Structure, mechanism, and regulation of polycomb-repressive complex 2

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Polycomb repressive complex 2 (PRC2) methylates lysine 27 in histone H3, a modification associated with epigenetic gene silencing. This complex plays a fundamental role in regulating cellular differentiation and development, and PRC2 overexpression and mutations have been implicated in numerous cancers. In this Minireview, we examine recent studies elucidating the first crystal structures of the PRC2 core complex, yielding seminal insights into its catalytic mechanism, substrate specificity, allosteric regulation, and inhibition by a class of small molecules. This is an open access article under the CC BY license.

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The abbreviations used are: PcG, Polycomb group; PDB, Protein Data Bank; KMT, lysine methyltransferase; EED, embryonic ectoderm development; EBD, EED-binding domain; SBD, SANT1-like binding domain; SAL, SET activation loop; AdoMet, S-adenosylmethionine; SMR, stimulation-responsive motif; BAM, β-addition motif; iSET, inserted SET; cSET, C-terminal SET; AdoHcy, S-adenosylhomocysteine.

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Polycomb group (PcG)3 proteins represent transcriptional repressors that are present in single-cell eukaryotes through multicellular organisms (1, 2). The genes encoding many of these proteins were initially characterized in Drosophila as key regulators of epigenetic silencing of homeotic genes (3, 4). Subsequent studies demonstrated that PcG proteins function in the context of large heteromeric complexes that repress gene expression within facultative heterochromatin. These PcG complexes include Polycomb repressive complexes 1 and 2 (PRC1 and PRC2), as well as the more recently identified Pho-repressive complex and Polycomb repressive deubiquitinase (1, 5–7). The PRCs possess intrinsic histone-modifying activities that contribute to their functions in transcriptional repression. PRC1 monoubiquitinates Lys-119 in histone H2A (H2AK119ub1) and can compact chromatin by binding to nucleosomes, whereas PRC2 is a lysine methyltransferase (KMT) that trimethylates Lys-27 in histone H3 (H3K27me3), a modification associated with PcG silencing (8–15). PRC1 has been shown to bind H3K27me3, whereas PRC2 can recognize H3K27me3 and H2AK119ub1, facilitating the recruitment of the PRCs to specific genomic loci (16–19). The interdependence of their enzymatic activities and chromatin localization illustrates how PRC1 and PRC2 can function in concert to epigenetically silence gene expression (20).

PRC2 comprises multiple subunits that facilitate its biological functions. The minimal core complex that exhibits methyltransferase activity comprises the core subunits embryonic ectoderm development (EED), Suppressor of Zeste 12 (SUZ12), and the catalytic subunit Enhancer of Zeste Homolog 1 or 2 (EZH1 or EZH2) that possess a conserved catalytic SET domain found in many KMTs (8–11, 20, 24, 25).

Many of these noncore subunits possess intrinsic histone or DNA-binding activity and can mediate recruitment of PRC2 to chromatin and promote H3K27 methylation (20, 26–29). Deletion of the genes encoding the PRC2 core subunits in mice results in morphological defects and embryonic lethality, underscoring their importance in regulating epigenetic programs that are essential to development and differentiation (22, 30, 31). PRC2 has also been shown to have context-dependent roles in cancer (32–35). Deletions and loss-of-function mutations of PRC2 core subunits have been characterized in certain tumors, whereas overexpression and gain-of-function mutations in EZH2 have been identified in a broad spectrum of cancers, illustrating that PRC2 can exhibit both tumor-suppressive and oncogenic activities. With respect to tumorigenesis, PRC2 has been shown to silence the expression of genes that regulate cell cycle checkpoints, differentiation, cell adhesion, and DNA damage response, thus promoting cancer cell growth and proliferation. Clinical data implicating PRC2 as an oncogenic driver in cancer have spurred the discovery and development of EZH2 inhibitors as a new class of chemotherapeutic drugs, several of which are in pre-clinical studies or clinical trials (36–46).

