notably, many of the genes identified in *Drosophila* have mammalian homologues with similar functions in development as well as disease.

Embryogenesis in *Drosophila* occurs through a distinct process of body segmentation as pluripotent cells differentiate to produce all tissues of a growing organism during organogenesis. These segments form along the anterior-posterior axis of the fly embryo and are specified by the activation or repression of a special class of developmental genes, called homeotic (*Hox*) genes (see Figure 1). The expression patterns of these genes are established early in embryogenesis by maternal, gap, and segmentation gene products that function at *cis*-regulatory sites proximally located to *Hox* promoters. Regardless, the maternal gene products are quickly degraded after deposition of the egg, and gap and segmentation genes are only transiently expressed. Although direct feedback mechanisms between *Hox* genes have been described,⁴ the Polycomb and trithorax groups of repressors and activators, respectively, are primarily responsible for the maintenance of lineage specific transcription for the remainder of the differentiation process. Intuitively, mutations within *Hox*, *PcG*, and *trxG* genes result in a variety of developmental phenotypes, most notably as morphogenic transformations between body segments.

Phenotypic screens for these transformations revealed two *Hox* gene clusters, named the bithorax complex (BX-C) and antennapedia complex (ANT-C) (see Figure 1), as well as several families of *PcG* and *trxG* protein complexes with regulatory functions within these sites, and these are discussed below.

The BX-C and ANT-C Are Crucial for Embryonic Development

The BX-C—The BX-C gene cluster contains three transcription factor encoding *Hox* genes (*Ubx*, *AbdA*, and *AbdB*) that control the developmental fate of the posterior two-thirds of the *Drosophila* embryo (see Figure 1). Calvin Bridges (1915) isolated the first BX-C mutant allele, named *bithorax* (*bx*), from a fly that displayed a morphogenic transformation (homeosis) of the third thoracic segment toward the second thoracic segment resulting in wing-like halteres.⁵ Subsequent phenotypic and complementation analysis identified nine classes of homeotic mutations within this group including *abx/bx*, *bx^d/pbx*, *iab-2* through *iab-7*, and *iab-8,9*.^{2,6} The characterized mutations in *abx/bx* and *bx^d/pbx* result in posterior to anterior transformations within the mesothorax and metathorax, while mutations in the *iab* loci cause similar transformations in the abdominal regions.

In a report detailing what would become his Nobel Prize-winning work, E.B. Lewis noted that the complete deletion of the chromosomal arm containing the BX-C resulted in a uniform homeotic transformation of the third thoracic segment and all abdominal segments toward the second thoracic segment.⁷ Using merely genetic information, Lewis postulated that the second thoracic segment represented a primitive level, and that regulated collinear expression of the BX-C along the anterior-posterior axis governed fly body patterning. The subsequent model by Lewis presupposed that previously discovered BX-C mutant alleles were representatives of genes and that a transcriptional repressor with varying specificity was present in each segment allowing for stepwise expression of each BX-C gene along the anterior-posterior axis. Although the eventual cloning of the BX-C gene cluster would reveal only three genes, *Ubx*, *Abd-A*, and *Abd-B*,^{8,9} this idiosyncrasy was resolved through *in situ* staining and transgenic reporter gene assays which unveiled a complex *cis*-regulatory system within the BX-C that was perturbed in the presence of each previously mapped homeotic mutation.⁶ The latter assumption by Lewis was validated with the discovery of the *Polycomb* (*Pc*) group of mutations that result in ectopic derepression of BX-C genes leading to anterior to posterior transformations in larval body patterns, *vide infra*.

The ANT-C—The ANT-C gene cluster is composed of five transcription factor encoding *Hox* genes (*lab*, *pb*, *Dfd*, *Scr*, and *Antp*) that control the development of the anterior fly body including the head and thoracic segments (see Figure 1).^{10–14} Four non-*Hox* genes are also present within this region and include *bicoid* (*bc*), a maternal-effect morphogen required for anterior-posterior embryo patterning, *fushi tarazu* (*ftz*), a segmentation gene, *zerknüllt* (*zen*), a gene required for dorsal-ventral patterning, and *z2*, a spatially regulated gene of unknown function.^{15–17}

The antennapedia (*Antp*) type mutations of the ANT-C have been well described in literature with communications detailing the distinctive antennae to mesothoracic leg phenotype as early as 1948.¹⁸ A variety of heterozygous dominant and homozygous lethal *Antp* alleles that cause this mutation were described over the following few decades, with the curious appearance of a heterozygous dominant and homozygous viable variant named *Nasobemia* (*Ns*).¹⁸ Hannah-Alava¹⁹ described another dominant allele, *Extra sex comb* (*Scx*), which resulted in a transformation of the meso and metathorax legs to prothorax legs. Despite the varying genetic and phenotypic properties of *Ns* and *Scx*, respectively, these alleles were

both determined to be allelic to *Antp*.¹⁸ This discrepancy was resolved in follow-up genetic and molecular studies that revealed a two-promoter system at the *Antp* locus that is spatially and temporally regulated in a similar fashion as genes of the BX-C.^{20–23} Cytogenetic and genetic analysis conducted contemporarily to these studies would also map homeotic mutants *Scr*, *Dfd* and *pb* to the same region as *Antp*.^{10,11,13} The most proximal gene of the ANT-C, *lab*, was fully described much later in several reports.^{12,14} For further insight into ANT-C functionality in *Drosophila* as well as higher organisms we refer you to the following reviews.^{24–26}

The Discovery of the PcG

The *Drosophila* PcG genes encode for a diverse family transcriptional repressors that control the expression of *Hox* genes during development and differentiation. The discovery of a single male fly with ectopic sex combs present on each of its six legs led to the isolation of the first PcG mutant, *extra sex combs* (*esc*), a recessive mutation mapped to the left arm of the second chromosome.²⁷ The presence of the ectopic extra sex comb phenotype was further observed in a heterozygous dominant and homozygous lethal mutant variant, called *Polycomb* (*Pc*), by P.H. Lewis in 1947.²⁸ Additional mutant alleles discovered over the following four decades include *Polycomblike* (*Pcl*),²⁹ *super sex combs* (*sxc*),³⁰ *Additional sex combs* (*Asx*),³¹ *Posterior sex combs* (*Psc*),³¹ *Sex combs on midleg* (*Scm*),³¹ *Sex combs extra* (*Sce*),³² and *polyhomeotic* (*ph*).³³ The molecular function of PcG genes was first realized with the observation of a dramatic homeotic transformation of the thoracic and first seven abdominal segments toward the eighth abdominal segment in hemizygous and homozygous *Pc* embryos.⁷ The direction of this transformation was antagonistic to mutations of the BX-C, suggesting that *Pc* encodes a BX-C repressor that controls *Hox* gene expression in each segment through a *cis*-regulatory mechanism. This paradigm was further validated in studies of BX-C dosage which showed a decrease in homeotic transformation severity with increased homeotic gene copy number,^{29,34} and later in molecular studies examining *Hox* gene mRNA^{35,36} and protein distribution throughout embryogenesis in *PcG* mutant flies.^{37–42}

Interestingly, many of these studies also showed that the PcG proteins do not influence early stages of *Hox* gene expression, but rather, they function in maintaining appropriate gene repression late in development and throughout terminal differentiation. Notably, Zink and Paro⁴³ showed PcG binding at sixty distinct sites along salivary gland polytene chromosomes, with many of the binding sites being located outside of the BX-C and ANT-C clusters.⁴⁴ These observations along with other studies using immunostaining^{45–48} suggested a more generalized role of PcG proteins in transcriptional regulation outside of development. Moreover, observed co-occupancy of PcG proteins at multiple chromosomal loci suggested the formation of PcG complexes.

PcG Proteins Form Histone Modifying Complexes

The dominant phenotypic interactions of PcG proteins, including synthetic lethality, had already led early investigators to propose the existence of one or several PcG protein complexes prior to immunolocalization studies.^{31,49} Using epitope tagged copies of *ph*, Shao et al.⁵⁰ successfully purified the first *Drosophila* PcG complexes containing *ph*, *Pc*, *Psc*, and

substoichiometric levels of scm. This complex inhibited the remodeling activity of the hSWI/SNF complex in a non-histone tail-dependent manner and was subsequently called Polycomb repressor complex 1 (PRC1). Further analysis of PRC1 composition revealed dRING1/sce as a stable component of the complex along with the DNA binding Zeste transcription factor and several TATA-binding protein (TBP) associated factors (see Figure 2).⁵¹ A second complex composed of E(z), ESC, NURF-55, and Su(z)12, named Polycomb repressor complex 2 (PRC2), was isolated from *Drosophila* by several groups,^{52,53} and found to have methyltransferase capability specific for H3K27, and to some extent H3K9, *in vitro* (see Figure 2 and Table 1) Given that the Pc subunit of PRC1 was shown to have sequence homology to the HP1 chromodomain,⁶⁴ and HP1 was shown to bind methylated H3K9,^{65,66} Cao et al.⁶⁷ examined the relationships between H3K27 methylation and PRC1 binding at the *Ubx* gene locus in *Drosophila*. These studies showed that PRC2 activity and methylation was correlated with PRC1 binding and repression at Polycomb response elements (PREs) in the *Ubx* gene. Nevertheless, this general paradigm is not ubiquitous and the chromatin and epigenetic landscape required for site specific PRC1 and PRC2 docking is still a matter of debate in *Drosophila* as well as higher organisms. The two prevailing models as they occur in mammals are illustrated in Figure 4(c) and (d).

