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Recent structural insights into PRC2 regulation and substrate binding

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Abstract

Polycomb group proteins are transcriptional repressors controlling gene expression patterns and maintaining cell type identity. The chemical modifications of histones and DNA by the regulated activity of chromatin modifying enzymes such as Polycomb help establish and maintain such expression patterns. Polycomb repressive complex 2 (PRC2) is the only known methyltransferase specific for histone H3 lysine 27 (H3K27) and catalyzes its tri-methylation leading to the repressive H3K27me3 mark. Structural biology has made important contributions towards understanding the molecular mechanisms that ensure the spatiotemporal regulation of PRC2 activity and the establishment of inactive chromatin domains marked by H3K27me3. In this review, we discuss the recent structural studies that have advanced our understanding of PRC2 function, in particular the roles of inter-subunit interactions in complex assembly and the regulation of methyltransferase activity, as well as the mechanism of local H3K27me3 spreading leading to repressive domains.

INTRODUCTION

Polycomb group proteins were discovered in *Drosophila melanogaster* almost a century ago as general repressors of homeotic (Hox) genes involved in proper body segmentation[1]. In the decades following their discovery, biochemical characterization identified them as essential epigenetic factors mediating gene silencing during cell differentiation and development[2, 3]. They function through the posttranslational modification of histone tails as part of multi-subunit chromatin modifying complexes: polycomb repressive complexes 1 and 2 (PRC1 and PRC2)[4]. PRC1 represses transcription by the ubiquitination of histone H2A at lysine 119 and is also involved in the compaction of chromatin marked by the repressive trimethylation of lysine 27 of histone H3 (H3K27me3)[5, 6]. PRC2 is the only known histone methyltransferase (HMTase) specific for the mono-, di- and trimethylation of H3K27

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(H3K27me1/2/3)[7]. PRC2 is indispensable for development, as deletions of genes coding for PRC2 components in mice result in embryonic lethality[8].

The function and subunit composition of PRC2 is conserved from *Drosophila* to mammals and has been shown to comprise four core components: Enhancer of Zeste Homolog 1 or 2 (EZH1/EZH2), Embryonic Ectoderm Development (EED), Suppressor of Zeste 12 (SUZ12), and Retinoblastoma Binding Protein 46 or 48 (RBAP46/RBAP48)[7, 9]. Early biochemical studies identified EZH2, which contains the SET (Su(var)3–9, Enhancer-of-zeste and Trithorax) domain, as the catalytic subunit responsible for H3K27 methylation[3, 7]. These studies also showed that SUZ12 and EED are essential for the catalytic activity of EZH2, with EZH2, EED, and the VEFS-domain of SUZ12 defining the minimal complex showing methyltransferase activity[3, 7].

In vivo studies have identified several cofactors, such as Adipocyte Enhancer-Binding Protein 2 (AEBP2), Jumonji and AT-Rich Interaction Domain 2 (JARID2), Metal Response Element Binding Transcription Factor 2 (MTF2), Plant Homeodomain Finger Protein 1 or 19 (PHF1/PHF19), Elongin BC and Polycomb Repressive Complex 2 Associated Protein (EPOP), or PRC2 associated LCOR isoform 1/2 (PALI1/2) that associate with PRC2 to form variant complexes[3, 10–14]. Among these cofactors, JARID2 and AEBP2 have been implicated in the recruitment and regulation of PRC2 activity[3, 15–17], and have been shown to coexist in the same PRC2 complexes *in vivo* and to act synergistically to stimulate methyltransferase activity[10, 11, 15, 18]. The genetic disruption of either AEBP2 or JARID2 impairs PRC2 recruitment to its targets, leading to defects in embryonic stem cell differentiation[16, 17, 19]. JARID2 has been proposed to help recruit PRC2 to genomic loci through its interactions with long non-coding RNAs (lncRNAs)[20, 21] and, importantly, is itself a substrate of PRC2[22]. LncRNAs, as well as nascent RNA transcripts, have recently gained considerable attention as determinants of PRC2 targeting and regulation *in vivo*[23–25]. Fundamentally, two aspects need to be understood to explain the function of PRC2 or any other chromatin modifier: its recruitment to genomic targets and its enzymatic regulation. For a comprehensive summary of the studies on PRC2 addressing these questions, we refer the readers to recent reviews[26–28].

In this review, we highlight the structural studies that have helped unveil the interplay between the cofactors and the core PRC2 components in the regulation of methyltransferase activity, as well as the interactions of PRC2 with chromatin substrates that shed light on the capacity of the complex to spread the H3K27me3 repressive mark, mechanisms that are both central to PRC2's biological function (Figure 1).

A. Early Structural Studies on PRC2

The first structural information on PRC2 resulted from studies focusing on individual subunits and their pairwise interactions. The crystal structure of human EED, which has a ß-propeller structure with seven WD40 repeats, in complex with a 30 aa helical segment of EZH2, termed the EED-binding domain (EBD), revealed how this stretch constitutes the minimal EED interacting region of EZH2[29]. The role of EED in the allosteric stimulation of PRC2 activity was subsequently uncovered in a study reporting crystal structures of human EED bound to trimethylated histone peptides[30]. EED bound repressive trimethyl marks

(H3K27me3, H3K9me3) with much higher affinity than H3K4me3, H3K36me3 and H3K79me3, all of which are linked to active transcription[30]. Importantly, allosteric stimulation of PRC2 by H3K27me3, its own catalytic product, suggested a feed-forward mechanism of H3K27 methylation.