Since its discovery, there has been widespread interest in understanding the architecture of the PRC2 complex and how its structure facilitates chromatin association and histone methylation. Ciferri et al. (47) made initial strides toward this goal by determining a low-resolution electron microscopy (EM) struc-
Structure and function of PRC2

Figure 1. Crystal structures of PRC2 core subunits. A, ribbon rendering of the β-propeller domain of mouse EED bound to the EBD motif of EZH2 (PDB accession code 2QXV). For clarity, proteins, domains, and motifs are uniformly color-coded throughout the figures (color key provided in Fig. 2A). B, human EED β-propeller domain bound to an H3K27me3 peptide (PDB code 3IIW). The H3K27me3 peptide and the side chains of interacting residues in EED are depicted in stick form, with the EED residues that compose the aromatic cage highlighted in orange. Hydrogen bonds are indicated by orange dashes. EED is rotated −180° relative to its orientation in A. C, ribbon diagram of the isolated catalytic domain of human EZH2 (PDB code 4MI0). Zinc ions are illustrated as gray spheres.

Initial studies of the PRC2 core subunits

Structural and biochemical studies of individual PRC2 core subunits paved the way for the subsequent structural determinations of the core complex. Among the first subunits characterized was the WD40 repeat protein EED. Using pulldown assays with a series of EZH2 constructs of varying lengths, Han et al. (53) identified a 30-residue motif (amino acids 39–68) that mediated association with EED, which they termed the EED-binding domain (EBD). To understand this interaction at the molecular level, they determined the crystal structure of mouse EED bound to an EBD peptide. As observed in other WD40 proteins, EED adopts a β-propeller structure with each of its seven WD40 repeats forming a propeller blade (Fig. 1A). The EBD peptide binds to a complementary channel in the larger circular surface of the EED β-propeller. The majority of the EBD motif forms an α-helix that spans nearly the full diameter of the EED β-propeller, with its C terminus adopting an extended coil conformation. The helix and extended coil are anchored to EED through an array of hydrogen bonds and van der Waals interactions. Mutations of the residues forming the EBD–EED interface either impaired or abolished binding, illustrating their importance in protein–protein recognition. Corroborating these results, a sequence alignment of the human, mouse, and Drosophila EBD motifs illustrates that the residues that interact with EED are highly conserved, underscoring the evolutionary role of the EBD in facilitating EZH2–EED association in metazoans.

Following the characterization of EED and the EBD motif, Margueron et al. (54) reported that recognition of repressive histone lysine methylation marks by EZH2 allosterically activates H3K27 methylation by the PRC2 complex. Isothermal titration calorimetry and fluorescence binding assays with trimethylated histone peptides revealed that human EED physically binds to histone methylation sites implicated in gene silencing, including H1K26me3, H3K9me3, H3K27me3, and H4K20me3. Conversely, EED exhibited either weak or negligible interactions with peptides mimicking methylation sites linked to transcriptional activation, such as H3K4me3, H3K36me3, and H3K79me3. Crystal structures of human EED bound to H1K26me3, H3K9me3, H3K27me3, and H4K20me3 peptides illuminated the molecular basis for the selective recognition of repressive methyl-lysine marks. The trimethylated histone peptides bind in the center of the smaller circular surface of the EED β-propeller, opposite to the side that recognizes the EBD motif (Fig. 1, A and B). The trimethyllysine is bound within an aromatic cage composed of Phe-97, Tyr-148, and Tyr-365 via cation–π interactions that are observed in other methyl-lysine–binding domains (Fig. 1B) (55). EED methylation site selectivity is achieved through recognition of the residues that are either two amino acids N- or C-terminal (−2 or +2 positions, respectively) to the methyl-lysine. In H3K9, H3K27, and H1K26, an alanine in the −2 position of these methylation sites binds in a small pocket on the surface of EED, whereas in H4K20, Leu-22 (+2 position) interacts with a hydrophobic pocket of the protein’s surface. Notably, H3K4, H3K36, and H3K79 lack an alanine or a bulky hydrophobic residue in the −2 or +2 positions of their methylation site sequences, respec-
tively, illustrating how EED discriminates against recognition of methyl-lysines linked to transcriptional activation.