The Discovery of the *trxG*

The *Drosophila* *trxG* genes encode transcriptional activators that function in opposition to the PcG proteins. Consequently, *trxG* proteins promote the transcriptional activation of *Hox* genes during development and differentiation. Prior to identifying *trxG* alleles, many researchers predicted the existence of a family of *Hox* gene activators in which mutant variants would resemble BX-C or ANT-C deletions. This hypothesis was validated upon the discovery of a spontaneous mutant with a transformation of the prothorax toward the mesothorax.⁶⁸ In extreme variants, this mutant allele produced flies with three sets of wings and was subsequently named *trithorax* (*trx*). Complementation analysis of the original *trx* mutant revealed that it was not a part of the BX-C and several works demonstrated the cytological location of the mutant allele to be located outside that of the BX-C and ANT-C. Follow-up studies also indicated that *trx* activates *Scr* of the ANT-C and genes of the BX-C acting antagonistically to PcG genes.^{69,70} This activity is further underscored by the ability of *trx* mutants in suppressing PcG mutant phenotypes.⁷¹ In a screen for dosage-dependent interactions with *trx* and *pcl*, Kennison and Tamkun⁷² expanded the family of trithorax like proteins revealing *moira* (*mor*), *osa* (*osa*), and *brahma* (*brm*) as suppressors of Pc phenotypes. Molecular cloning and sequencing of the *brm* gene by Tamkun et al.⁷³ revealed distinct homology to the yeast transcriptional activator, SNF2/SWI2. This included a 57% sequence identity to the SNF2/SWI2 helicase domain as well as a conserved bromodomain.⁷⁴ Functional redundancy of the SNF2/SWI2 and *brm* ATPase domain was observed in *swi* deficient yeast cells expressing a SNF2/SWI2 subunit with a *brm* ATPase domain.⁷⁵ Although the chimeric SNF2/SWI2-*brm* protein rescues yeast in this background, *brm* alone is not a sufficient replacement indicating different hierarchical function.

The first yeast *trxG* family of proteins to be fully characterized were discovered independently of those in *Drosophila* in two genetic screens in *Saccharomyces cerevisiae* and eponymously named for their biological phenotype.⁷⁶ The first of these screens

identified several mutated genes that repress expression of *HO*,⁷⁷ a gene encoding an endonuclease required for yeast mating-type switching,⁷⁸ and were referred to as switching defective (*SWI*) genes. In the second screen, another group of genes were shown to be necessary for expression of *SUC2*,⁷⁹ an invertase necessary for sucrose metabolism,⁷⁶ and were subsequently referred to as sucrose non-fermenting (*SNF*) genes. The culmination of these studies revealed the essential nature of SNF2/SWI2 (both SNF2 and SWI2 were determined to be identical), SWI1, SWI3, SNF5, and SNF6 for the transcriptional activation of a large set of genes involved in cell homeostasis.

trxG Proteins form Chromatin Remodeling and Modifying Complexes

The trxG Chromatin Remodelers—The trxG family of chromatin remodeling complexes include SWI/SNF, CHD, ISWI, and INO80 (see Table 2). This review will focus primarily on the SWI/SNF complex. For more information on the other chromatin remodeling groups we direct the reader to the following reviews.^{94–96}

Much like the PcG family of proteins in *Drosophila*, redundant phenotypes were recognized between single and multiple *SWI* and *SNF* mutant variants⁹⁷ and eventually led to the revelation that *SWI* and *SNF* gene products form an approximately 1-MDa SWI/SNF complex composed of between 9 and 12 subunits. Cote et al.⁹⁸ were the first group to biochemically purify a yeast SWI/SNF complex including SWI1, SWI2/SNF2, SWI3, SNF5, SNF6, SWP29, SWP73, SWP82, ARP7, and ARP9 (see Figure 3 and Table 2). A stoichiometry study and scanning transmission electron microscopy structure of the yeast SWI/SNF complex would follow nearly a decade later.⁹⁹ The predicted nucleosome remodeling ability of the SWI/SNF complex was realized with the discovery of suppressor mutations in chromatin components and the observation of increased nuclease sensitivity at the *SUC2* promoter region during activation.¹⁰⁰ Furthermore, purified yeast SWI/SNF was observed to disrupt mono-nucleosomes in vitro in an ATP-dependent manner.¹⁰¹

The homology between brm and SNF2 suggested that there might be an equivalent complex in *Drosophila*. Cloning and biochemical analysis of a *Drosophila* homologue of human INI1/SNF5, named Snr1, revealed a SWI-SNF like brm-Snr1 complex of ~2-MDa.¹⁰² Papoulas et al.¹⁰³ further characterized eight proteins that interact with brm, referred to as brm associated proteins (BAPs), two of which, not including brm and Snr1, were conserved in yeast and human SWI/SNF, BAP155 a homologue of BAF155/170 (later to be unequivocally identified as mor), and BAP60 a homologue of BAF60A/B/C. These studies also suggested the presence of two other trxG complexes, a 2-MDa ASH1 complex and a 500-kDa ASH2 complex.¹⁰³ The ARID-domain-containing osa, discovered by Kennison and Tamkun,⁷² was also identified as a functional subunit of a subset of BAP complexes in both genetic and biochemical studies.^{104,105} The resolution of these BAP complexes was complete with the characterization of an additional BAP complex containing subunits polybromo (PB) and BAP170, but lacking osa, using mass spectrometry and was subsequently named PBAP (see Figure 3 and Table 2).¹⁰⁶

The chromodomain helicase DNA-binding (CHD) family is characterized by the presence of a SNF2-like ATPase domain and a N-terminal tandem chromodomain.⁹⁵ The founding member of the CHD family, CHD1, was discovered serendipitously while probing libraries

derived from mouse lymphoid cell mRNA for transcription factors that bind immunoglobulin promoters.¹⁰⁷ There are currently nine known CHD family members in mammals, each split into sub-groups based on the presence of additional domains: Sub-group I (CHD1 and CHD2), Sub-group II (CHD3 and CHD4), and Sub-group III (CHD5, CHD6, CHD7, CHD8, and CHD9).¹⁰⁸ CHD1 has been observed to have numerous binding partners, all of which are known to be involved in chromatin and/or epigenetic function including transcriptional co-repressor NCoR, SAGA, and HDAC1.¹⁰⁸ CHD3 and CHD4, also known as Mi-2a and Mi-2b, respectively, were discovered to be part of 2-MDa chromatin remodeling complex called nucleosome remodeling and histone deacetylation (NuRD) complex (see Table 2).¹⁰⁹ The functionality of the sub-group III CHD family proteins has only recently been studied, and while their general role appears to also be at the chromatin and transcriptional control level, this sub-group represents a facet of chromatin biology that remains largely unexplored.

The identification of the SWI2/SNF2 related transcriptional activator, called imitation switch (ISWI),^{75,110} led to three ISWI sub-complexes being identified including the nucleosome remodeling factor (NURF) complex, ATP-dependent chromatin assembly and remodeling factor (ACF) complex, and chromatin accessibility complex (CHRAC).^{111–113} These complexes contain an ISWI family ATPase with two to four additional subunits (see Table 2). The observed structural diversity of the ISWI family of complexes impart functional diversity in terms of nucleosome spacing optimization (ACF and CHRAC) as well as nucleosome spacing randomization (NURF).¹

The INO80 subfamily is the most recently described member of the SNF2 family of chromatin remodeling complexes. Co-purification of the INO80 ATPase, a gene product required for inositol biosynthesis in yeast,¹¹⁴ yielded an approximately 1–1.5-MDa complex composed of Act1, Arp4, Arp5, Arp8, Rvb1, Rvb2, and other ancillary proteins (see Table 2).¹¹⁵ Arp proteins (Arp7 and Arp9) had already been identified as stable components of the yeast SWI/SNF complex, and Rvb1 and Rvb2 were previously observed to have DNA helicase capability that is essential in yeast,¹¹⁶ strongly suggesting function as a chromatin remodeler. INO80 has been extensively studied in the context of DNA repair as it is recruited to the sites of DNA breaks, as well as, the maintenance of telomere structures.¹

The trxG Chromatin Modifiers—The trxG family of chromatin modifiers includes COMPASS, COMPASS-like, TAC1, and ASH1 complexes. Similar to the mammalian SWI/SNF (BAF and PBAF) chromatin remodeling complexes, COMPASS and COMPASS-like share several core subunits including ASH2L, DPY30, HCF1, RBBP5, and WDR5 (see Figure 3 and Table 3). Furthermore, all of these complexes include a SET-domain methyltransferase with specificity for H3K4 (SET1A/B, MLL1–4), or H3K36 (ASH1L).