In the structures, the trimethylated lysine is bound by an aromatic cage in the central cavity of EED containing three conserved residues[30] (Figure 2A). In addition, analyses of these structures suggested that the amino acids in the +2 or −2 position relative to the trimethylated lysine aided in discriminating activating from repressive marks[30] (Figure 2A). Active chromatin marks such as H3K4me3 and H3K36me3 were shown to inhibit PRC2 HMTase activity in *cis*, i.e. when present on the same histone tail as the substrate H3K27[31]. The same study showed that the *Drosophila* homolog of RBAP48, Nurf55, preferentially binds to the N-terminus of histone H3 when unmethylated at position 4 (H3K4)[31]. H3K4 methylation, however, did not alter the affinity of nucleosome binding to PRC2[31]. The precise molecular mechanism of H3K4me3 mediated inhibition therefore remains enigmatic.

Defining the complete subunit organization of PRC2 is an important step towards understanding its function and regulation. The first structural study of a complete PRC2, in complex with the cofactor AEBP2, used negative-stain electron microscopy (EM) to reveal the subunit organization within the full complex, pointing out important aspects of their functional interplay[32]. 3D-EM reconstruction was combined with genetic tagging, cross-linking mass spectrometry (CX-MS) and the approximate docking of known crystal structures of individual subunits, to map the complex architecture. PRC2 was shown to be organized into four major density regions, making up a top and bottom lobe (Figure 2B). The top lobe was proposed to contain several domains of EZH2 (SANT1, SANT2, SET), EED, and the VEFS domain of SUZ12, while the bottom lobe comprised RBAP48 and the rest of SUZ12[32]. AEBP2 was found to bridge the two halves, increasing the stability and rigidity of the complex[32]. The proximity of EED to the catalytic SET domain of EZH2 suggested that their direct communication may mediate the stimulation of HMTase activity. This arrangement, together with preexisting biochemical data, led to a proposed model of nucleosome engagement by PRC2[32].

High-resolution structural information is needed to decipher molecular mechanisms underlying the stimulation of methyltransferase activity by cofactors such as JARID2 and AEBP2, the local spreading of the repressive H3K27me3 mark facilitated by EED, or the inhibitory effect of active transcription marks or RNAs. Below, we summarize the most recent structural studies that have addressed some of these questions.

B. Structures of the catalytic top lobe of PRC2: EZH2-EED-SUZ12(VEFS)

In 2015, Jiao et al. published a landmark study on the structure of the top lobe of the yeast *Chaetomium thermophilum* PRC2, containing EZH2, EED, and the SUZ12 VEFS domain[33]. Structures of both a basal and a stimulated state revealed how a stimulatory signal is structurally transmitted from EED to the SET domain of EZH2[33] (Figure 2C). In both states, a substrate peptide was bound to the active site of EZH2, while the stimulated state contained an additional H3K27me3 bearing peptide bound to the allosteric site on EED

(Figure 2C). This work was soon followed by two similar studies of the top lobe of human PRC2 with largely consistent findings on the structure and activation mechanism[34, 35].

One of the structures of human PRC2 showed the top lobe with an EED-bound stimulatory peptide of JARID2 trimethylated at K116me3, closely resembling H3K27me3 binding[35]. The active site of EZH2 was occupied by a substrate H3 peptide with the oncogenic K27M mutation found in pediatric glioblastoma[35–37]. The other structure consisted of the human EED, SUZ12 (VEFS) and the *Anolis carolinensis* EZH2 bound to a pyridone-based PRC2 inhibitor[34]. All three studies explained how a catalytically competent conformation of the EZH2 SET domain is only achieved in the presence of both EED and SUZ12 (VEFS), while the isolated EZH2 SET domain is in an auto-inhibited state[33–35, 38]. EED and SUZ12 (VEFS) act as structural scaffolds for EZH2, which wraps around EED like a belt and requires the SUZ12 (VEFS) domain for the proper folding of its SET domain[33–35]. In this context, the SET-I helix, a classical element of SET domains, exposes the substrate-binding pocket that would otherwise be occluded[38, 39].

The above studies revealed two major structural changes upon peptide-mediated stimulation vs the basal/pyridone-inhibited states of PRC2. The first is the absence of the so-called stimulatory-response motif (SRM) in the basal and inhibitor-bound states (Figure 2D). The second difference is the conformational variability of a region of EZH2 comprising both the SANT1 and EBD domains (Figure 2D). The structures suggested that the conformation of the EBD-SANT1 module and the ordering of SRM are hallmarks of the stimulated state in which the allosteric signal is transmitted from EED to EZH2 to stimulate methyltransferase activity. The binding of a stimulatory peptide to EED helps stabilize the SRM, which then interacts with the SET-I helix[33, 35]. The structures indicate that the SRM-SET-I interaction stabilizes contacts of SET-I with residues surrounding the substrate-binding pocket[33, 35].

