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Structural basis for PRC2 engagement with chromatin

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The polycomb repressive complex 2 (PRC2) is a conserved multiprotein, repressive chromatin complex essential for development and maintenance of eukaryotic cellular identity. PRC2 comprises a trimeric core of SUZ12, EED and EZH1/2, which together with RBBP4/7 is sufficient to catalyse mono-methylation, di-methylation and tri-methylation of histone H3 at lysine 27 (H3K27me1/2/3). These histone methyltransferase activities of PRC2 are deregulated in several human cancers and certain developmental disorders, such as Weaver Syndrome. Core PRC2 associates with several accessory proteins, which organise to define two main subassemblies, PRC2.1 and PRC2.2. Here we review new biochemical and structural studies that are providing critical insights into how core and accessory PRC2 subunits coordinate the faithful deposition of H3K27 methylations genome-wide.

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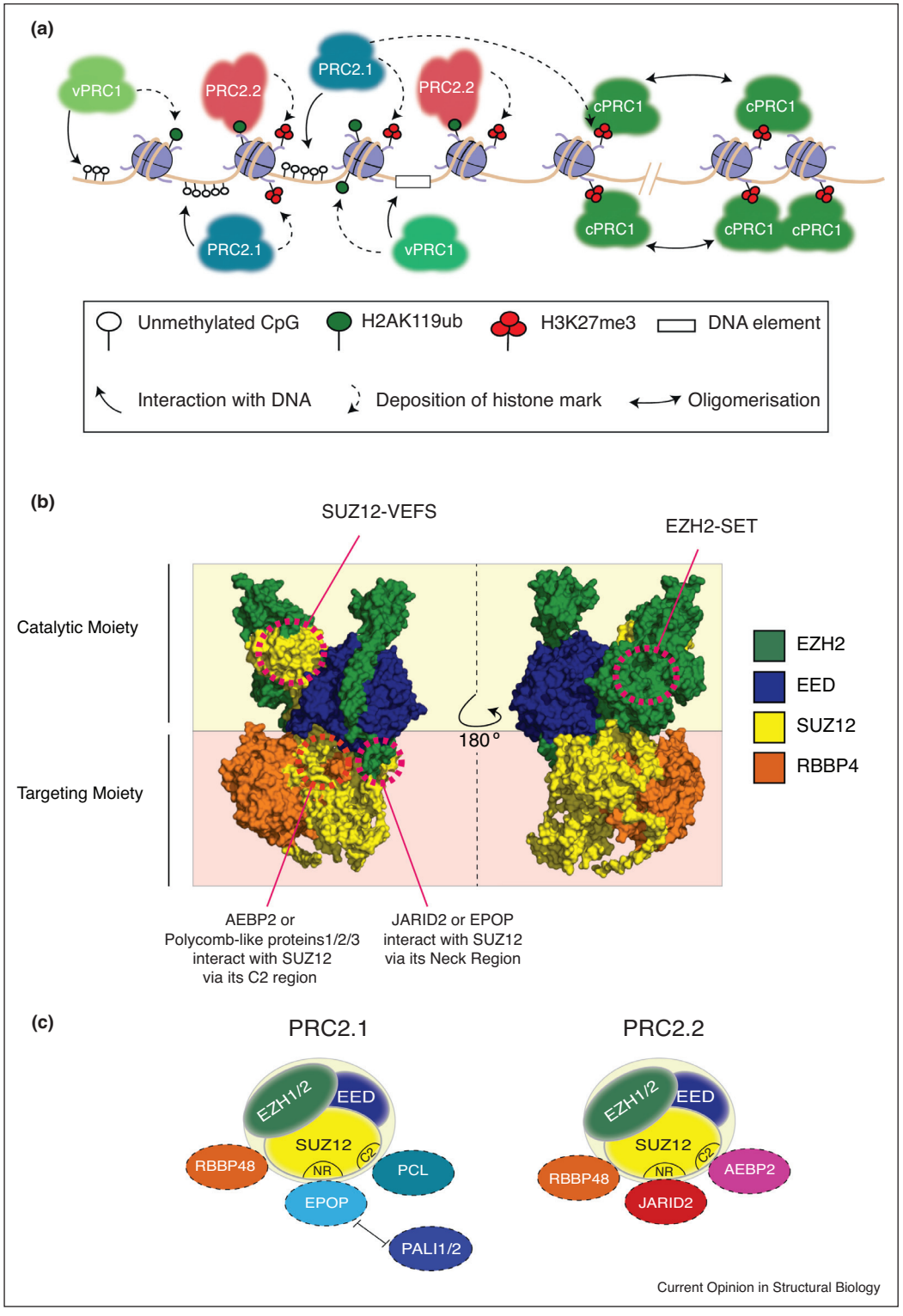
Introduction