After characterizing methyl-lysine recognition by EED, Margueron et al. (54) investigated whether association with methylated histone peptides allosterically regulates the activity of a PRC2 complex comprising EZH2, EED, SUZ12, and RBAP48. In the presence of an H3K27me3 peptide, PRC2 methylation of recombinant oligonucleosomal substrates was allosterically stimulated by 7-fold compared with its basal activity with an unmodified H3K27 peptide. Kinetic analysis of this allosteric regulation demonstrated that the $V_{\text{max}}$ value of PRC2 was increased by H3K27me3 peptide, whereas the $K_m$ value for oligonucleosomes was essentially unaltered. Methylation assays performed in the presence of H3K27me1 and H3K27me2 peptides showed only modest or no stimulation relative to PRC2 basal activity, consistent with the observation that EED preferentially binds H3K27me3. Furthermore, H3K9me3, H4K20me3, and H1K26me3 peptides only modestly increased PRC2 activity compared with the larger stimulatory effect observed for H3K27me3, whereas H3K4me3 and H3K36me3 peptides exhibited no appreciable enhancement of oligonucleosome methylation. An independent study largely corroborated these results, with the exception that H1K26me3 was reported to inhibit nucleosome methylation by PRC2 (56).

In agreement with these findings, mutations of the aromatic cage residues in EED abolished H3K27me3-mediated stimulation of PRC2 in vitro and resulted in developmental defects and lethality in Drosophila (54). Together, these results demonstrate that the H3K27me3 recognition by EED mediates chromatin interactions and allosteric activation of PRC2, stimulating the propagation of this modification within chromatin.

In 2013, two groups independently determined crystal structures of the isolated catalytic domain of human EZH2 (57, 58). These structures reveal that the N-terminal region of the catalytic domain comprises a CXC domain possessing two Zn$_2$Cys$_6$ motifs, whereas the C-terminal region is composed of the SET domain with an inserted SET (iSET) motif and a C-terminal SET (cSET) motif that follows the SET domain (Fig. 1C). The EZH2 SET domain possesses a $\beta$-sheet topology that is structurally conserved across the SET KMT family (59), but it adopts an autoinhibited conformation that renders it catalytically inactive (57, 58). Notably, comparisons with the ternary complexes of other SET domain KMTs bound to peptide substrates and cofactors illustrate that the isolated EZH2 SET domain adopts a conformation that is incapable of binding the methyl donor S-adenosylmethionine (AdoMet) and the protein substrate. Specifically, the cSET region is partially disordered and oriented away from the cofactor-binding site in the isolated EZH2 catalytic domain, eliminating interactions that are essential for AdoMet recognition. Moreover, the cSET motif is positioned within the substrate-binding cleft, occluding protein substrate binding. Consistent with these observations, the isolated EZH2 catalytic domain was unable to bind AdoMet or an H3K27 peptide substrate and was inactive in methyltransferase assays, whereas the PRC2 core complex containing EZH2, EED, and SUZ12 displayed robust activity with the substrates, concurring with earlier studies (9, 10, 23, 58). Collectively, these findings illustrate that the isolated EZH2 SET domain adopts an autoinhibited conformation that is incapable of substrate binding and catalysis, underscoring the importance of the PRC2 core subunits in endowing the enzymatic activity of EZH2.

**Chaetomium thermophilum PRC2 structure**

Crystal structures of the thermophilic fungus *C. thermophilium* PRC2 (CtPRC2) core complex offered the first insights into its atomic level structure, catalytic mechanism, and allosteric regulation (48, 49, 51). Jiao and Liu (48, 49) determined the following two CtPRC2 structures composed of EED, EZH2, and the SUZ12 VEFS domain: 1) the basal state bound to the methyl transfer product S-adenosylhomocysteine (AdoHcy), and 2) the stimulated state bound to AdoHcy and an allosteric H3K27me3 peptide. The overall architecture of CtPRC2 comprises regulatory and catalytic lobes, with EZH2 spanning between the two lobes (Fig. 2A). EED forms the center of the regulatory lobe and is surrounded by a belt composed of the N-terminal domains of EZH2, including the SANT1-like binding domain (SBD), EBD, $\beta$-addition motif (BAM), SET activation loop (SAL), stimulation-responsive motif (SRM), and SANT1-like (SANT1L) domains. The SBD associates with the SANT1L domain, completing the belt-like structure that encircles the EED $\beta$-propeller domain. In the structure of the CtPRC2-stimulated state, the H3K27me3 peptide interacts with the SRM motif and occupies the allosteric binding site in EED, analogous to the human EZH2–H3K27me3 complex (Figs. 1B and 2A).