The structural changes of the EBD-SANT1 region of EZH2 are less consistent. The EBD helix is bent with the EBD-SANT1 module tacked in for the stimulated states, while it is straight in the pyridone-inhibited state (Figure 2D). However, this is not the case for the basal or the auto-inhibited states of the fungal structures, which lack the structured SRM but do not exhibit differences in EBD-SANT1 conformation, possibly due to crystal contacts in this region[33, 39]. The use of EZH2 from different species (fungal vs human vs *Anolis carolinensis*), complicates the interpretation of these conformational changes. Interestingly, in the cryo-electron microscopy (cryo-EM) study of the full, human PRC2 that we discuss below, both the bent and straight conformations of the EBD-SANT1 module were visualized for the stimulated states of the complex[40] (Figure 3A).

The crystal structures of the PRC2 top lobe enabled mapping of disease-associated mutations within EZH2 (SRM, SET-I), EED and SUZ12 (VEFS) and provided insights into their functional impact[33, 35]. While these studies were extremely informative providing a possible mechanism for the allosteric activation of EZH2, they used a reduced system comprising a partial complex, as well as peptide substrates. This limited our understanding of the role of core subunits such as RBAP48 or SUZ12, or cofactors such as JARID2 and AEBP2, in the assembly of the full PRC2 complex and the allosteric stimulation of its

methyltransferase activity. Furthermore, mechanisms of chromatin engagement and stimulation in the context of nucleosomes remained unknown.

To address these questions, structures of a fully functional PRC2 holo-complex including cofactors, as well as chromatin-bound PRC2, were needed. Two recent cryo-EM studies, as well as a crystal structure of the bottom lobe of human PRC2, have provided significant advances towards a comprehensive understanding of the structure-function relationship in this key epigenetic complex[40–42].

C. Cryo-EM studies of human PRC2

C. 1. Structures of the full human PRC2 in complex with cofactors AEBP2 and JARID2—

Our recent cryo-EM study of human PRC2 in complex with AEBP2 and a JARID2 fragment (aa 106–450) showed two co-existing states[40] (Figure 3A). Both states contained the stimulatory JARID2 K116me3 bound to EED and a substrate JARID2 K116 bound to the EZH2 active site, since the co-expression of all subunits in insect cells results in methylation of JARID2 by EZH2. Consequently, both states represent “active” complexes and are a direct confirmation that JARID2 acts both as a substrate and as an allosteric stimulator of PRC2. These states, named compact and extended active states, show specific differences in the conformation of the EBD-SANT1 module and the SRM (Figure 3A).

In the compact active state, the EBD-SANT1 is bent and the SRM ordered, mirroring the stimulated state of the crystal structure of the isolated human PRC2 top lobe[35] (Figure 3A). In contrast, the extended state shows a straight EBD-SANT1 conformation, but the SRM is disordered (Figure 3A). Intriguingly, the straight EBD-SANT1 conformation in the extended active state is not only observed in the pyridone-inhibited top lobe structure[34] but also in the active state conformation of the PRC2 top lobe when bound to a dinucleosome substrate[42] (see below). This opens up the possibility that the conformational plasticity of the EBD-SANT1 together with the SRM could be important in the regulation of PRC2 activity (Figure 3B). Besides the JARID2 K116me3 interaction with EED resembling H3K27me3 binding, the cryo-EM structures show that the C-terminus of AEBP2 interacts with RBAP48 in a way that is similar to unmodified histone H3K4[31, 40]. Thus, both JARID2 and AEBP2 mimic histone tail binding to PRC2 at different sites.

The atomic model obtained from the cryo-EM structures highlights the central role of SUZ12 in the assembly and stability of the full PRC2 complex[40]. SUZ12 interacts with all other core subunits (EZH2, EED, RBAP48) and both cofactors AEBP2 and JARID2. In particular, SUZ12 integrates RBAP48 into the full assembly by wrapping around its WD40 domain in a manner similar to EZH2 wrapping around the EED WD40 domain (Figure 3C)[33–35, 40]. The bottom lobe of PRC2 encompasses RBAP48, the C-terminus of AEBP2, and a beta-sheet rich domain of SUZ12 that is potentially involved in RNA binding[40, 43], providing an RNA binding surface in addition to the reported binding sites of EZH2 in the top lobe[44]. The two lobes are stapled together through intricate interactions between the SUZ12 zinc finger, AEBP2 and the JARID2 trans-repression domain (Figure 3D). AEBP2 plays a key role, as both the SUZ12 zinc finger and the JARID2 trans-repression domain do not appear to fold in its absence, resulting in dramatic conformational flexibility between the top and bottom lobes[32, 40]. The architecture of the bottom lobe has also been described in

the crystal structure of a partial human PRC2 complex containing RBAP48, the beta-sheet rich region of SUZ12 (aa 76–546), AEBP2 and JARID2[41].

Mechanistically, the structures of the fully functional human PRC2 complex provided a structural basis for the synergistic stimulation of methyltransferase activity by cofactors, through the allosteric stimulation by JARID2 and the structural stabilization provided by AEBP2, while also highlighting the structural role played by SUZ12 in the assembly of the full complex.