Polycomb proteins are chromatin repressors required for maintaining cellular identity during differentiation and development [1]. They assemble into two distinct multiprotein complexes, called polycomb repressive complex-1 (PRC1) and complex-2 (PRC2). Taking into account their association with various substoichiometric components, these are further subdivided into distinct biochemical subassemblies; canonical and variant PRC1 (cPRC1 and vPRC1), as well as PRC2.1 and PRC2.2 [2]. The core of PRC2 contains EED, SUZ12 and one of two histone methyltransferases (HMT), EZH1 or EZH2, and, in association with RBBP4/7 is sufficient to mediate all mono-methylation, di-methylation and tri-methylation of lysine 27 on Histone H3 (H3K27me1/2/3) [3–5]. The PRC2-mediated H3K27me3 contributes to gene repression by promoting the recruitment of cPRC1 to chromatin via specific binding of chromodomains within its CBX subunits [6,7]. Once associated, the PHC and CBX2 components of cPRC1 are thought to promote stable repression via long range chromatin interactions [8,9]. The vPRC1 complexes deposit H2AK119ub1, which in turn promotes association of PRC2.2, and to a lesser extent PRC2.1 [10–13,64] (Figure 1a). However, it is becoming increasingly clear that vPRC1-mediated H2AK119ub1 also has a role in gene repression that is independent of PRC2 and cPRC1 [13–16]. While, the various PRC2 and PRC1 complexes are highly abundant at CpG islands located near developmentally repressed gene promoters, the majority of global H3K27me3 deposition occurs elsewhere in the genome, in the absence of stable PRC2 binding [4,12,17]. Similarly, H3K27me2 is observed throughout the genome. It is possible that these dispersed H3K27me2 and H3K27me3 depositions act as a repressive blanket to prevent aberrant transcriptional activity [3,18]. Importantly, this pervasive deposition of H3K27me2 and H3K27me3 implies that PRC2 also engages with chromatin outside of traditional Polycomb target genes, potentially in a less stable, more transient fashion.

PRC2 folds into two functionally distinct lobes, a catalytic lobe comprising EZH2, EED and the VEFS domain of SUZ12, as well as a targeting lobe composed of the N-terminal region of SUZ12, RBBP4/7 and various associated accessory proteins [19,20,21••] (Figure 1b). These PRC2 accessory subunits compete for interaction with the N-terminal region of SUZ12 to define the PRC2.1 and PRC2.2 subcomplexes [19,22] (Figure 1b–c). PRC2.1 contains one polycomb-like protein (either PHF1, MTF2 or PHF19) together with either PALI1/2 or EPOP [23–25], while PRC2.2 contains JARID2 and AEBP2 [26,27] (Figure 1c). The most recently described eutherian-specific EZHIP/CATACOMB accessory protein is believed to interact with core PRC2 to inhibit its histone methyltransferase activity [28–31]. The subdivision of PRC2 is conserved in *Drosophila*, which have mutually exclusive Pcl–PRC2 and Jarid2–PRC2 subcomplexes [2].

The PRC2.1-specific and PRC2.2-specific accessory proteins modulate the targeting and enzymatic activity of core PRC2 at repressed gene promoters [4,12,17], and some may also act to bridge PRC2 with other transcriptional regulators [24,32,33]. While a minimal catalytically active PRC2 comprising EZH2, EED and the SUZ12–

Figure 1



How polycomb repressive complexes are targeted to chromatin and the structure of PRC2.
(a) Polycomb repressive complexes are targeted to chromatin by multiple pathways. vPRC1.1 binds CpG islands and vPRC1.3/5/6 harbour sequence specific DNA binding proteins to promote targeting and deposition of H2AK119ub1. Components within PRC2.1 (polycomb-like proteins and PALI1) also interact with DNA, while PRC2.2 components (AEBP2 and JARID2) directly bind H2AK119ub1. PRC2.1 and PRC2.2 mediate H3K27me3 deposition, facilitating recruitment of cPRC1 through CBX proteins which bind this modification. cPRC1 can subsequently compact chromatin through polymerisation.