The PRC2 catalytic lobe comprises the SUZ12 VEFS domain and the C-terminal domains of EZH2, including the motif connecting SANT1L and SANT2L (MCSS) and the SANT2L, CXC, and SET domains (Fig. 2A) (48). AdoHcy is bound within the AdoMet-binding cleft of the SET domain and adopts a conformation that is homologous to the cofactor-binding modes observed in the structures of other SET KMTs (60, 61). In addition, an extended loop from a SUZ12 molecule in an adjacent asymmetric unit in the crystal lattice interacts with the protein substrate-binding cleft of the SET domain, mimicking a bound substrate (49, 51). The interface between the two lobes is primarily composed of the EED, SRM, and SAL domains in the regulatory lobe, and the SUZ12 VEFS and SET domains and iSET motif in the catalytic lobe. The subunit interactions at this interface have an essential role in stabilizing and allosterically stimulating the EZH2 SET domain in CtPRC2. Finally, a comparison of the CtPRC2 crystal structures and the human holo-PRC2 EM structure reveals an overall high degree of structural similarity, illustrating the conservation of the complex’s architecture between fungi and animals (47, 48).

The SAL motif plays an essential role in stabilizing the SET domain in a catalytically productive conformation in the basal and stimulated states of CtPRC2 (48). This motif sits at the junction between the regulatory and catalytic lobes and interacts with the EED $\beta$-propeller, SUZ12 VEFS domain, and the EZH2 MCSS and SET domains (Fig. 2B). Deletion and mutations of residues 310–315 in the SAL motif, which interact with the N-terminal region of the SET domain, abolished CtPRC2 catalysis but do not affect complex assembly. Furthermore, substitutions in a subset of these residues (313–315) impaired CtPRC2 activity, illustrating that a few key amino acids in the
SAL domain are critical for maintaining the SET domain in a catalytically productive conformation.

A comparison of the basal and stimulated states of CtPRC2 provides insight into the mechanism by which H3K27me3 binding to EED induces allosteric activation (48). A structural alignment of these states reveals that the SRM motif is mobile and disordered in the basal state but becomes fully ordered in the stimulated state through direct interactions with the EED-bound H3K27me3 peptide (Fig. 2C). Binding of the peptide to the allosteric site induces a conformational change in the PRC2 complex, rotating the catalytic lobe in a counterclockwise direction toward the SRM motif. The SRM structure is composed of a coil followed by an α-helix that mediates interactions between the H3K27me3 peptide and the iSET motif within the SET domain (Fig. 2D). Residues Ala-24 through Ser-28 in the H3K27me3 peptide interact with the SRM through a network of hydrogen bond and van der Waals interactions. Notably, the packing of the SRM motif against the H3K27me3 peptide sterically occludes the binding of residues with large side chains in the −3 position of the methylation site, corresponding to Ala-24 in the H3K27 sequence. This observation concurs with previous studies showing that H1K26me3, which harbors a lysine in the −3 position, binds to EED but does not appreciably stimulate PRC2 activity (54).

The α-helix of the SRM motif packs adjacent to the iSET α-helix through a series of hydrophobic interactions, facilitating allosteric communication between the regulatory and catalytic lobes of CtPRC2 (Fig. 2D) (48). These interactions stabilize the iSET motif, as evidenced by the ordering of the C-terminal residues in the iSET motif that are disordered in the basal state...
This stabilization presumably contributes to the allosteric stimulation of the SET domain. Consistent with these observations, mutations or deletions of the SRM residues that interact with the H3K27me3 peptide or the iSET motif, including Pro-325, His-326, Asp-329, and Leu-350, disrupted PRC2 stimulation, underscoring their importance in allosteric regulation within the complex (Fig. 2D). In summary, the structure of the CtPRC2-stimulated state reveals that the SRM motif functions as an allosteric sensor that detects the binding of the repressive trimethyl-lysine marks to EED and transmits the conformational signal through the iSET motif to the SET domain, thus stimulating PRC2 activity.