C. 2. PRC2-AEBP2-dinucleosome structure—Cryo-EM has also allowed the visualization of PRC2 in complex with dinucleosome substrates of functional significance, in which one substrate nucleosome contained unmodified H3 and a modified nucleosome contained H3K27me3, thus serving as an allosteric stimulator of PRC2[42] (Figure 4). The study showed PRC2 interacting mainly with the nucleosomal DNA (Figure 4A, B) rather than the histone core. This binding mode is distinct from that reported for most of other chromatin modifiers, which interact with the acidic patch in the globular histone core of nucleosomes[45]. The modified nucleosome is bound through positively charged patches on EED and the EBD-SANT1 domain, in a range of orientations that remain compatible with H3K27me3 recognition by EED (Figure 4B, C). On the opposite side of the top lobe, Lys and Gln residues of the CXC domain of EZH2 (Figure 4D) mediate substrate nucleosome binding, and position the substrate H3 tail near the active site of the SET domain (Figure 4E).

The simultaneous engagement of PRC2 with two adjacent nucleosomes allows the binding of histone tails from the modified and substrate nucleosomes to EED and the EZH2 SET domain, respectively. The geometry of the PRC2-dinucleosome interactions agrees well with biochemical data indicating that dinucleosomes are better PRC2 substrates than single nucleosomes or histone peptides[46, 47]. Most importantly, the structure shows how PRC2 recognition of H3K27me3 present on one nucleosome can stimulate its methyltransferase activity and simultaneously result in the trimethylation of a neighboring substrate nucleosome, thus leading to a straightforward mechanistic model of how PRC2 spreads the H3K27me3 mark from one nucleosome to the next.

Compared to the cryo-EM structures of the full PRC2, the EBD-SANT1 module adopts only a straight conformation, together with an ordered SRM. This straight/ordered configuration of the EBD-SANT1/SRM is distinct from the straight/disordered and bent/ordered conformations observed for the extended and compact active states, respectively. The mostly positively charged surface of the EBD helix interacts with the phosphate backbone of the nucleosomal DNA. This interaction serves as an anchor that facilitates binding of the modified nucleosome, even when changes in linker DNA lengths impose geometrical constraints on the complex[42]. The observed interactions with the modified nucleosome likely stabilize the straight EBD-SANT1 conformation, while H3K27me3 binding to EED stabilizes the SRM.

Around the substrate nucleosome binding site, an unassigned density was observed that likely corresponds to otherwise flexible regions of EZH2 or AEBP2 (Figure 4E). Both

regions are rich in positively charged amino acids and would therefore be good candidates for DNA binding. In agreement with this prediction, XL-MS results suggest that a stretch of AEBP2, identified by a recent study to mediate nucleosome interactions[48], is located in the vicinity of this unassigned density. Intriguingly, H3K36, which is trimethylated during active transcription and was shown to inhibit PRC2 activity[31], is located close to the unassigned density, hinting at a potential role of this region in responding to H3K36 methylation (Figure 4E). Further experiments are required to identify this region of EZH2 or AEBP2, as well as its potential role in PRC2 regulation.

Technical limitations in the cryo-EM study of the PRC2-dinucleosome complexes prevented visualization of the bottom lobe of PRC2. Although present in the sample, this region becomes disordered and/or partially unfolded during cryo-EM grid preparation, likely due to interaction with the air-water interface. While the cryo-EM study of free PRC2 benefited from stabilization by chemical crosslinking, such conditions proved incompatible with nucleosome binding, most likely due to the titration of lysines needed for DNA interactions. However, in negatively-stained samples, the full PRC2 structure is preserved without crosslinking[42]. Reference-free 2D class averages of such samples allowed the assessment of nucleosome binding by the bottom lobe, a likely scenario given the known interaction of RBAP48 with unmodified histone H3K4 as well as the potential role of SUZ12 in nucleic acid interactions[31, 40, 43]. These results indicate that while one nucleosome, most likely the substrate nucleosome, consistently binds near the SET domain, the other, likely the modified nucleosome, exhibited a wide range of positions. This variability indicated potential interactions both with EED and RBAP48, or the SUZ12 region located in the bottom lobe.

While the X-ray crystallographic study of the RBAP48-SUZ12 sub-complex included biochemical data in support of nucleosome binding to the bottom lobe[41], the cryo-EM study of dinucleosome-PRC2 complexes strongly suggest that the top lobe of PRC2 is the major site of nucleosome interaction in this context. An additional interaction site of PRC2 with chromatin involves two C2H2 zinc fingers of AEBP2, which have recently been suggested to preferentially interact with methylated CpG DNA[49]. The cryo-EM reconstructions of PRC2 bound to dinucleosomes lacked discernable density for this region, which could be explained by the absence of methylated cytidines in the linker DNA or the geometry of the linker DNA in these assemblies.