VEFS domain is sufficient to maintain global levels of H3K27me1/2/3, the N-terminal region of SUZ12 is required to interact with RBBP4/7 and additional accessory proteins to accurately target PRC2 to polycomb-repressed gene promoters [4,12,17]. PRC2.1-specific and PRC2.2-specific accessory proteins co-occupy the majority of polycomb target genes, where they synergise to coordinate deposition of H3K27me3 [12]. The polycomb-like proteins in PRC2.1, and JARID2 in PRC2.2, are key for targeting their respective subcomplexes and loss of these accessory proteins leads to mis-localisation of PRC2 [12,17,34]. In this review, we will focus on recent structural studies highlighting how core PRC2 binds DNA, senses histone modifications, and how the respective architecture of PRC2.1 and PRC2.2 facilitate engagement with chromatin by independent, but complimentary mechanisms.

Core PRC2 assembly and interaction with chromatin

The core members of PRC2 directly engage with chromatin via interactions with histone tails [35–38] and DNA [39••,40••]. Initial studies revealed that core PRC2 member EED binds to H3K27me3 and this interaction allosterically promotes PRC2 histone methyltransferase (HMT) activity [34,36,41]. The structural basis for this allosteric activation was initially described in *fungi* (*Chaetomium thermophilum*) by X-ray crystallography [35]. This revealed that the stabilisation of the EZH2–SRM domain is a key defining characteristic towards the achievement of a stimulated state [35]. The structural organisation within core PRC2 and its allosteric activation have been comprehensively reviewed previously [42,43].

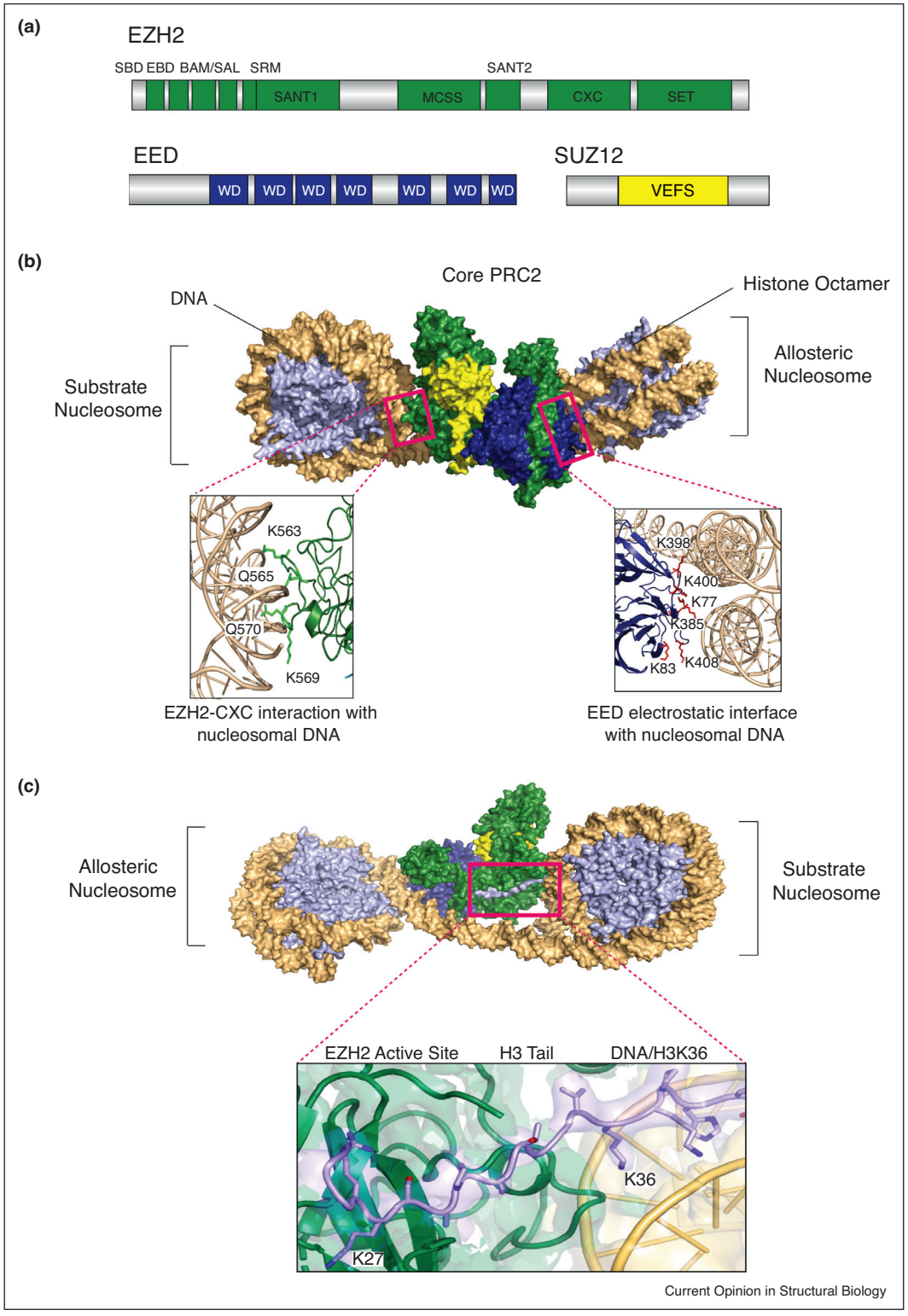
Recent cryo-EM structures of PRC2 together with mononucleosomes or di-nucleosomes revealed an extensive three-way interaction between CXC/SET domains of EZH2, nucleosomal DNA and the H3 N-terminus, which serve to position the H3 tail into the methyltransferase active site (Figure 2a–b) [39••,44••]. It is clear that core PRC2 members are involved in several electrostatic interactions with nucleosomal and linker DNA [39••,40••,45•,46]. Structural characterisation of these interactions has highlighted their critical role in regulating HMT activity and nucleosome binding (Figure 2b). For example, interaction between specific lysine and arginine residues within the EZH2–CXC domains, and the substrate nucleosomal DNA are critical for PRC2 activity [39••]. While a lysine patch within EED provides an electrostatic interface for interaction with the allosteric nucleosomal DNA (Figure 2b). These data provide new insights into specific interactions between core PRC2 members and nucleosomal DNA, which regulate two key aspects of PRC2 biology — chromatin engagement and methyltransferase activity.