Human PRC2 structure

Following the publication of the CtPRC2 complex, Justin et al. (50) reported the crystal structure of the stimulated state of the human PRC2 (HsPRC2) core complex bound to AdoHcy, an allosteric JARID2-K116me3 peptide, and an H3K27M inhibitor peptide (Fig. 3A). The allosteric peptide is based upon the trimethylation of Lys-116 in JARID2 by PRC2, which has been shown to bind to EED and activate the complex, analogous to H3K27me3 (54, 56, 62). The H3K27M peptide is derived from a missense mutation first identified in a subset of pediatric glioblastomas and inhibits PRC2 activity by associating with the protein substrate-binding cleft in the EZH2 SET domain (63, 64). Superimposition of CtPRC2 and HsPRC2 structures illustrates their overall homology, highlighting the evolutionary conservation of the core complex from fungi to mammals (Fig. 3B). Nonetheless, there are notable variations between the two structures. In the regulatory lobe, C. thermophila EED possesses an inserted motif in the sixth blade of the /H9252-propeller, which is absent in the human protein. In addition, the EZH2 SBD and SANT1 domains are shifted relative to each other in the CtPRC2 and HsPRC2 structures. The EBD domains also differ between the two complexes. In HsPRC2, the EBD forms an /H9251-helix that nearly spans the diameter of the EED /H9252-propeller, analogous to the mouse EED–EBD structure, whereas in CtPRC2, the EBD adopts a short /H9251-helix followed by an extended coil that connects to the BAM (Figs. 1A, 2A, and 3, A and B). In the catalytic lobe, the SRM /H9251-helix of HsPRC2 is shorter than the corresponding helix in CtPRC2, whereas the iSET helix is longer and exhibits a slightly bent conformation in human EZH2 compared with CtPRC2. Furthermore, the C terminus of the human MCSS motif possesses an additional /H9251-helix that serves as a docking site for nascent RNAs that bind PRC2 and inhibit its activity (65). In contrast, the MCSS motif of CtPRC2 lacks this helix, implying differences in how RNA may modulate the activities of fungal and vertebrate PRC2.
A comparison of structures of the isolated human EZH2 catalytic domain and the stimulated state of HsPRC2 reveals the interactions within the core complex that stabilize and allosterically activate the SET domain (50, 57, 58). In HsPRC2, the SAL motif engages in hydrophobic interactions with residues in the N-terminal region of the SET domain, causing it to adopt a conformation distinct from the structure of the isolated catalytic domain (Figs. 1C and 3C). The SAL motif also buttresses the residues in the SET domain flanking the iSET α-helix, inducing a counterclockwise rotation of the helix compared with the helix in the isolated protein. Further stabilizing this rotation, the SRM and iSET α-helices pack anti-parallel to each other through a network of side chain-mediated van der Waals interactions. Most notably, the cSET motif of EZH2 adopts strikingly different conformations in the isolated catalytic domain and HsPRC2 structures. In the isolated protein, the cSET region is mostly disordered and occupies part of the protein substrate-binding cleft, contributing to EZH2 autoinhibition (Figs. 1C and 3C). In contrast, the cSET motif is flipped in the opposite direction in the HsPRC2 structure, enclosing the AdoMet-binding pocket. Correlatively, the cSET motif adopts a structurally homologous conformation and interactions in the stimulated state of CtPRC2 (Figs. 2, A and C, and 3B) (48, 49). This conformational change induces a dramatic repositioning of the residues in the C terminus of the SET domain and cSET motif that completes the formation of the substrate-binding clefts, as highlighted by the alteration in the orientation of Tyr-728 between the isolated EZH2 and HsPRC2 structures (Fig. 3C). In summary, the SAL, SRM, and cSET motifs promote a catalytically productive conformation of the SET domain in PRC2, relieving the autoinhibited state of the isolated EZH2 catalytic domain.