The Set1/COMPASS complex was the first complex with H3K4 methyltransferase activity to be described. Miller et al.¹²⁰ first purified this complex in *Saccharomyces cerevisiae* using tandem affinity purification of Set1. This complex, named Set1/COMPASS, is an eight-subunit complex with individual deficiencies in six subunits (including Set1) resulting in similar growth delay on rich medium as well as sensitivity to hydroxyurea (an indicator of gene regulatory function). Furthermore, inactivation of many of the Set1/COMPASS

subunits relieves silencing of *URA3* genes located near telomeres, which is phenotypically similar to Set1 deficiency. The H3K4 methyltransferase ability of Set1/COMPASS was described in several studies and shown to be required for maintenance of telomere length.^{121,122} Interestingly, deletion of *Set1* along with *Cps50* and *Cps30* results in defective mono-, di-, and tri-methylation, whereas *Cps40* deletion primarily reduces H3K4 tri-methylation (>80%) with minimal effect on di- and tri-methylation.^{119,123} Deletion of *Cps25* or *Cps60* also results in diminished H3K4 tri-methylation, but with greater reduction in H3K4 di-methylation.^{119,123} For additional details regarding Set1/COMPASS subunit biological activities see Table 3.

A homologous Set1/COMPASS complex is also present in *Drosophila melanogaster*, containing dSet1 and harnessing H3K4 mono-, di-, and tri-methylation ability.^{124,125} However, during evolution this complex diverged into two additional COMPASS-like complexes that are characterized by the presence of either trx or trr.¹²⁵ These complexes, while compositionally similar to the dSet1/COMPASS complex, appear to have unique functionality as knockdown in flies revealed minimal change to bulk H3K4 di- and tri-methylation.¹²⁵ It is thus possible that these complexes have locus-specific functionality. The trx protein has also been shown to associate with dCBP and Spf1 to form TAC1.¹²⁶ The presence of both trx and dCBP enables TAC1 to function as both a H3K4 methyltransferase as well as a H3K27 histone acetyltransferase. The ASH1 complex, composed of Ash1 and dCBP, has a similar combined HMT/HAT activity, however its HMT activity is specific for H3K36.¹²⁷ Subsequently, both TAC1 and ASH1 are suspected to play prominent roles in directly antagonizing PcG mediated silencing.

POLYCOMB AND TRITHORAX COMPLEXES IN MAMMALS

The Mammalian PRC1

The *Drosophila* PRC1 complex is composed of Pc, Ph, Psc, dRING/Sce, and Scm.^{50,51} The mammalian PRC1 complex contains homologues of each of these subunits, with the canonical PRC1 complex consisting of CBX, PHC, PCGF, RING, and SCMH proteins, respectively (see Figure 2 and Table 1). Throughout evolution, each of these subunits has expanded into several distinct paralogue families allowing for multiple permutations of the mammalian PRC1 complex. Furthermore, additional complexity in PRC1 assembly has more recently been defined with the observation of non-canonical complexes that include RYBP, or the homologue YAF2, in place of CBX proteins. RYBP/YAF2 complexes are further categorized by the presence or absence of a number of ancillary subunits such as KDM2 and E2F6 that are implicated in providing functional diversity and specificity. The primary method of transcriptional repression by PRC1 has long been attributed to its H2AK119 monoubiquitinase ability, however, the mechanism by which this histone mark perturbs transcription is not understood. Moreover, the newly appreciated structural diversity of the mammalian PRC1 has further complicated mechanistic studies as described sequentially below.

The human Psc homologues, cumulatively named the PcG RING finger (PCGF) family, exhibit the most diversity among PRC1 subunits, including six variants, BMI1 (PCGF4), MEL18 (PCGF2), MBLR (PCGF6), NSPC (PCGF1), PCGF3, and PCGF5 (see Table 1).

BMI1 was discovered in two independent screens attempting to identify factors that work synergistically with c-Myc to promote lymphoma in mouse models.^{128,129} The later observation of senescent phenotypes in BMI1 deficient cells revealed its function as a repressor at the *INK4a* locus.^{130,131} BMI1 was eventually shown to be necessary for H2AK119 monoubiquitination (H2AK119Ub1) activity of the E3 ligase proteins, RING1A and RING1B.¹³² Interestingly, while knockdown of BMI1 or either of the RING proteins reduces H2AK119 ubiquitylation, H3K27me3 is not significantly affected suggesting independent functionality between PRC1 and PRC2. This is further conferred in PRC2 deficient mouse ESCs that maintain H2AK119Ub1 levels through a non-canonical RYBP-PRC1 complex.¹³³

However, whether or not the H2AK119Ub1 mark is a ubiquitous facilitator of repression currently remains an unanswered question. While H2A ubiquitination is necessary for viability early in embryogenesis in *Drosophila*, it is not necessary for maintaining transcriptional repression at known PRC1 target genes at later stages.¹³⁴ Intriguingly, knockdown of RING1A and RING1B and consequent loss of H2AK119Ub1 in mouse ESCs demonstrates a loss of higher order chromatin compaction resulting in aberrant expression of RING1A and RING1B occupied genes.¹³⁵ It is thus possible that there is a temporal distinction in which the H2AK119Ub1 mark is integral in orchestrating a dynamic hierarchical chromatin structure during development, but plays a less prominent role in terminally differentiated tissues. Furthermore, deubiquitination of H2AK119 is a domain of research that is currently less well understood. At least four H2A deubiquitinases are known including 2A-DUB, Ubp-M, USP21, and USP7 (see Figure 4(a)). Interestingly, the USP7 deubiquitinase has been recently identified as a necessary component of both BMI1 and MEL18 type PRC1 complexes.¹³⁶ For further reading in deubiquitinases we refer you to several reviews.^{137–139}

Although BMI1 and MEL18 type complexes share similar PRC1 complex subunit composition (RING1A/B, PHC1/2/3, and CBX proteins) and H2AK119Ub1 function,¹⁴⁰ MEL18 has been shown in several studies to down regulate BMI1 and c-Myc expression resulting in activation of the *INK4a* locus.^{141–144} Paradoxically, ChIP-seq analysis demonstrates BMI1 and MEL18 overlapping at specific genomic loci, possibly suggesting direct antagonistic activity among MEL18 containing PRC1 complexes. One explanation for this phenomenon is that proper repressive or activation activity at a given locus is dictated by fine-tuning the levels or binding of these proteins. Nevertheless, further investigation into the antagonistic functions of BMI1 and MEL18 PRC1 complexes is needed.

MBLR was originally identified in a PRC1 complex containing RING1A, RING1B, and BMI1 as a transcriptional repressor that is phosphorylated during mitosis.¹⁴⁵ In separate study, this complex was shown to have H3K9 methyltransferase capability through associations with several SET domain-containing proteins.¹⁴⁶ Alternatively, Lee et al.¹⁴⁷ have shown a functional association between MBLR and the H3K4 demethylase JARID1d suggesting multiple mechanisms of repression by the MBLR containing PRC1 complex. MBLR has also been shown to interact with core ESC regulator Nanog¹⁴⁸ and L3MBTL2,¹⁴⁰ the latter recently being revealed to be crucial in ESC processes.¹⁴⁹ Similar

to MBLR, the PCGF homologue NSPC forms a PRC1 complex with H3K36 demethylase activity conferred by KDM2B (FBXL10).¹⁵⁰

Although the physical associations of PCGF3 and PCGF5 have been well characterized by proteomics,¹⁴⁰ the functionality of these variants still remains poorly understood. A recent report by Gao et al.¹⁵¹ demonstrates transcriptional activation by PCGF5 in a GAL4-luciferase assay suggesting a divergent function of these complexes in general transcriptional activation. Activation *in vivo* is also suggested by the occupancy of AUTS2, a member of PCGF3/5-PRC1 complexes, at sites enriched with H3K4me3 and RNA polymerase.

The human homologues of *Drosophila* Pc, named the chromobox (CBX) family, include CBX2, CBX4, CBX6, CBX7, and CBX8. Each of the mammalian CBX homologues contains a chromodomain that is implicated in providing binding specificity to its respective PRC1 complex. Histone H3 binding studies using all five mouse Cbx homologues have demonstrated Cbx4 specificity for H3K9me3, and Cbx2 and Cbx7 specificity for both H3K9me3 and H3K27me3.¹⁵² This group also observed the localization of all Cbx members, with the exception of Cbx4, to inactivated X chromosomes during differentiation in female ESCs suggesting CBX mediated PRC1 repressive activity plays a role in this process. With the identification of RYBP-containing PRC1 sub-complexes that can mono-ubiquitylate H2AK119 independent of H3K27me3,^{140,153} it has been proposed that CBX containing PRC1 complexes constitute a PRC2 dependent sub-population (see Figure 4(c)).