The core members of PRC2 can also sense the methylation status of H3K4 and H3K36, which in turn influence HMT activity [37,38,39••]. The abovementioned recent structural studies illustrating how H3 is threaded into the PRC2 active site hint at a mechanism through which EZH2 senses the methylation status of the histone H3 tail [39••]. These findings supported previous biochemical evidence that active chromatin marks, including H3K4me3 and H3K36me2/3, competitively inhibit PRC2 HMT activity [37,38,47]. The presence of H3K36me3 inhibits PRC2 in *cis* and thereby prevents accumulation of H3K27me3 [37,38]. Therefore, the H3K27me3 and H3K36me3 modifications never co-localise across the genome [48,49]. The correct balance between their deposition profiles is essential for maintenance of gene expression patterns during development [39••]. Recent work by Finogenova *et al.* [39••], highlighted the role of unmodified H3K36 in feeding the substrate histone tail into the PRC2 active site via contact with the DNA backbone, which is less favourable in the presence of H3K36me3 [39••] (Figure 2c). It is likely that the structural constraints of the nucleosome also contribute to the allosteric inhibition by H3K36me3 as the inhibition was lost on peptide substrates [39••]. Although H3K36me3 and H3K27me3 never co-localise, it is interesting to consider that perhaps H3K36me2 could still allow for some, albeit lower, PRC2 activity. Supporting this theory, the inhibition of H3K27me3 and H3K27me2 deposition was less pronounced on H3K36me2 nucleosomes than on H3K36me3 nucleosomes [37]. Taken together, these data could help rationalise why genome-wide low levels of H3K27me2, and to a lesser extent, H3K27me3 can exist in the presence of H3K36me2. It is likely that these effects result from changes in the dynamics of the H3 tail alignment on

(b) Cryo-EM structure of PRC2 containing core complex members EZH2, SUZ12 and EED (PDB: 6C24, EMDB-7335 Ref. [21••]). Shown is SUZ12 (yellow) which threads through both the catalytic and targeting moieties of PRC2. EZH2 (green) folds around EED (dark blue) and interacts with VEFS region of SUZ12 (yellow) to form the catalytic moiety. RBBP4 (orange) and the N-terminal region of SUZ12 (yellow) form the targeting moiety. Dashed red circles indicate regions of special interest, including the SUZ12–VEFS domain (minimal region of SUZ12 required for PRC2 catalytic activity), the EZH2 SET domain (catalytic domain), and the SUZ12 C2 and NR regions within the targeting moiety that facilitate interaction with PRC2.1 and PRC2.2 specific accessory proteins.

(c) Schematic representation of the compositions of PRC2.1 and PRC2.2. Mammalian PRC2.1 contains one of three PCL proteins (PHF1, MTF2 or PHF19) in complex with either EPOP or PALI1/2. PRC2.2 contains JARID2 and AEBP2. PRC2.1-specific accessory protein EPOP and PRC2.2-specific accessory protein JARID2 likely compete for the neck region (NR) of SUZ12, while PRC2.1-specific PCL proteins and PRC2.2-specific AEBP2 compete for SUZ12 C2 region. It is not yet known structurally where PRC2.1-specific PALI1 interacts with SUZ12.