SUMMARY

Structural studies have enabled detailed insights into some of the regulatory mechanisms of H3K27 trimethylation by PRC2, as well as its interaction with chromatin substrates. Such studies explain the molecular basis for the co-occupancy of PRC2 core components at genomic loci, the requirement of SUZ12 and EED for HMTase activity and *in vivo* function of PRC2, and the local spreading of H3K27me3 important for establishing repressive domains or restoring them after replication. The intricate structural interplay of PRC2 subunits with each other and with chromatin illustrates the role of distinct substructures within this multi-subunit chromatin modifier for its regulated function. The structural studies to date, together with biochemical data, have led to a mechanistic model of how recognition of the repressive H3K27me3 allosterically stimulates PRC2 methyltransferase activity. The

role played by JARID2 and AEBP2 in synergistically stimulating PRC2 activity can be explained by how the structural effects of their interaction with PRC2 influence both the stability and activity of the full complex, while also serving as substitutes for specific histone tail interactions. The molecular basis of the local spreading of H3K27me3, as well as the ability of PRC2 to functionally engage chromatin in diverse conformational environments, have now become more clear through the visualization of PRC2 interactions with dinucleosomes.

The structure-derived mechanistic insights described above now serve as a platform for future functional studies to address specific aspects of PRC2 function. For example, a recent study showed that JARID2 and MTF2 are important for the initial deposition of H3K27me3, which in turn is sufficient for the local spreading of repressive H3K27me3 marks[50]. Structural work on EED and the catalytic top lobe has enabled the targeted disruption of the allosteric activation mechanism by designing small molecule inhibitors which target either the trimethyl-lysine binding pocket in EED or the EBD interaction surface on EED[51, 52]. Structural insights into the architecture of the full human PRC2, as well as its interaction with chromatin, are expected to promote further efforts towards the development of anti-cancer therapeutics.

OUTLOOK

Despite the advances presented here, several key questions remain unanswered that should be the focus of future structural and functional studies. In contrast to allosteric activation by trimethyl-lysine binding to EED, the mechanisms underlying the inhibition of PRC2 by active transcription marks remain unexplained. While the regulatory response to H3K36me3 may involve parts of EZH2 or AEBP2, it is not known where, if at all, H3K4me3 is recognized by PRC2 and how it could inhibit catalytic activity. Other cofactors have been shown to regulate PRC2 activity in certain biological settings. For example, PHF1/19 are known to bind the active transcription mark H3K36me3 and override its inhibitory effect on PRC2, utilizing a yet to be determined mechanism. The impact of interactions with regulatory DNA elements such as CpG islands, nascent RNA transcripts or lncRNA on PRC2 activity are also still incompletely understood and need mechanistic clarification. Also, the reported role of cofactors such as JARID2 or AEBP2 for chromatin binding await mechanistic elucidation. Further efforts aimed at visualizing the interactions of PRC2 with DNA and RNA, as well as the interactions of other cofactors with PRC2 by cryo-EM or X-ray crystallography will be particularly helpful in answering these questions. Taken together, structural studies have been very informative in providing mechanistic insight of epigenetic phenomena as summarized here for the case of PRC2. Genetic, cell biological and biochemical work will continue to inspire structural efforts, and vice versa, in a multidisciplinary effort to expand our understanding of the complexities underlying the plasticity and stability of gene expression patterns.

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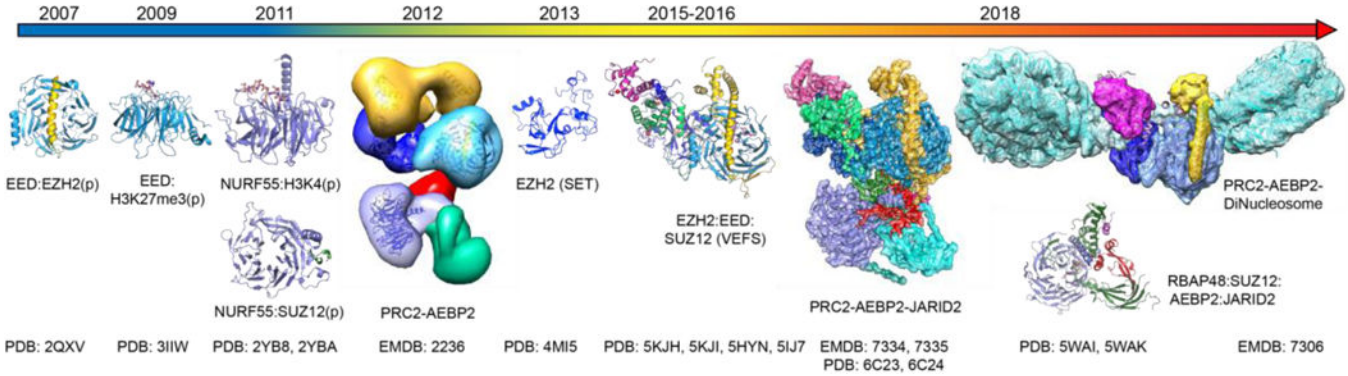

Figure 1.
Schematic overview of the various structural studies on PRC2.