The HsPRC2 structure yields key insights into its inhibition by the oncogenic H3K27M mutation (50, 63, 64). The H3K27M peptide binds in an extended conformation in the protein substrate-binding cleft formed by the SET domain and iSET motif, analogous to the substrate-binding modes observed in other SET KMT structures (Fig. 3D) (59, 66). The backbone of the H3K27M peptide is anchored in the substrate-binding cleft through a network of hydrogen bonds and van der Waals interactions with the residues composing the cleft. Recognition of the H3K27 methylation site occurs through hydrogen bonding between the side chains of Arg-26 in the peptide and Asp-652 in the iSET motif, as well as hydrophobic interactions with the side chains of Ala-25 and Ala-29 in histone H3. Together, these interactions deposit the K27M side chain of the peptide into the H3K27M peptide diminished its binding affinity and inhibition of PRC2, demonstrating that residues flanking Lys-27 contribute to site recognition (50, 70). Based on these results, Justin et al. (50) proposed a model for H3K27M-mediated inhibition wherein the EZH2 SET domain and EED bind to nucleosomal H3K27M and to repressive methyl-lysine marks, such as H3K27me3, respectively. These multivalent interactions would serve to anchor PRC2 to chromatin, thus sequestering and inhibiting the propagation of H3K27 methylation in heterochromatin (50).

**Structure of a PRC2–inhibitor complex**

Overexpression and somatic mutations of EZH2 and other PRC2 subunits have been implicated as oncogenic drivers in multiple cancers, rendering PRC2 an attractive target for the design of new chemotherapeutic drugs (32, 33). Multiple groups have reported the development of small molecules that exhibit potent inhibition of EZH2 in vitro and in vivo, with several of these compounds in preclinical studies or clinical trials (36). The majority of these EZH2 inhibitors possesses a pyridone core that is key to inhibiting PRC2, although the molecular mechanism by which these compounds target PRC2 had remained poorly understood. To gain insight into their mechanism of action, Broun et al. (52) determined the structure of a chimeric PRC2 core complex composed of human EED, the VEFS domain of human SUZ12, and Anolis carolinensis (Carolina anole) EZH2 bound to inhibitor-1, a pyridone-containing EZH2 inhibitor (Fig. 4, A and B). A. carolinensis EZH2 displays 95% sequence identity with human EH22 and was selected for these studies based on its greater enzymatic activity compared with the human protein and its propensity to crystallize in the context of the PRC2 core complex (for additional information, see Ref. 52). In the chimeric PRC2 complex, the inhibitor occupies a relatively hydrophobic pocket formed by EED and the SAL motif and the SET domain of EZH2 (Fig. 4C). The pyridone core is recognized via hydrogen bonding with Trp-624 and van der Waals interactions with the side chains of Trp-624, Phe-665, Arg-685, Phe-686, and Asn-688 in the SET domain. The fused 2-piperidinone and 1,4-dichlorobenzeno rings of inhibitor-1 form van der Waals contacts with the side chains of the SAL residues Ile-109 and Tyr-111, Phe-665 in the SET domain, and Tyr-661 and Cys-663 in the iSET motif. In addition, the Tyr-111 engages in a hydrogen bond with the 2-piperidinone moiety. Finally, the piperidine and hydroxypropanoyl moieties of inhibitor-1 participate in van der Waals interactions with the side chains of Tyr-111 in the SAL motif and Val-657, Tyr-658, and Tyr-661 in the iSET motif. The hydroxypropanoyl group also forms a hydrogen bond with Arg-222 in EED. Consistent with these findings, a recently reported structure of HsPRC2 bound to a different pyridone inhibitor...
reveals a similar binding mode and interactions with the SET domain and the iSET and SAL motifs of EZH2 (71). Importantly, the pattern of hydrogen bonds and van der Waals contacts with the pyridone core is analogous between the two inhibitor-containing complexes, revealing a common mechanism by which these compounds target PRC2.