The human homologues of Ph (PHC1, PHC2, and PHC3) and Scm (SCMH1, SCML1, and SCML2) are the least understood components functionally. Nevertheless, both PHC and SCMH proteins contain a conserved sterile- α motif that is present in various proteins that are important in development and differentiation and has been shown to facilitate polymerization of Ph and more recently suggested to bind RNA.^{154,155} Given recently reported ncRNA recruitment mechanisms for PRC2¹⁵⁶ it is possible that these proteins function in a similar fashion to provide combinatorial specificity with chromatin and DNA binding motifs found in other PRC1 subunits. Proteomic studies with biochemical validation will be needed to fully resolve the specific roles of CBX proteins in PRC1 targeting, complex composition, and function.

The Mammalian PRC2

The core of the *Drosophila* PRC2 complex is composed of E(z), Su(z)12, ESC, and NURF55.^{52,53} Additional subunits that are not essential for the H3K27 methyltransferase activity of *Drosophila* PRC2 include Pcl and Jing, both of which possibly play a role in modulating targeting specificity.^{157,158} The mammalian PRC2 complex varies less from its *Drosophila* counterpart compared to PRC1, with the E(z) homologue being the only core subunit to have multiple paralogues, EZH1 and EZH2. The mammalian counterparts for Su(z)12, ESC, and NURF55 are Suz12, EED, and RbAp46/48, respectively (see Figure 2 and Table 1). Compositional diversity of the mammalian PRC2 core is conferred at least in part by the presence of PCL1, PCL2, and PCL3 (homologues of *Drosophila* Pcl), and AEBP2 (a homologue of jing). In a recent report, Kalb et al.¹⁵⁹ have implicated AEBP2 and JARID2 in H2AK119Ub1 binding (see Figure 4(d)). Both *Drosophila* and mammalian PRC2

complexes facilitate transcriptional repression via the deposition of H3K27 trimethylation (H3K27me₃) (see Figure 4(b)). The interplay between PRC2 and PRC1 in initiating transcriptional repression has been the subject of much debate among researchers. Although H3K27me₃ has been observed to function as a docking site for PRC1 through the chromodomain of CBX proteins, suggesting PRC2 as a frontier complex, several recent reports have challenged the generality of this model. In these cases, the catalytic activity of PRC1 was found to be required for recruitment of PRC2 and consequent methylation of H3K27.^{159–161}

EZH1 and EZH2 are members of the SET-domain methyltransferase superfamily that includes all known chromatin methyltransferases with the exception of DOT1L.¹⁶² As the PRC2 catalytic subunits, EZH1 and EZH2 are responsible for the SAM-dependent mono-, di-, and tri-methylation of H3K27. Intriguingly, while these proteins are necessary for the stepwise methylation of H3K27, they are not sufficient on their own to propagate this mark and must exist in a minimal core complex with SUZ12 and EED for *in vitro* activity.⁶³ While both EZH1 and EZH2 function with other PRC2 components to facilitate transcriptional repression via H3K27me₃ methylation, they are differentially expressed. Although EZH1 is ubiquitously expressed at a constant level throughout the cell cycle, EZH2 expression is primarily associated with actively dividing cells.¹⁶³ Moreover, although both PRC2-EZH1 and PRC2-EZH2 complexes have H3K27 methyltransferase ability, PRC2-EZH2 activity is 20-fold greater. Non-redundancy between these components is further demonstrated by PRC2-EZH2 lacking the direct chromatin compacting capability exhibited by PRC2-EZH1. Differences at the molecular level have been further extended to biological function in the context of differentiation with the observation of adult hematopoietic stem cell reduction post EZH1 deletion,¹⁶⁴ a phenotype not associated with EZH2 deletion.¹⁶⁵ More recently, the presence of a GATA mediated EZH1/2 switch in erythroid specification has been identified, further implicating non-redundant functionality.¹⁶⁶

EED, the mammalian homologue of *Drosophila* ESC, contains a WD40 repeat domain that has been shown to bind H3K27me₃ resulting in modulation of PRC2 activity (see Figure 4(b)).¹⁶⁷ Specifically, this binding event increases the catalytic ability of the PRC2 complex between three to seven fold, functioning similarly to HP1 mediated positive feedback of the H3K9me₃ mark.^{65,66} In mice, EED knockout results in post-implantation embryonic lethality.¹⁶⁸ Furthermore, EED knockdown in ESCs maintain limited self-renewal capability, but are prone to differentiation.¹⁶⁹ NURF55 homologue RbAp46/48 is another WD40 repeat domain containing protein that binds selectively to H3K36me₃ resulting in negative regulation of the PRC2 complex.¹⁷⁰ The other core component, SUZ12, contains a putative zinc finger domain that might function as a DNA or RNA binding motif, however, no reports have validated this hypothesis.⁶³ As an essential PRC2 component, SUZ12 inactivation in mice is embryonic lethal and results in impaired differentiation in ES cells.¹⁷¹

Additional subunits including JARID2, AEBP2, and several PCL proteins have been found to associate with the core PRC2 complex and provide modularity and specificity.^{157,172,173} JARID2, while a member of the JumonjiC domain family of lysine demethylases, paradoxically improves PRC2 binding to target loci to help propagate the H3K27me₃

mark.¹⁷⁴ A recent work has suggested that ncRNA binding at JARID2 is responsible for this phenomenon.¹⁵⁶ AEBP2 is suspected to confer specificity to DNA targets in conjunction with histone and nucleosome binding proteins through its zinc finger domain.^{172,175} The three mammalian homologues of *Drosophila* Pcl are particularly intriguing in that PCL1 and PCL3 interact with H3K36me3 through their single Tudor domain, but promote H3K27me3 by PRC2. On the other hand, PCL2 also interacts with the H3K36me3 mark yet can promote or diminish the activity of PRC2 in a tissue dependent manner.¹⁷⁶ The seemingly idiosyncratic role of these post-translational modifications in PRC2 targeting and activity stress the need to further evaluate how these proteins carry out specific functions. Elucidation of an underlying combinatorial histone-docking environment would provide insight into epigenetic mechanisms as well as reveal novel therapeutic targets in treating diseases driven by these complexes. The latter goal is of particular importance given that abnormally high levels of the PRC2 complex components and the H3K27me3 mark are commonly observed in a number of malignancies. In addition, further research in identifying proteins that facilitate the removal of H3K27 methylation will also be beneficial in understanding these epigenetic mechanisms. To date, only two H3K27 demethylases are known, UTX and JMJD3. However, the catalytic activity of UTX and JMJD3 only allows for demethylation of H3K27 from tri-methyl to di-methyl, and di-methyl to mono-methyl, which is sufficient for allowing transcriptional activation (see Figure 3(b)). A demethylase capable of completely removing methyl marks from this locus in a similar fashion as LSD1 (a H3K4 and H3K9 demethylase), remains to be discovered.

The Mammalian SWI/SNF (BAF) Complex

The size and subunit complexity of the mammalian SWI/SNF complex easily surpasses that of PRC1 and PRC2. During evolution from single cell to multicellularity the BAF complex has not only gained novel subunits, but it has also diverged into at least two distinct complexes: BAF and PBAF (BAP and PBAP in *Drosophila*) (see Figure 3). Subsequently, the mammalian BAF/PBAF complexes are larger than their orthologous fly and yeast counterparts at approximately 2-MDa and containing up to 15 subunits. Moreover, many of these subunits have multiple paralogues including BAF45A/B/C/D, BAF60A/B/C, BAF155/170, BAF250A/B, BAF53A/B, and SS18/CREST.¹⁷⁷ Additional subunits without paralogues include BAF57, BAF47, and the ubiquitous ATPase β -actin. PBAF complexes are distinguished from BAF complexes by the presence of BAF200 (ARID2), and BAF180 (PBRM1), but the lack of BAF specific BAF250A/B (ARID1A/B).¹⁷⁸ Regardless, BAF and PBAF complexes each contain one of two mutually exclusive ATPases, Brg and Brm, both of which share a high degree of homology to the yeast SNF2/SWI2 and *Drosophila* Brm.

Although Brg and Brm are homologous ATPases of BAF/PBAF their individual roles in development and cellular homeostasis are still somewhat uncertain. Both recombinant Brg and Brm can remodel nucleosomes in an ATP dependent manner *in vitro*, however, Brg has approximately four fold higher activity over Brm.¹⁷⁹ In more biologically relevant settings, the ES cell BAF complex (esBAF) is characterized by the sole presence of Brg along with ARID1A, BAF60A/B, and BAF155, whereas Brm, ARID1B, BAF60C, and BAF170 become expressed during differentiation.^{180,181} Moreover, deletion of *Brg1* in ES cells results in loss of both renewal and pluripotency, and deletion of *Brg1* in mice results in peri-

implantation lethality.^{180,182} On the other hand, mice with homozygous deletions of *Brm* have been reported to be viable and fertile, albeit slightly larger in size.¹⁸³ Nevertheless, the latter observations have been recently questioned with the identification of a functional truncated form retained in the knockout mice used for these studies.¹⁸⁴ The full impact of Brm loss during development thus remains an open question to be addressed. Intriguingly, both Brg and Brm contain C-terminal bromodomains suggesting an ancillary histone binding function in parallel to ATPase activity. However, small molecule inhibition of Brg and Brm bromodomains did not reveal a notable change in binding or proliferation.¹⁸⁵ It is likely that these results further exemplify a combinatorial histone/chromatin binding mechanism imparted by the presence of multiple DNA and histone binding proteins in the BAF complex. This is suggestive that BAF/PBAF complexes must be viewed holistically in terms of subunit composition, and specifically with regard to the paralogue variants incorporated, to fully understand their biological function.