Figure 2



Core PRC2 members interact with nucleosomal DNA to direct the H3 tail into its active site.
(a) Domain architecture of the catalytic moiety of core PRC2 included in the cryo-EM structures of panels (b) and (c). The domains in EZH2 are highlighted in green, EED in dark blue and SUZ12–VEFS domain in yellow.
(b) Cryo-EM reconstruction of the catalytic moiety of PRC2 engaged with a dinucleosome separated by 35 base pair of linker DNA (EMDB-7306 Ref. [40••]). Density is coloured as in (A) to show PRC2 subunits, DNA (cream) and histone octamers (purple). The DNA exiting the substrate

the EZH2 surface during the enzymatic cycle, and differences in the residence time of the tail in a conformation where K27 can enter the catalytic site.

How PRC2.2 reads vPRC1-mediated H2AK119ub1 and interacts with DNA

The PRC2.2-specific accessory proteins JARID2 and AEBP2 interact with chromatin and contribute to directing H3K27 methylations. The deposition of H2AK119ub1 is essential for Polycomb-mediated gene repression and serves, at least in part, to stabilise PRC2.2 at target genes [12–16]. A recent cryo-EM study of PRC2.2 bound to a H2AK119ub1-modified nucleosome structurally confirmed the interaction between ubiquitin and the putative ubiquitin interacting motif (UIM) in the N-terminus of JARID2 [44••,50,64], while also revealing for the first time that AEBP2 can engage ubiquitin [44••]. The JARID2–UIM is sandwiched between the histone core and the ubiquitin molecule and interacts with both the ubiquitin molecule and the H2A–H2B acidic patch [44••] (Figure 3b). Two C2H2 zinc-fingers in AEBP2 interact with ubiquitin and the H2A–H2B surface [44••] (Figure 3b–c). Interestingly, the relatively large distance between ubiquitin and the NR region of SUZ12 that is known to interact with JARID2 implies the structure may have partially captured the engagement of two separate JARID2 molecules [44••,51]. This could support previous reports of PRC2 existing in a dimer formation [52,53]. The cryo-EM structure also highlighted the interaction between AEBP2 and nucleosomal DNA [44••] (Figure 3b–c), supporting previous biochemical and functional data which uncovered a lysine–arginine motif (KR-motif) within AEBP2 capable of boosting PRC2 HMT activity [54]. Future studies that succeed in including the N-terminal region of JARID2 and C-terminal region of AEBP2 not yet structurally characterised may further elucidate their roles in PRC2's engagement with chromatin.