Structural and functional studies of inhibitor-1 provide insights into its mechanism of PRC2 inhibition and how mutations in EZH2 confer resistance to the pyridone class of inhibitors (52). Prior kinetic studies had shown that the pyridone compounds function as AdoMet-competitive inhibitors of PRC2 (37, 72). The structure of PRC2 bound to inhibitor-1 offers an explanation for this mode of inhibition. Structural alignment of PRC2–inhibitor-1 complex and the HsPRC2–AdoHcy–JARID1K116me3–H3K27M peptide complex reveals that the pyridone core of the inhibitor occupies the region of the cofactor-binding pocket that binds to the methionyl moiety of AdoMet, thus directly occluding substrate binding (Fig. 4C). In particular, a Y111L missense mutation reduced PRC2 inhibition by inhibitor-1 by 70-fold in vitro and conferred resistance to a related pyridone inhibitor in tumor cell proliferation assays (74). Taken together, these studies elucidate the binding mode and AdoMet-competitive mechanism of the pyridone inhibitors and provide a molecular explanation for EZH2 somatic mutations that impart resistance to these compounds.

**Future directions**

The PRC2 crystal structures have yielded fundamental insights into subunit interactions, substrate recognition, and allosteric activation of the core complex and will provide a foundation for ongoing efforts to determine new structures of PRC2 complexes (48, 50). Indeed, while this article was under review, Bratkowski et al. (75) reported novel structures of the CtPRC2 apoenzyme and a CtPRC2–AdoMet complex, illustrating that the apoenzyme adopts an autoinhibited conformation that is relieved upon binding to AdoMet. Complementing these X-ray structures, the low-resolution EM structure of holo-PRC2 illustrates the spatial arrangement of the core subunits bound to AEBP2 and RBAP48 (47). Building upon these studies, high-resolution EM combined with X-ray crystallography...
The ability to determine crystal structures of PRC2 in complex with inhibitors represents a milestone in the design and optimization of potent and selective EZH2 inhibitors (52, 71). For example, JARID2 and AEBP2 harbor DNA-binding domains, whereas NURF55 and EED have been shown to recognize the N-terminal tails of core and linker histones (1, 20, 25, 26, 29, 54, 56, 78). Conversely, modifications associated with transcriptionally active chromatin, including H3K4me3 and H3K36me2/3, have been shown to interact with subunits in PRC2 and inhibit its enzymatic activity (29, 79, 80). Together, these findings have been instrumental in the development of models of holo-PRC2 bound to nucleosomes, explaining the molecular basis of its chromatin recruitment, substrate specificity, and allosteric regulation (47). Higher resolution structures of holo-PRC2 bound to di- or oligonucleosomes will be essential in obtaining an atomic-level view of how its subunits mediate multivalent engagement with nucleosomal substrates and regulate its enzymatic activity.

The pyridone core occupies the methionyl portion of the AdoMet-binding cleft, where it is recognized through an analogous set of interactions with residues in the SET domain (Fig. 4, C and D). It is conceivable that the pyridone core could be further derivatized to incorporate pendant groups that occupy the remainder of the cofactor-binding site, potentially mimicking the adenosyl moiety of AdoMet. Outside of the pyridine core, there is considerable variability in the structures of the pyridone inhibitors that have been reported by various groups (36). This variability suggests plasticity in the region of the inhibitor-binding pocket composed of the iSET and SAL motifs and EED that could be exploited to further enhance potency (Fig. 4C). In addition, a separate class of non-pyridone EZH2 inhibitors possessing a tetramethylpiperidinyl benzamide core has been discovered that inhibits PRC2 activity in vitro and blocks B cell lymphoma proliferation (81, 82). These compounds exhibit AdoMet-competitive inhibition of EZH2, but their interactions with PRC2 are not well-understood at the molecular level. Crystal structures of PRC2 in complex with these inhibitors would be informative in understanding how their binding modes compare with that of the pyridone inhibitors. In summary, PRC2 structures bound to EZH2 inhibitors will accelerate the development of these compounds as a new class of chemotherapeutics, offering novel avenues for the treatment of cancer.

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