The role of differential BAF subunit paralogue assembly has been demonstrated elegantly in a report observing BAF complex composition during neuronal development from pluripotent ESCs to post-mitotic neurons.¹⁸⁶ In this study, BAF complexes of ES cells were characterized as containing Brg, BAF53A, BAF45A/D, and a heterodimer of BAF155 among other canonical subunits. However, as ES cells differentiate to neurons, BAF complexes begin to incorporate Brm, BAF53B, BAF45B/C, and BAF170. From these results, it is inferred that the biological function BAF155 and BAF170 is not entirely redundant. Subsequently, BAF155 depletion in ES cells has been demonstrated to result in proliferative defects and diminished Oct4 expression that is not rescued by ectopic expression of BAF170.¹⁸⁰ Similarly, deletion of *BAF53B* in mice results in lethal defects in neuronal development that cannot be rescued by BAF53A.¹⁸⁷ Interestingly, in this study it BAF53B was found to associate specifically with CREST (an SS18 paralogue) in neuron specific BAF (nBAF) complexes and direct the complex to genes important in dendritic growth. More recently, a report monitoring the kinetics of the neural progenitor BAF (npBAF) to nBAF transition during differentiation have captured the respective subunit switching from SS18 and CREST.¹⁸⁸ Knockdown of BAF45A, or BAF53A, in E13.5 cortical cultures has been demonstrated to impair neural progenitor proliferation. Conversely, BAF53A expression under control of the neural specific *Nestin* promoter was shown to produce between two and four fold increase in neural progenitor cells, while BAF53B expression in the same system showed no proliferation effects.¹⁸⁶ Although much of the work conducted in BAF complex assembly and function has been focused on neural development this phenomenon has also been expanded to other tissues. The reshuffling of BAF paralogues also occurs during muscle cell differentiation where BAF complexes in muscle progenitor cells contain BAF60A/B, while fully differentiated muscle tissues have BAF complexes with BAF60C.¹⁸⁹

Although the compositional differences between BAF and PBAF have become increasingly well defined since their discovery, the functional distinctions between them have been sparsely examined (see Figure 5) Nevertheless, PBAF has been recently implicated as a facilitator of DNA double-strand break repair.¹⁹⁰ Interestingly, this study suggests that PBAF is required for transcriptional silencing at the site of DNA double-strand breaks in a mechanism that is dependent on ATM phosphorylation of BAF180. This apparent non-

canonical function of PBAF further outlines a dire need for functional characterization of these complexes especially given their prominent roles in human disease. The assembly pathway of both BAF and PBAF complexes is also currently unknown. Although mammalian SWI/SNF complexes share a common core of subunits, how this core is directed to form either BAF or PBAF complexes has yet to be defined. Uncovering BAF/P-BAF assembly dynamics as well as functional specificity is a necessary hurdle in solving BAF driven disease mechanisms.

The Mammalian COMPASS Complex

The mammalian counterparts of the Set1/COMPASS complex have been well conserved from *Saccharomyces cerevisiae* to humans with most of the subunits having close homologues (see Table 3). The human homologues of yeast Set1 include Set1A/B, MLL1, MLL2, MLL3, and MLL4.¹¹⁹ Subsequently, each of these proteins were identified to form COMPASS-like complexes with yeast Cps homologues.^{191–199} The shared core subunits ASH2L, RBBP5, WDR5, and DPY30 are related to Cps60, Cps50, Cps30, and Cps25, respectively.¹¹⁹ Similar to other complexes in the trxG family, the mammalian COMPASS complexes exhibit both structural and functional diversity. Structurally, COMPASS complexes containing SET1A/B contain CXXC and WDR82 (homologues of Cps40 and Cps35, respectively) along with the common core of subunits. This complex has been shown in several studies to function as the primary H3K4 methyltransferase complex as depletion of WDR82 results in global loss of methylation at this locus.^{197,200} MLL1/2 have been shown to interact specifically with menin, a tumor suppressor protein involved in the pathogenesis of familial multiple endocrine neoplasia type 1, and LEDGF.^{119,191} Both MLL1 and menin deletion have been shown to diminish H3K4 methylation at *Hox* loci, with the most dramatic decreases in total *Hox* loci methylation occurring with loss of menin.²⁰¹ Importantly, loss of MLL3/4 or Set1 type complexes did not result in alterations in H3K4 methylation in *Hox* loci suggesting a *Hox* specific role for the MLL1/2 containing COMPASS-like complexes.²⁰¹ The MLL3/4 COMPASS-like complexes associate specifically with UTX, NCOA6, PA1, and PTIP.¹¹⁹ These COMPASS-like complexes have been demonstrated to play a prominent role in nuclear transport signaling, adipogenesis, and immunoglobulin class switching.¹¹⁹

DISEASE MECHANISMS

Polycomb and Trithorax Deregulation in Developmental Disease

Weaver Syndrome—Weaver syndrome is a rare congenital disorder that is characterized by dysmorphic facial features, learning disability, and tall stature. Two independent whole-exome sequencing studies were conducted in patients with Weaver syndrome revealing a spectrum of mutations in the PRC2 catalytic subunit EZH2.^{202,203} Further analysis characterized a total of 48 individuals with Weaver syndrome that carry mutant variants of EZH2.²⁰⁴ Notably, there is some overlap of missense mutations with common somatic variants found in myeloid malignancies (D664V, R684C, and Y733X),²⁰⁵ however, whether or not patients with Weaver Syndrome have a predisposition for malignancy is still uncertain due to the rarity of the disease. In a brief communication published last year,²⁰⁶ a patient with overgrowth phenotypes associated with Weaver syndrome and Sotos syndrome (a

similar disorder attributed to NSD1 deficiency) was found to carry a *de novo* missense mutant variant of EED, but no pathogenetic mutations in EZH2 or NSD1. It is thus possible that deregulation of the PRC2 complex is a general pathogenic mechanism for Weaver syndrome.

Ataxia-Telangiectasia—Ataxia telangiectasia (AT) is a multisystem disease, but is characterized primarily by its neurological symptoms which arise from CNS degeneration, including ataxia, choreoathetosis, and loss of ambulation.^{207,208} Mutations within the AT mutated (ATM) gene, a gene that encodes for a serine/threonine kinase responsible for mediating cell cycle arrest during DNA damage,²⁰⁹ are the causative factor in AT.²⁰⁸ The observation that loss of ATM leads to nuclear accumulation of HDAC4 led researchers to subsequently assess the role of PRC2 and H3K27me3 in ATM deficient models.²¹⁰ In this analysis it was shown that H3K27 is hypermethylated in both AT human cerebellar tissue and ATM deficient mice. EZH2 protein levels were also elevated in ATM deficient brain extracts without a change in mRNA level suggesting post-translational regulation. This mechanism was further elaborated after observing an extended half-life of nonphosphorylated S652 and S734 that correlated with ATM deficiency. Lastly, EZH2 knockdown in AT mouse models rescued neurodegeneration phenotypes in Purkinje cells. These data suggest a downstream role of EZH2 in AT pathogenesis.

Autism Spectrum Disorders—One of the most compelling instances of PcG protein deregulation in autism spectrum disorders (ASD) was reported after identifying an association between AUTS2, a commonly disrupted protein in neurological disorders,²¹¹ and the PRC1 complex.¹⁵¹ Interestingly, this complex was shown to be a transcriptional activator in cell based reporter gene assays which is further confirmed by AUTS2-PRC1 co-occupancy at genomic sites enriched in H3K4me3 and deficient in H3K27me3. Most striking are the AUTS2 heterozygous and homozygous mouse phenotypes, which recapitulate clinical low birth weight and stature in humans carrying a disrupted allele of AUTS2. Further analysis into the downstream effectors of the AUTS2-PRC1 complex will hopefully shed light on the pathogenic consequences of a variety of developmental disorders.

Several core components of the BAF complex including BAF155, BAF170, and BAF180, have also recently been identified in large scale *de novo* mutation analyses.^{212,213} Intriguingly, the lesser-studied CHD family proteins, CHD7 and CHD8, have also been implicated in autism pathogenesis. Through sequencing efforts Vissers et al.²¹⁴ initially identified mutations in the CHD7 gene in 10 of 17 patients with CHARGE syndrome, which primarily consisted of stop-codon mutations and deletions, suggesting a haploinsufficiency mechanism similar to that recently described for CHD8 in an AD model.²¹⁵

Coffin-Siris Syndrome—Coffin-siris syndrome (CSS) is a rare genetic disorder characterized by developmental delay, intellectual disability, microencephaly, dysmorphic facial features, and hypoplasia of the fifth finger and toenails. Recently, whole-exome sequencing efforts aimed at identifying causal genes in CSS pathogenesis have identified several mutations in BAF complex subunits. In an communication by Tsurusaki et al.,²¹⁶ two *de novo* heterozygous mutations in BAF47 were identified among five individuals

(p.Lys364del and p. Arg377His). Follow-up screening in a larger cohort of 23 individuals revealed *de novo* p.Lys364del BAF47 mutations in two additional patients. The low mutation detection rate of BAF47 mutations led researchers to screen for other BAF subunit mutations further characterizing *de novo* mutations in Brg, Brm, BAF57, ARID1A, and ARID1B. Interestingly, all mutations annotated in ARID1A and ARID1B have been predicted to result in protein truncation and/or nonsense-mediated mRNA decay suggesting haplosufficiency at these loci as a potential mechanism. Whole-exome sequencing by another group²¹⁷ similarly showed recurrent deletion and truncation mutations in ARID1B further reinforcing this hypothesis. Nevertheless, the causative nature of the identified BAF mutations in CSS remains to be clearly demonstrated.