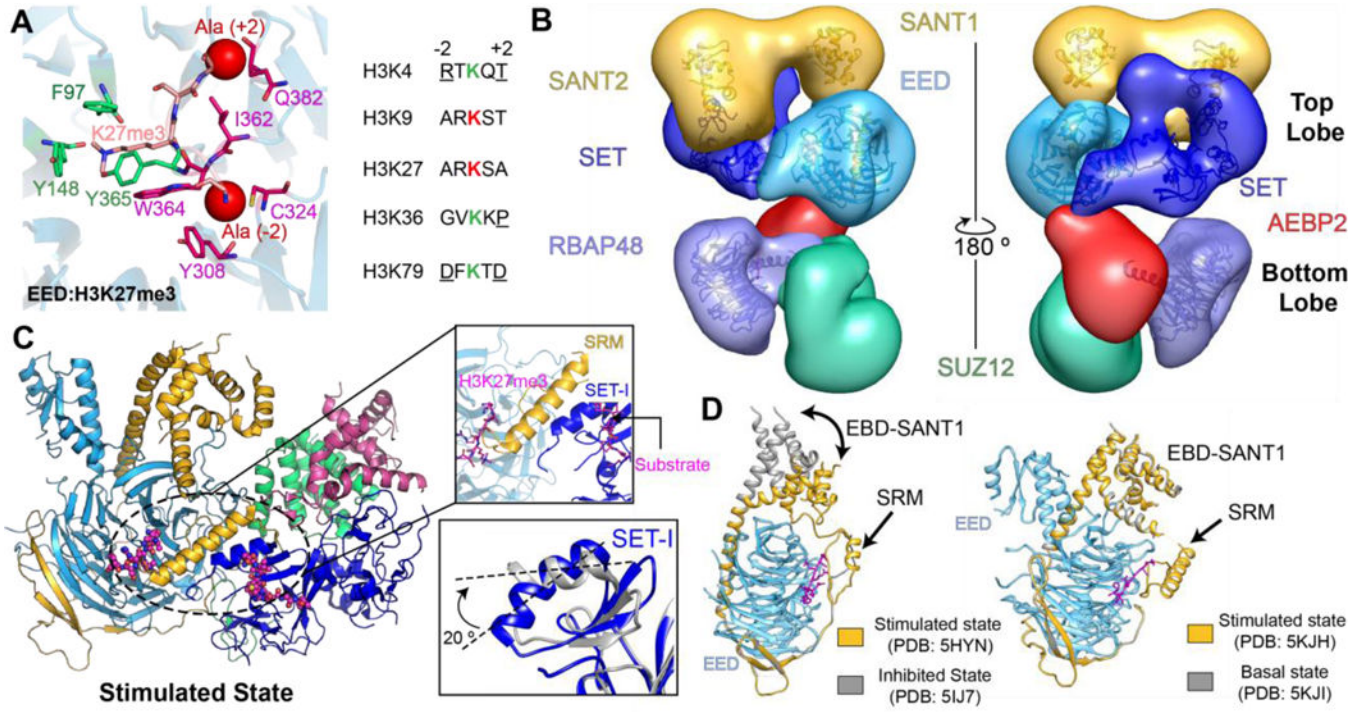

Figure 2. PRC2 architecture and structural changes upon allosteric stimulation.

(A) (Left) Residues in EED involved in binding of histone H3 peptide containing H3K27me3. Red spheres represent the side-chain methyl group of the +2, −2 alanine residues in histone H3K27me3 peptide (PDB: 3IIW). (Right) Sequence comparison of different H3 peptides suggests potential steric clashes due to bulky side chain groups (underlined) in the +2, −2 positions in peptides containing active chromatin marks. (B) Negative-stain EM reconstruction of the PRC2-AEBP2 complex, with docked crystal structures of known domains at the time (EMDB: 2236). (C) Ribbon representation of the stimulated state of a fungal PRC2 top lobe. (Top inset) The SRM helix (gold) links the SET-I helix (blue) of the catalytic SET domain to the binding of stimulatory peptide (H3K27me3, magenta) to EED. (Bottom inset) Overlay of the SET domain in the stimulated (blue, PDB: 5HYN) and the auto-inhibited state (gray, PDB: 4MI0) showing a 20º rotation of the SET-I helix. (D) (Left) Comparison of the crystal structures of the top lobe of PRC2 indicates differences in the EBD-SANT1 module and the presence or absence of the SRM helix for stimulated vs inhibitor-bound states of human PRC2. (Right) Apart from the SRM, there is no apparent difference visible in the fungal basal versus stimulated states.