Polycomb-like protein of PRC2.1 can recognise DNA and histone modifications

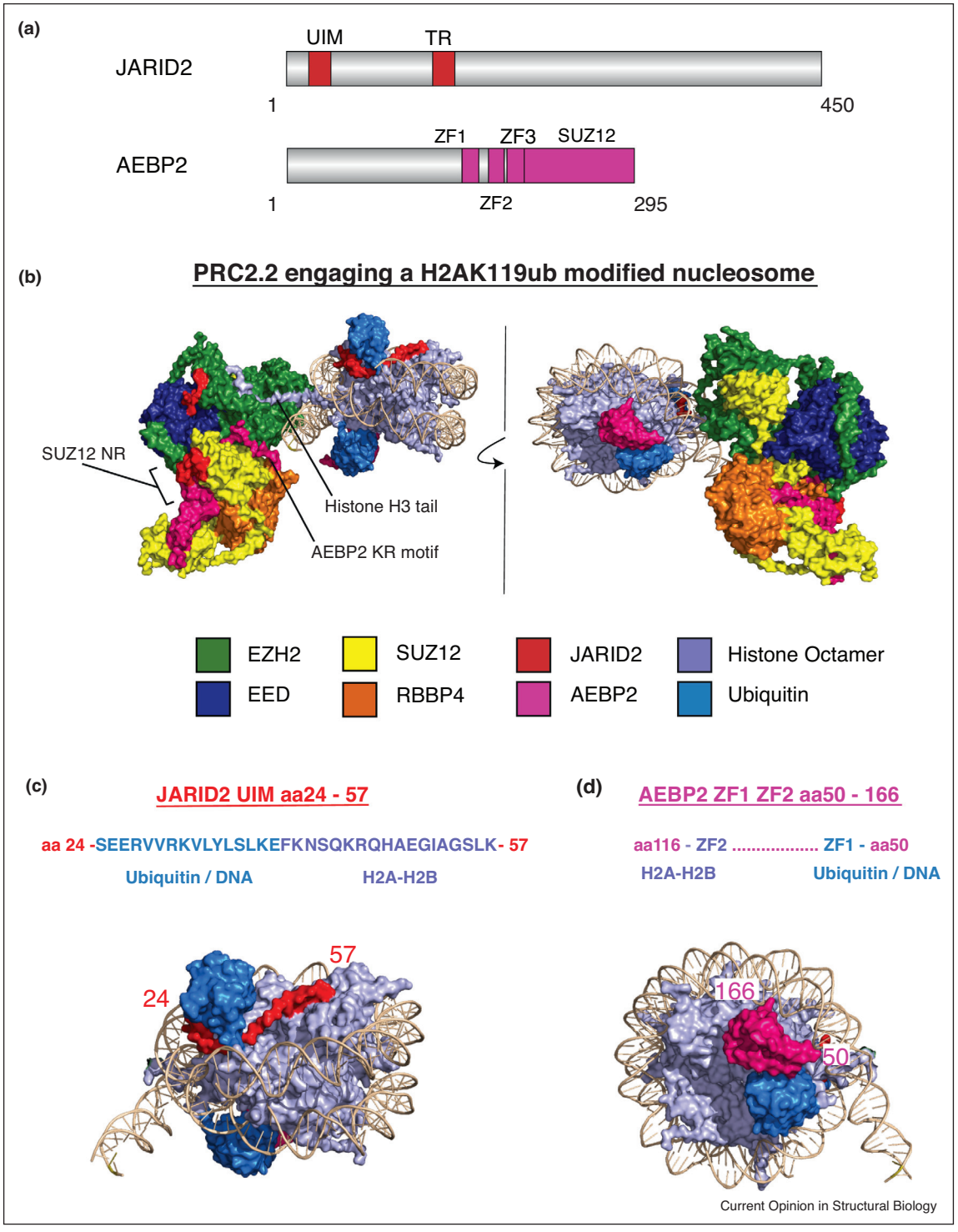
Polycomb-like proteins are vital for the interaction of PRC2.1 with chromatin [12,17,45•,55•,56]. However, to date, efforts to study the structure of the polycomb-like protein (PCL) containing PRC2.1 have proven challenging. In fact, PCL proteins are difficult to purify due to their largely unstructured C-terminal region [57] (Figure 4a). Even when successfully purified, it has been difficult to precisely localise the PCL component within the electron density of structures of PCL–PRC2.1 containing complexes [39••]. Despite these challenges, the Liu group determined the crystal structure of the C-terminal region of PHF19 interacting with SUZ12, reporting an analogous interaction to that seen for AEBP2–SUZ12 [52]. In addition, work from the Müller and Wang labs, presented the crystal structure of the N-terminal region of *Drosophila* (Dm) Pcl [45•], human (Hs) PCL1/PHF1, and Hs-PCL2/MTF2 [55•]. These studies revealed an extended homologous region (EH) that adopts a winged helix-like structure capable of binding DNA with high affinity (Figure 4b) [45•,55•]. While, polycomb-like proteins are thought to interact with DNA and stabilise PRC2.1 on chromatin by increasing the residency time, their ability to recognise specific DNA sequences is disputed [45•,55•]. Since the addition of PHF1/PCL1 increases the PRC2 residency time on nucleosomal substrates [45•], it is possible to conceive that stable PRC2.1 binding occurs via an avidity-based mechanism in which a minimum threshold of DNA binding must be reached. Polycomb-like proteins can also bind H3K36me2/3 via an aromatic cage in the N-terminal TUDOR domain [58–60]. While the functional relevance of this interaction remains unknown, one possibility is that PCL–PRC2 engages with and reads H3K36me2/3 throughout the genome and that this contributes to the demarcation of H3K27 and H3K36 methylation boundaries on chromatin.

JARID2 and PALI1 interact with EED through a strikingly similar structural mechanism to promote enzymatic activity

The JARID2 (PRC2.2) and PALI1 (PRC2.1) accessory proteins stimulate PRC2 activity *in vitro* and are direct substrates for PRC2 [24,26,61,62•]. JARID2 is methylated by PRC2 at K116 *in vitro* and *in vivo*, which has an allosteric stimulatory effect on PRC2 HMT activity though recognition by EED [61]. A similar accessory protein-induced mechanism of allosteric activation for PRC2 has now been described for the first time in the context of PRC2.1 [62•]. PALI1–K1219 and PALI1–K1241 are substrates for EZH2, and when methylated can allosterically activate the complex when bound to EED [62•], through a similar mechanism to that proposed for JARID2-K116me3 [61] (Figure 4c). However, the mode by which methylated JARID2 and PALI1 proteins