Nicolaides-Baraitser Syndrome—Nicolaides-Baraitser syndrome (NBS) is clinically similar to CSS with characteristics that include intellectual disability, limited speech, dysmorphic facial features, and brachytachdylly.²¹⁸ However, NBS patients typically have prominent finger joints and broad distal phalanges instead of the CSS hallmark hypoplasia of the fifth finger and toenails.²¹⁹ In a whole exome analysis of 44 NBS patients, 36 members were determined to carry *de novo* heterozygous missense mutations in Brm (SMARCA2).²²⁰ Interestingly, most of these mutations reside in the ATPase domain of Brm suggesting a dominant effect due to alteration of catalytic activity. Mutations in ARID1B and BAF47 have also been described suggesting a pathological commonality between CSS and NBS, but further mechanistic studies are needed.

Polycomb and Trithorax in Human Cancer

Polycomb Repressor Complex 1—Amplification and overexpression of BMI1 has been observed in a number of human malignancies including mantle cell lymphoma, acute myeloid leukemia, colorectal carcinoma, liver carcinoma, non-small cell lung cancer, breast carcinoma, prostate cancer, head and neck cancer, medulloblastoma, and glioblastoma.^{58,59} BMI1 overexpression drives oncogenesis by repressing transcription of the p16 and p14arf tumor suppressors at the *INK4a* locus.^{130,131} CBX7 overexpression has also been implicated in driving human malignancies by similarly down regulating expression at the *INK4a* locus.^{55,221,222} Although both of these proteins are canonical PRC1 subunits and have overlapping function in regulating *INK4a*, they appear to exist in different PRC1 variants.^{221,222} Intriguingly, although CBX8 does functionally associate with BMI1 at the *INK4a* locus,²²³ its recent oncogenic role in MLL-AF9 driven leukemia appears to be independent from PRC1 repressor activity and *INK4a* mediated senescence.⁵⁷ The mechanisms that lead to upregulation of these components are still undetermined, however, insight into a novel regulatory mechanism by Yap et al.⁵⁶ implicates ANRIL ncRNA as a regulator of CBX7 in prostate cancer. Whether this mechanism applies to other CBX homologues, or other PRC1 subunits in general, remains to be seen. CBX4 has also been identified as an oncogenic driver in the case of hepatocellular carcinoma, functioning as a promoter of angiogenesis through regulation of HIF-1.⁵⁴ KDM2B is overexpressed in breast and pancreatic cancer as well as a variety of leukemias.⁶⁰

Polycomb Repressor Complex 2—The upregulation of PRC2 subunits has also been reported in numerous studies over the last decade. In particular, the catalytic subunit EZH2

is overexpressed in an array of cancers including prostate, colon, lung, endometrial, and breast tumor types.²²⁴ Furthermore, in a recent genome-wide sequencing effort aimed at identifying mutations that contribute to non-Hodgkin lymphoma subtypes, a recurrent missense mutation in exon 15 of the histone methyltransferase EZH2 was discovered in 21% of germinal center B-cell diffuse large B-cell lymphomas (GCB-DBCLs) and 7% of follicular lymphomas.²²⁵ This missense mutation occurs at Y641 within the catalytic SET-domain of EZH2 with Y641F, Y641S, Y641N, Y641H, and Y641C mutants all characterized in this study. Although mutations at this locus were initially believed to be disabling due to the inability of EZH2 mutants to catalyze the mono-methylation of H3 peptide substrate, follow-up studies revealed that while the catalytic rate of mono-methylation is indeed diminished, the rate of di- to tri-methylation is augmented as much as 20-fold.^{226,227} From these studies, a paradigm emerged that explained the heterozygosity of these missense mutations and disease pathogenesis. Although mutant forms of EZH2 are catalytically more efficient at depositing tri-methylation at H3K27, they require a wild-type EZH2 allele to mono- and di-methylate in advance. Another genome-wide sequencing study²²⁸ discovered a similar missense mutation at A677 that gives EZH2 equal catalytic ability for di- and tri-methylation compared to mono-methylation although this mutation appears to be present in a much smaller subset of tumor types. PRC2 loss (via loss of function somatic alterations in SUZ12 or EED) has also been noted in PNS tumors, high-grade gliomas, and melanomas.^{61,62} In this case, PRC2 loss appears to cooperate with the loss of NF1, a GTPase stimulating protein that activates Ras, resulting in unchecked proliferation.

BAF/PBAF Complex—The recent discovery of a myriad of mutations in BAF complex components in exome and whole genome sequencing efforts has signaled a paradigm shift in the epigenetics of cancers. Overall, mutations in BAF complex subunits are present in approximately 20% of human cancer.¹⁷⁷ As examples, these data have shown ARID1A inactivation in 57% of ovarian clear cell carcinomas,⁸¹ and 83% of gastric carcinomas with microsatellite instability.⁸² Losses of both ARID1A and ARID1B have been identified in 11% of neuroblastomas.⁸³ Loss of function mutations in BAF57 have recently been identified in spinal meningiomas.⁸⁵ BAF180 is mutated in 40% of renal cancers.⁸⁴ Moreover, somatic loss of both Brg and Brm are present in small-cell lung cancer (23% and 26%, respectively) and non-small-cell lung cancer (76% and 77%, respectively).⁸⁰ BRD7 deletion in p53 wild-type breast cancers has also been observed.⁹³ One theme that emerges from these recent data is that of tissue- (and subsequent cancer-) specificity. Specific subunits are mutated in specific malignancies, likely underscoring the tissue-specific protective functions.

The earliest link of BAF complexes to cancer came from studies demonstrating bi-allelic inactivation of BAF47 in ~100% of MRT, an especially aggressive subset of pediatric tumors.⁸⁶ BAF47 is a special case in that the severity of bi-allelic inactivation in mouse models results in 100% of mice developing tumors at 11 weeks, making it the most potent tumor suppressor known to date.⁸⁷ Loss of BAF47 is also observed in a subset of epithelioid sarcomas.⁸⁸ Molecular studies have revealed that BAF47 loss drives oncogenesis at least in part by upregulation of EZH2 and consequent repression of *INK4a* among other lineage

specific PcG targets.²²⁹ These data are suggestive of both a loss of function aspect of BAF47 inactivation as well as a subsequent synthetic lethality effect through the perturbation of EZH2 function. Subsequently, a recent report has identified EZH2 inhibitors as selective drivers of apoptosis in MRT cell models.²³⁰ Another report has also identified EZH2 inhibitors as effective in causing regression in ARID1A null tumors in mice.²³¹

While the previously described mutations of BAF complex subunits are inactivation or truncation mutations, synovial sarcoma is unique in that it appears to be driven by the formation of fusion protein resulting from a precise translocation between the SS18 subunit and SSX1/2/4.^{89–91} This translocation results in a fusion protein formed by 78 amino acids of the SSX C-terminus being appended to the C-terminus of SS18. Notably, outside of this fusion event, synovial sarcoma genomes do not appear to undergo genomic instability. The oncogenic function of this fusion protein has been recently shown to eject BAF47 from the BAF complex⁹² leading to proteosomal degradation of the freed subunit. This finding is further reinforced in histology reports showing diminished staining for BAF47 in synovial sarcoma tissue samples. Although the direct impact of the SS18-SSX fusion protein is known, the mechanism by which it directs oncogenesis is currently on going. It is interesting that BAF47 loss in MRT is also characterized by a lack of genomic instability possibly suggesting a strong loss of function component to synovial sarcoma disease progression. However, reported aberrant transcriptional activation of SOX2 upon SS18-SSX expression is suggestive of a concurrent gain of function mechanism in these tumors.⁹² Furthermore, altered genomic targeting imparted by the presence of the SSX C-terminal domain has not yet been ruled out.