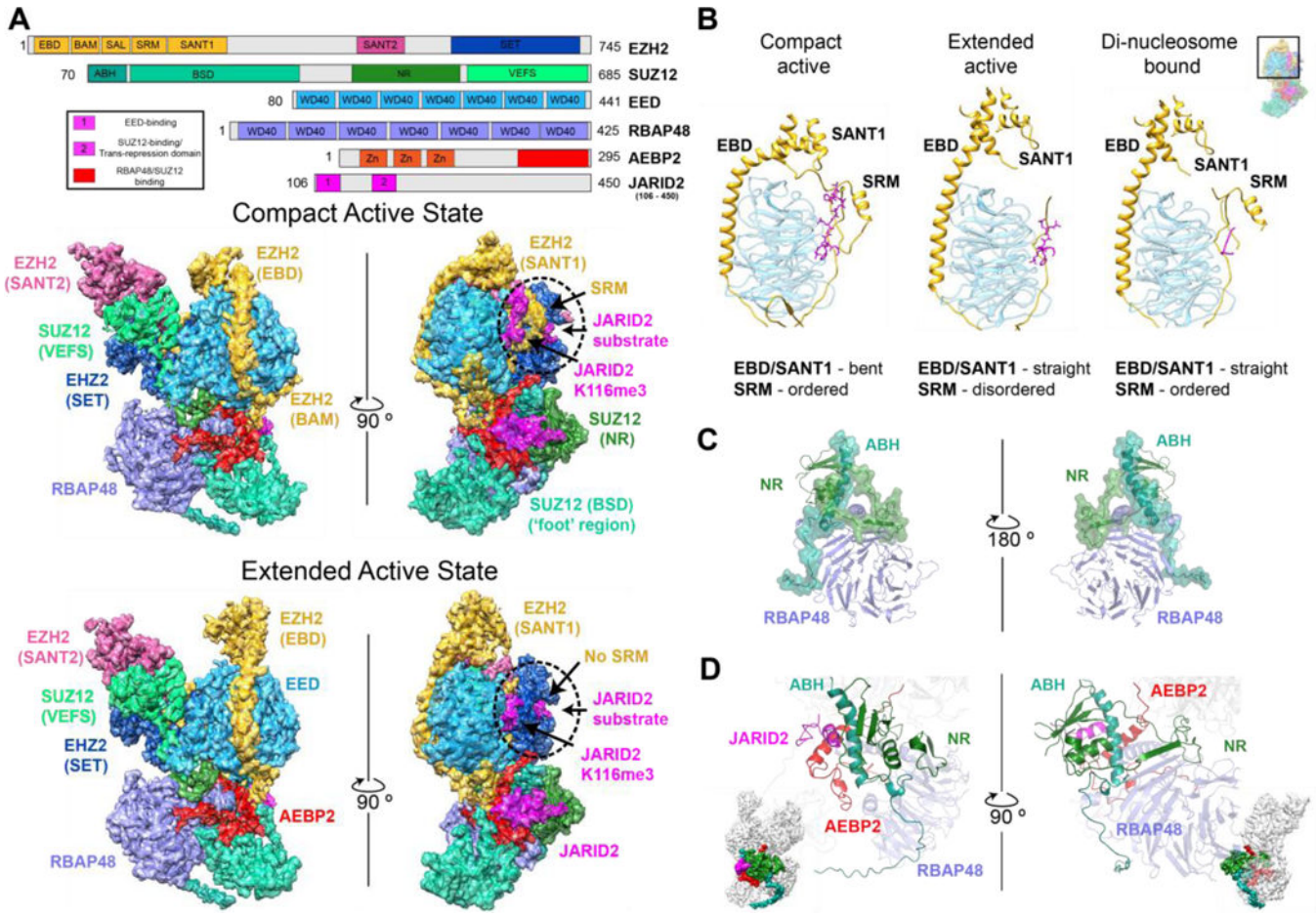

Figure 3. Cryo-EM structures of the human PRC2-AEBP2-JARID2 complex.

(A) (Top) Schematic representation of protein domains within the PRC2-AEBP2-JARID2 complex. Truncations of EZH2 and EED were used to eliminate unstructured regions lacking known functions. For JARID2, a minimal construct (aa 106–450) known to be sufficient for chromatin binding and EZH2 stimulating activities was used. (Bottom) Cryo-EM reconstructions of two active states of the complex, with their corresponding refined models shown as ribbons. Changes in EBD-SANT1 conformation are visible, and changes in SRM conformation are highlighted within the dashed black circle. (B) Comparison of the EBD-SANT1 module and SRM configuration in the cryo-EM structures of the PRC2-AEBP2-JARID2 and the fitted top lobe structure of PRC2 in the PRC2-dinucleosome complex. All structures represent active states and contain both stimulatory peptide/histone H3K27me3 tail (magenta) bound to EED, and substrate peptide bound to SET domain (not shown). Inset shows the black boxed region of the structure for which the comparisons are shown. (C) SUZ12 (green) interacts extensively with and wraps around RBAP48 (purple), resulting in its incorporation in the full PRC2 complex (PDB: 6C23, 6C24). (D) A complex hetero-trimeric junction, consisting of AEBP2 (red), JARID2 (magenta) and SUZ12 (green), staples the top and bottom lobe of PRC2. Inset shows the location of the respective view in the full PRC2 complex.