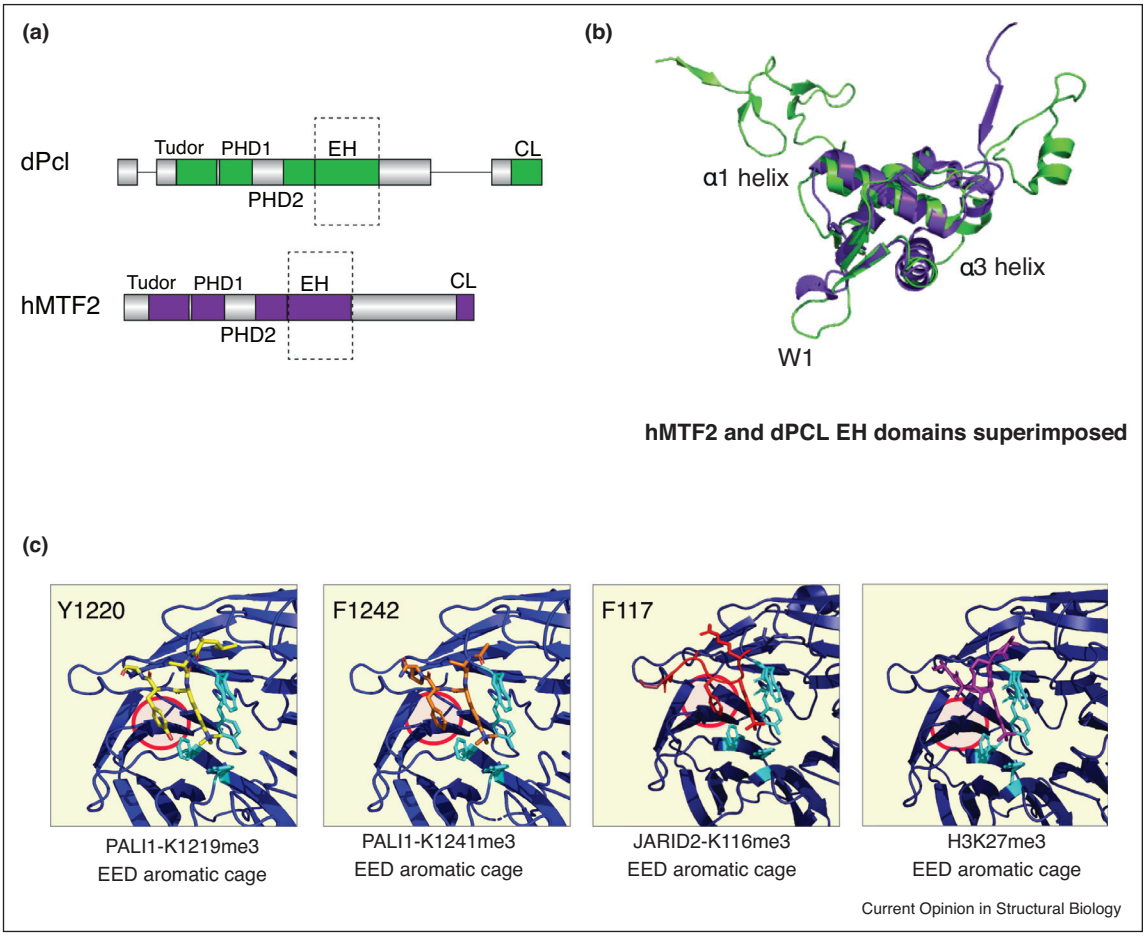
(Figure 2 Legend Continued) nucleosome on the left interacts with the CXC domain of EZH2 (green). On the left, a zoomed in ribbon representation of positively charged amino acids in EZH2–CXC (green) capable of forming electrostatic interactions with nucleosomal DNA are highlighted. On the right, a zoomed in ribbon representation of allosteric nucleosomal DNA interacting with EED (dark blue) creating an electrostatic interface. Positively charged residues of EED are highlighted (red) as likely interactors with the negatively charged DNA backbone. **(c)** Cryo-EM reconstruction of the catalytic moiety of PRC2 engaged with a dinucleosome separated by 35 base pair of linker DNA [39••] (structure kindly provided by the Müller lab). Density is coloured as in A and B. A zoomed in view of the H3 tail (purple) threading into the EZH2 active site (green) illustrates how an unmodified H3K36 facilitates feeding of the H3 tail (purple) into EZH2 active site (green) through interaction with the DNA backbone (pale yellow).