These human genetics and functional studies have underscored the fact that BAF complexes have acquired new functions over the course of evolution. Intriguingly, the most commonly mutated BAF complex subunit, ARID1A, is not required for *in vitro* chromatin remodeling on chromatinized nucleosomal templates. However, studies above suggest indirectly that it is required for opposition with PcG complexes, either genome-wide or in a locus-specific manner.^{231,232} New methods and experimental systems that faithfully reflect mammalian biology and the complexity of chromatin *in vivo* will be required to identify the full spectrum of newly gained functions of BAF complexes. For an overview of current paradigms regarding BAF perturbation in oncogenesis see Figure 6 and for further reading in BAF complexes in cancer we refer you to the following review.²³³

PRC1/2 and BAF Complex Opposition—Research on transcriptional regulation of the *INK4a* locus is perhaps the most studied example of direct PRC1/2 and BAF antagonism. The PRC1/2 and BAF complexes are responsible for the transcriptional regulation of the *INK4a* locus. While PRC1 is necessary to maintain transcriptional repression of *INK4a*,¹³⁰ it is also dependent upon the prior deposition of H3K27me3 by PRC2 as shown in SUZ12 knockdown studies.²³⁴ The role of the BAF complex in *INK4a* activation was first noted in malignant rhabdoid tumor (MRT) models by Betz et al.²³⁵ during BAF47 (SNF5) ectopic expression studies. In this work, BAF47 null cell lines were found to undergo cell cycle arrest during re-expression of BAF47 as a result p16ink4a induction, RB phosphorylation, and cyclinA down regulation. Furthermore, the recapitulation of the wild-type BAF complex at the *INK4a* gene correlated with loss of both PRC1 and PRC2 at this locus. More recently,

in *Caenorhabditis elegans* the BAF complex has been shown to regulate key cell cycle inhibitors during muscle differentiation in a manner that is antagonistic to Polycomb mediated repression.²³⁶ Nevertheless, the exact molecular order of events that lead to PRC1/2-BAF switching remain to be determined.

MLL/COMPASS Complexes—Exome sequencing of over 3000 tumors has revealed the KMT2 family as being among the most frequently mutated genes in human cancer.²³⁷ Rearrangements involving *MLL1* (*KMT2A*) are the most comprehensively studied of this group with an estimated 10% of all human leukemias harboring translocations, duplications, or amplifications at this locus. Translocation induced protein fusions between MLL1 and EAP complex components alone account for at least 70% of MLL1 acute myeloid leukemia and acute lymphoblastic leukemia cases.²³⁸ Members of the EAP complex are known to interact with transcriptional elongation and chromatin modifying factors including pTEFb and DOT1L, respectively.²³⁹ MLL1-EAP complex component fusions thus facilitate oncogenic transformation by ectopically activating genes that are important in hematopoietic development including *Hoxa9* and *Meis1*.²⁴⁰ More recently a series of nonsense and frameshift mutations have been identified in a spectrum of solid tumor cancer types including lung, colon, bladder, and breast cancer.²⁴¹ Intriguingly, 18% of truncation mutations reside in the PHD region, and 9% reside in the CXXC domain.²⁴¹ Nevertheless, *MLL1* loss of function studies in mice do not indicate that haploin-sufficiency at this locus is not sufficient to drive oncogenic transformation.²⁴²

A spectrum of frame shift, nonsense, and missense mutations have also been identified in *MLL2* (*KMT2D*) and *MLL3* (*KMT2C*). These two groups are the most frequently mutated KMT2 family members in human cancer.²³⁷ Similar to mutations of *MLL1*, protein truncations are the most common with mutations residing in PHD and SET domains for both MLL2 (60% and 37%, respectively) and MLL3 (25% and 28%, respectively).²⁴¹ The mutation frequencies in SET1A are notably lower than other members of the KMT2 family, however, they have recently been suggested to have an oncogenic function in breast and colorectal cancer.^{117,118}

CONCLUSIONS AND FUTURE OUTLOOK

In this review, we present a historical perspective of the Polycomb and trithorax groups of chromatin modulators and further outline the mechanisms by which they function to control cell identity processes and drive diseases ranging from cancer to neurodevelopmental disorders. Through spatial and temporal opposition these groups form a unique regulatory system that has only begun to be demystified with the aid of next-generation technologies. With an evolving understanding of these systems, we can finally begin to probe the underlying mechanisms at the trx-PcG axis that drive mammalian disease.

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FIGURE 1.

Polycomb and trithorax group proteins control Hox gene expression. Appropriate spatial and temporal expression of Antennapedia Complex (ANT-C) and Bithorax Complex (BX-C) genes in *Drosophila melanogaster* is required for proper anatomical patterning in developing embryos. Intriguingly, the expression pattern of each of these clusters along the embryonic anterior-posterior axis mirrors their collinear arrangement along their respective chromosomal location. The BX-C, comprised of *Ubx*, *abdA*, and *AbdB*, controls the development of the posterior two-thirds of the embryo. Selected mutations mapped to these loci (shown above the each gene in red) cause homeotic transformations toward more anterior segments. The ANT-C, comprised of *lab*, *pb*, *Dfd*, *Scr*, and *Antp*, controls the development of the anterior segments of the fly embryo including the head and prothorax (non-homeotic genes including *zen*, *ftz*, and *z2* have been omitted from this figure). Mutant alleles in this locus (also shown above the respective genes in red) cause a variety of homeotic transformations in the anterior portion of the fly. A subclass of these mutations, including the *Ns* mutation, result in the eponymous antennapedia transformation in which antennae develop as leg structures.

FIGURE 2.

The evolution of Polycomb PRC1 and PRC2 complexes occurred similarly with both groups obtaining greater structural and functional diversity in mammals. Polycomb repressor complex 2 (PRC2) is the least altered over evolutionary time with the catalytic subunit E(z) and the ancillary subunit Pcl being the only subunits to expand into multiple paralogs, EZH1/2 and PCL1/2/3, respectively. Greater complexity is exhibited in the mammalian polycomb repressor complex 1 (PRC1) compared to its *Drosophila* counterpart. While all mPRC1 variants appear to have a stable core of RING1A/B and one of the six PCGF paralogs, two general sub-groups exist as defined by the presence of other protein subunits. The first is the canonical sub-group that is characterized by one of three PHC paralogs and one of five CBX paralogs. The second is the non-canonical subgroup that is characterized by the absence of PHC and CBX type proteins and the presence of either RYBP or YAF2. Dashed outline indicates an ancillary subunit. *Indicates that both PCGF and CBX have several paralogs within these complexes.

FIGURE 3.

Throughout evolution the BAF complex diverged into two distinct complexes: the BAF complex (BAP complex in *Drosophila*) and the PBAF complex (PBAP complex in *Drosophila*). Furthermore, in mammals several subunits obtained multiple paralogues including BAF45A/B/C/D, BAF60A/B/C, BAF155/170, BAF250A/B, BAF53A/B, Brd7/9, and the catalytic subunits Brg/Brm. The COMPASS complex also developed greater structural and functional diversity during evolution. While the Set1/COMPASS complex was maintained in both *Drosophila* and mammals, additional COMPASS-like complexes have been identified including the trx/COMPASS-like complex (MLL1/2/COMPASSlike in mammals), and the trr/COMPASS-like complex (MLL3/4/COMPASS-like in mammals).

FIGURE 4.

Transcriptional repression by polycomb repressor complex 1 (PRC1) and polycomb repressor complex 2 (PRC2) is multi-faceted. (a) PRC1 mono-ubiquitinates histone 2A at lysine 119 (H2AK119Ub1) at genes targeted for transcriptional repression. In the absence of histone methyl marks (i.e., H3K27me3) PRC1 can bind to DNA via RYBP, YAF2, and other ancillary factors in order to propagate repressive H2AK119Ub1. Deposition of H2AK119Ub1 can be reversed by deubiquitinase (DUB) proteins including 2A-DUB, Ubp-M, USP21, and USP7. (b) The catalytic subunit of PRC2, EZH2, is capable of mono-, di-, and tri- methylating H3K27, with the trimethyl mark being the stable mark of heterochromatin formation and subsequent transcriptional repression. Both UTX and JMJD3 demethylases are capable of antagonizing H3K27me3 mediated repression by reducing H3K27me3 to H3K27me1 through sequential demethylation. (c) The canonical PRC1 can bind to H3K27me3 through its CBX chromodomain allowing for H2AK119Ub1 deposition and further reinforcing a repressive environment. (d) Alternatively, non-canonical PRC1 has recently been suggested to function as a frontier complex by binding to DNA through KDM2B allowing for primary H2AK119Ub1. This is followed by PRC2 mediated H3K27me3 via interactions with ubiquitin that are mediated by JARID2 and AEBP2.

FIGURE 5.

Two sub-families of BAF chromatin remodelers: BAF and PBAF. Both BAF and PBAF complexes have ATP-dependent chromatin remodeling ability, and both complexes can recruit ancillary protein factors and transcriptional machinery. Although the general chromatin remodeling functionality of these complexes is understood, genomic targeting specificities during development and disease are still uncertain. Furthermore, how these complexes are formed, and how the core subunits are apportioned to maintain a balance between BAF and PBAF remains to be determined.

FIGURE 6.