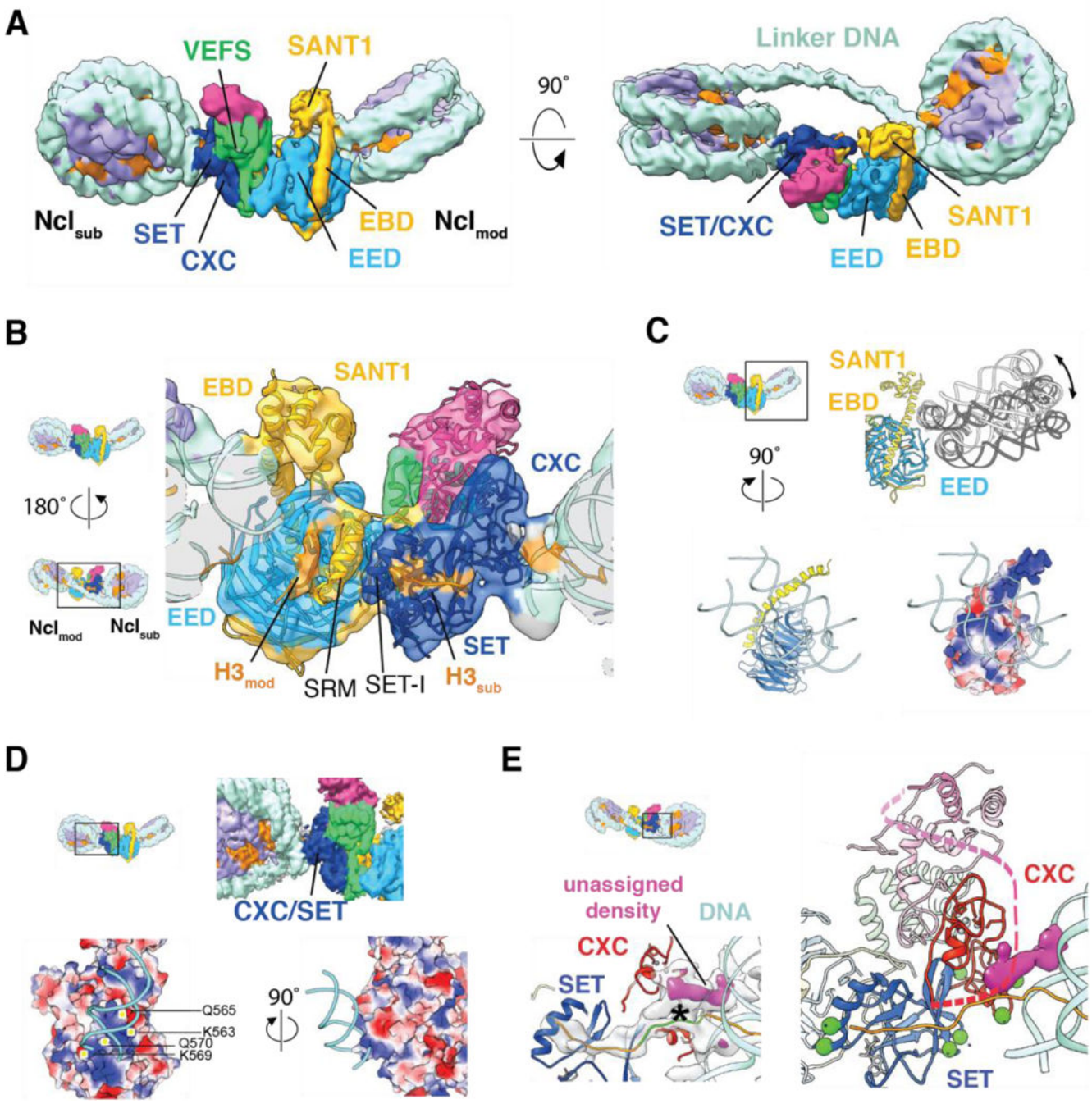

Figure 4. Cryo-EM reconstruction of a PRC2-dinucleosome (PRC2-DiNcl) complex.

(A) Overview of the catalytic top lobe of PRC2 bound to a hetero-dinucleosome with 35 bp of linker DNA. Ncl_{sub} = unmodified substrate nucleosome, Ncl_{mod} = nucleosome carrying the H3K27me3 modification. (B) Enlarged back view of the PRC2-DiNcl map showing densities in the H3 tail binding sites of EED and the SET domain of EZH2, and for the ordered SRM helix. (C) Interaction of the modified nucleosome with EED/EBD-SANT1. Top right, variability in the orientation of Ncl_{mod} (grey) relative to EED. Bottom, interaction surface of the nucleosomal DNA with PRC2, shown in ribbon representation (left) and with

the electrostatic surface potential (right; red = negative, blue = positive). The positively charged surface of the top part of EBD tightly interacts with the nucleosomal DNA, while two bands of positive surface potential on EED allow flexible association with Ncl_{mod}. (D) Top right, improved map of the Ncl_{sub} interacting region of PRC2. Bottom, DNA interacting interface of the CXC domain of EZH2 showing the electrostatic surface potential. Four residues are likely to mediate Ncl_{sub} binding via electrostatic interactions with the DNA backbone of Ncl_{sub}. (E) Left, enlarged view of the CXC/SET domains of EZH2 and interactions with Ncl_{sub}. EM density corresponding to the substrate histone tail reaching into the active site of the SET domain, with an unassigned density (purple) sitting on top of where H3K36 is expected (black asterisk). Right, candidate regions contributing to the unassigned density. The purple dashed line shows a potential path for the connection of the CXC and SANT2 domains of EZH2, which was not resolved in previous crystal or cryo-EM structures lacking nucleomsomes. Green spheres indicate previously reported cross-links of AEBP2 to this region of EZH2[32].