Figure 3



Both JARID2 and AEBP2 of PRC2.2 can interact directly with H2AK119ub1.
(a) Domain architecture of PRC2.2-specific accessory proteins JARID2 (red) and AEBP2 (pink). UIM; ubiquitin interaction motif, TR; trans repression domain, ZF; zinc finger.
(b) Cryo-EM structure of PRC2.2 bound to H2AK119ub1 modified nucleosome [44••] (structure kindly provided by the Nogales Lab). Subunit colours are highlighted, EZH2 (green), EED (dark blue), SUZ12 (yellow), JARID2 (red), AEBP2 (pink), Histone octamer (purple), Ubiquitin (light blue). Both JARID2 (red) and AEBP2 (pink) interact with ubiquitin (light blue). AEBP2 (pink) has addition contacts with nucleosomal DNA via a lysine arginine rich region.

Figure 4



PRC2.1 members can interact with DNA and also allosterically activate PRC2.
(a) Domain architecture of *Drosophila* Pcl (green) and human MTF2 (PCL2) (purple). Dashed box highlights the region of the solved crystal structures of the extended helix (EH) domain.
(b) Superimposition of the crystal structure of dPcl WH (green) (PDB: 5OQD Ref. [45•]) and human MTF2 (purple) (PDB: 5XRF Ref. [55•]). While there is high structural conservation between the two domains their reported regions of interactions with DNA differ. hMTF2 is reported to interact with DNA major groove via the w1 region [55•], while dPcl is reported to interact with DNA by insertion of the α three helix in the DNA major groove [45•].
(c) PRC2 can be allosterically activated by the PALI1 and JARID2 accessory proteins in PRC2.1 and PRC2.2, respectively. Crystal structures of EED (dark blue) with either PALI1-K1219me3 (orange) (PDB 6V37; [62•]), PALI1 K1241me3 (yellow) (PDB 6V3X [62•]), JARID2–K116me3 (red) (PDB 4X33; [61]) or H3K27me3 (Purple) (PDB3IIW; [36]) are represented in cartoon formation. The structure of the PALI1–K1219me3 and PALI1–K1241me3 peptides was kindly provided by the Davidovich Lab. EED aromatic cage amino acids are highlighted in cyan. The aromatic residue +1 amino acid to the methylated peptides for JARID2 (PRC2.2) and PALI1 (PRC2.1) provides an extra contact with EED, a feature not present in the H3K27me3–EED interaction.

(c) JARID2 (red) interacts with ubiquitin (light blue) through its UIM domain in its N-terminus. Amino acids 24–40, corresponding to the previously annotated UIM [50], interact directly with the ubiquitin molecule (light blue), while amino acids 41–57 engage with acidic patch of H2A–H2B (purple).
(d) AEBP2 (pink) interacts with ubiquitin (light blue) through the first two of its three ZF domains. The first ZF domain at amino acid 50 interacts with the ubiquitin molecule (light blue) and DNA while the second ZF domain at amino acid 116 engages H2A–H2B (purple).

bind to EED is slightly different than that described for H3K27me3, whereby both utilise an additional aromatic residue [36,61], which increases the contact with EED (Figure 4c). The cryo-EM structure describing JARID2 bound to the allosteric and stimulatory sites of PRC2 advanced our understanding of allosteric activation of PRC2 [21••]. This revealed two co-existing stimulated states of PRC2: compact-active and extended-active, which differ in the conformation of the EZH2–SBD and EZH2–SRM. Therefore, it is possible these structures have captured different stages of activation; the initial binding of a stimulating repressive peptide and SRM stabilisation followed by bending of the SBD towards SANT1. Interestingly, independent of its allosteric activation function, PALI1 substantially increased the affinity of PRC2 for DNA relative to core PRC2 alone, in a non-sequence specific manner [62•].

Perspectives

Recent advances in mass spectrometry and structural biology techniques have led to an explosion of studies revealing novel insights into PRC2 organisation and engagement with chromatin. The emerging picture tells us that it is most likely a series of multivalent interactions including but not limited to, core PRC2 members interacting with nucleosomal DNA and histone tails, as well as the specific accessory proteins of PRC2.1 and PRC2.2, that serve to both direct and modulate enzymatic activity across the genome. These new insights will aid us in mapping key causative mutations in diseases where PRC2 function is perturbed, including cancer and developmental disorders [3,63]. This is an excellent example of how cross-disciplinary biology can marry functional, biochemical and structural information to bridge key conceptual gaps providing a framework for drug design and future mechanistic studies.

Author contribution

All three authors are equally responsible for the conceptualization of the ideas within.

Conflict of interest statement

C.C. is a Genentech/Roche employee and owns shares in the Genentech/Roche group.

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