Mutations of BAF subunits are thought to drive oncogenesis through both loss-of-function and gain-of-function mechanisms. In this figure, a BAF subunit (in green) is lost due to deletion (or nonsense) mutations resulting in haploinsufficiency or loss of heterozygosity, or carries truncation or missense mutations that affects a functional region (highlighted in yellow). Loss of this BAF subunit could possibly result in paralogue compensation (replaced by red BAF paralogue) leading to gain and/or loss of function at crucial regulatory genes (left and right pictures, respectively). Similarly, BAF subunit loss could also result in destabilization of BAF complexes with gain and/or loss of function outcomes due to residual aberrant complexes (top right and bottom left pictures, respectively). Truncation or missense mutations that affect a functional region could also lead to gain of function due to mistargeting to oncogenes (top left picture) or loss of function (bottom right picture) due to inability to target crucial tumor suppressor genes for activation.

TABLE 1

The PcG Repressive Complexes

<i>Drosophila melanogaster</i>	Mammals (Mouse/Human)	Domains	Epigenetic Function	Disease Relevance
<i>PcG Complexes</i>				
PRC1				
Pc	CBX2 CBX4 CBX6 CBX7 CBX8	Chromodomain	H3K27me3 binding (all) H3K9me3 binding (CBX4) RNA binding (CBX4 and CBX7)	Hepatocellular carcinoma (CBX4) ⁵⁴ Thyroid cancer (CBX7) ⁵⁵ Prostate cancer (CBX7) ⁵⁶ MLL (CBX8) ⁵⁷
Ph	PHC1 PHC2 PHC3	Sterile alpha motif (SPM domain)	Possible RNA binding	
dRING/Sce	RING1A RING 1B	RING-finger domain	Histone Ubiquitination (H2AK119Ub1)	
Psc	NSPC (PCGF1) MEL18 (PCGF2) PCGF3 BMI1 (PCGF4) PCGF5	RING-finger domain	Histone Ubiquitination (H2AK119Ub1)	Breast cancer (MEL18 and BMI1) ⁵⁸ Mantle cell lymphoma (BMI1) ⁵⁹ AML (BMI1) ⁵⁸ Lung cancer (BMI1) ⁵⁸ Medullocarcinoma (BMI1) ⁵⁸ Prostate cancer (MEL18 and BMI1) ⁵⁸
Scm	SCMH1 SCMH2	Sterile alpha motif (SPM domain)	Possible RNA binding	
dRYPB	RYPB YAF2	Zinc finger domain	DNA binding	
	KDM2B	Zinc finger domain JmjC domain	H3K36me3 demethylase unmethylated CpG island binding	T-ALL and B-ALL ⁶⁰ AML ⁶⁰ Pancreatic cancer ⁶⁰ Breast cancer ⁶⁰
PRC2				
E(Z)	EZH1 EZH2	SET domain SANT domain	H3K27 methyltransferase Histone binding	Various Non-Hodgkins lymphomas as well as a number of prostate, lung, endometrial, and breast cancers
SU(Z)12	SUZ12	Zinc-finger domain	Possible DNA/RNA binding	Malignant peripheral nerve sheath tumor ^{61,62} Melanomas ⁶¹ Breast cancer ⁶³
ESC	EED	WD40-repeat domain	H3K27me1/2/3 binding	Malignant peripheral nerve sheath tumor ^{61,62}
NURF55	RbAp46 RbAp48	WD40 domain	H3K36me3 binding	

Drosophila melanogaster	Mammals (Mouse/Human)	Domains	Epigenetic Function	Disease Relevance
Pcl	PCL1 PCL2 PCL3	PHD-finger domain Tudor domain	H3K36me3 binding	
Jing	AEBP2 JARID2	Zinc-finger domain ARID domain Zinc finger domain	H2AK119Ub1 binding RNA binding H2AK119Ub1 binding	

TABLE 2

The trxG Chromatin Remodeler Complexes

	<i>Saccharomyces cerevisiae</i>	<i>Drosophila melanogaster</i>	Mammals (Mouse/Human)	Domains	Epigenetic Function	Disease Relevance
<i>trxG Remodeling Complexes</i>						
SWI-SNF (BAF and PBAF)	Swi2/Snf2	BRM	BRM BRG	Helicase Bromodomain	ATPase function	Lung cancer ⁸⁰
	Swi1	OSA	BAF250A BAF250B	ARID domain	Possible DNA-binding	Ovarian carcinoma ⁸¹ Gastric carcinoma ⁸² Neuroblastoma ⁸³
	Swi3	MOR (BAF155)	BAF155 BAF170	SWIRM domain SANT domain Chromodomain	Possible DNA binding Possible histone binding	
		BAP170	BAF 180 BAF200 (ARID2)	Polybromodomain	Histone binding	Renal carcinoma ⁸⁴
	Swp73	BAP60	BAF60A BAF60B BAF60C	SWI-B domain		
		BAP111	BAF57	HMG domain	Possible DNA binding	Spinal meningioma ⁸⁵
	Snf5	SNR1	BAF47	Winged Helix domain		Malignant rhabdoid tumor ^{86,87} Epithelioid sarcoma ⁸⁸
	Swp82	SAYP	BAF45A BAF45B BAF45C BAF45D	PHD-finger domain		
	Arp7 Arp9	BAP55	BAF53A BAF53B	Actin-like		
		Actin	beta-Actin			Synovial sarcoma ⁸⁹⁻⁹²
NuRD			SS18 (SYT) SS18L1 (CREST)			
			BCL7A BCL7B BCL7C			
			BRD7 BRD9	Bromodomain	Histone binding	Breast cancer ⁹³
		dMi2	CHD3 CHD4	Chromodomain Helicase domain		
		dMBD2 dMBD3	MBD3		Methylated CpG binding	

	<i>Saccharomyces cerevisiae</i>	<i>Drosophila melanogaster</i>	Mammals (Mouse/Human)	Domains	Epigenetic Function	Disease Relevance
ISWI		dMTA	MTA1 MTA2 MTA3	SANT domain BAH domain Zinc finger		
		NURF55	RbAp46 RpAp48	WD40 domain	Histone binding	
		p66 p68				
		ISWI	SNF2L SNF2H	SANT domain Helicase domain	ATPase function DNA binding	
	Isw1/2		BPTF hACF1	Bromodomain PHD-finger domain	Histone binding	
	Icf1	NURF301 ACF1				
		NURF38				
		CHRAC14 CHRAC16	hCHRAC17 hCHRAC15			
	Ioc2/3/4	NURF55	RbAp46 RbAp48	WD40 domain	Histone binding	
	Ino80	dIno80	hIno80	Helicase domain	ATPase function DNA binding	
INO80	Rvb1,2	Reptin	RUVBL1,2	AAA+ domain	DNA binding	
	Arp5 Arp8	dArp5 dArp8	BAF53A Arp5 Arp8	Actin-like		

TABLE 3

The trxG Chromatin Modulator Complexes

	<i>Saccharomyces cerevisiae</i>	<i>Drosophila melanogaster</i>	Mammals (Mouse/Human)	Domains	Epigenetic Function	Disease Relevance
<i>trxG Modifying Complexes</i>						
COMPASS	Set1	dSet1	SET1A SET1B	SET domain	H3K4me1/2/3 methyltransferase	Breast cancer (SET1A) ¹⁷ Colorectal cancer (SET1A) ¹⁸
		HCF1	HCF1	Kelch domain		
	Cps35	WDR82	WDR82	WD repeat	Histone binding	
	Cps40	CXXC1	CXXC1	Zinc finger domain	DNA binding	
	Cps60	ASH2	ASH2L	Zinc finger domain	DNA binding	
	Cps25	Dpy30	Dpy30	WD repeat	Histone binding	
	Cps50	RBBP5	RBBP5	WD repeat	Histone binding	
	Cps30	WD5	WDR5	WD repeat	Histone binding	
MLL1/2 COMPASS-like		trx	MLL1 MLL2	Set domain	H3K4me 1/2/3 methyltransferase	Bladder, lung, and endometrial cancer (MLL2) ¹⁹ AML and ALL (MLL1) ¹⁹
		menin	menin			
MLL3/4 COMPASS-like		HCF1	HCF1	Kelch domain		
		trr	MLL3 MLL4	Set domain	H3K4me 1/2/3 methyltransferase	Bladder, lung, endometrial cancer (MLL3/4) ¹⁹ Renal clear cell carcinoma (MLL3) ¹⁹ Follicular lymphoma (MLL4) ¹⁹ Diffuse large B-cell lymphoma (MLL4) ¹⁹
	NCOA6	NCOA6	NCOA6			
	PA1	PA1	PA1			
	UTX	UTX	UTX	ImjC domain	H3K27 demethylase	Esophageal carcinoma ¹⁹ Multiple myeloma ¹⁹
TAC1		PTIP	PTIP	Set domain	H3K4me1/2/3 methyltransferase	
		trx				

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<i>Saccharomyces cerevisiae</i>	<i>Drosophila melanogaster</i>	Mammals (Mouse/Human)	Domains	Epigenetic Function	Disease Relevance
	dCBP		HAT domain Bromodomain	H3K27 acetyltransferase	
ASH1	SBF1	ASH1L	SET domain Bromodomain	H3K36 methyltransferase	
	dCBP		HAT domain Bromodomain	H3K27 acetyltransferase	

COMPASS subunits in bold are common core subunits also present in MLL1/2/COMPASS-like and MLL3/4/COMPASS-like complexes.