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Research Highlight

WD40 Repeats Arrange Histone Tails for Spreading of Silencing

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Direct binding of WD40 repeat of Embryonic Ectoderm Development (EED) in the Polycomb Repressor Complex 2 (PRC2) to the histone H3 tail regulates the H3K27 methyltrasnferase activity of PRC2. The binding activity is required for the methylation of H3K27 over long distance of a *Drosophila* chromosome from the UBX (Hox gene) promoter to the PBX upstream enhancer region, implying EED initiates propagation of this repressive mark.

Polycomb group (PcG) proteins contribute to maintenance of a repressive chromatin status. There are three distinct PcG complexes (PRC1, PRC2 and PhoRC) that assemble on chromatin and coordinate H3K27 methylation and H2AK119 ubiquitination (Schwartz and Pirrotta, 2007). In *Drosophila*, PRC1 consists of Polycomb (PC), Posterior sex comb (PSC), Polyhomeotic (PH), dRing and Sex comb on midleg (SCM), and it binds to H3K27me3 to maintain the transcriptional repression. PRC2 contains the Enhancer of *zeste* [E(Z)] H3K27 methyltrasnferase, extra sex combs/extra sex comb like (ESC/ESC like), Suppressor of *zeste* [SU(Z)] and p55. PhoRC contains pleiohomeotic/pleiohomeotic like (PHO/PHOL) proteins and binds to DNA (Schwartz and Pirrotta, 2007). Homologues of PcG proteins were also identified in humans. Recently, Margueron et al. demonstrate that Embryonic Ectoderm Development (EED), the human homologue of ESC, binds to histone bearing repressive methylation marks including H3K27, and this interaction of EED with histone facilitates the PRC2 methyltransferase activity on H3K27 (Margueron et al., 2009).

It has been observed that the flanking residue(s) of the lysine targeted by a methyltrasnferase plays crucial roles for the enzymatic activity of the complex. A well studied example is histone H3K4 methylation by Set1 in the mammalian MLL3/4 complex. Crystal structures of the WD40 repeats of WDR5, which is a component of the MLL3/4 complex, has shown that it directly interacts with A1, R2 and T3 of histone H3. Thus, interactions of WDR5 with residues adjacent to K4 may function in recognition of the H3 tail prior to methylation of K4 by the SET1/MLL complex(es) (Suganuma et al, 2008). Recently, Margueron et al. demonstrated specific binding of the WD40 repeats of EED with H3K27me3 through the formation of hydrogen bonds by its Arg414 and its aromatic cage corresponding to Phe97, Try364 and Try365. This small cage associated with residues at the -2 position from H3K27. The reconstituted PRC2 complex replaced with Phe97A or Tyr365A did not bind to either K27 methylated or unmodified nucleosomes. The binding specificity of H3K27 depended on these aromatic contacts (residues) in EED and required particular flanking residues at -2 or $+2$ position from the methylated lysines but not $-1/+1$ position (-2 position from H3K27 and $+2$ position from H4K20). The binding activity with methylated lysines in peptides displayed that the EED cage was able to bind any of silencing methyl lysines including H3K27me3, H3K9me3, H4K20me3 and H1K26me3, but not active methyl lysines marks including H3K4me3, H3K36me3 and H3K79me3 (Figure 1). This specificity is due to EED recognition of the amino acids adjacent to the methylated lysines which serve to guide the PRC2 methyltransferase complex to silenced rather than active regions where it can reinforce and/or spread silencing marks. Recombinant EED preferentially bound to reconstituted nucleosomes containing chemically trimethylated H3K27. Reconstituted PRC2 complex bound to H3K27me0, me1, me2 or me3 containing nucleosome (me0 $<$ me1 $<$ me2 $<$ me3), however, the complex replaced with EEDY365A bound to K27me1 and K27me2 nucleosomes but failed to bind to K27me3 nucleosomes. Hence, the aromatic cage in EED is critical for the binding of PRC2 to K27me3 nucleosomes but not to nucleosomes in general, which is likely due to other nucleosome-binding domains in the complex.

The authors asked whether binding of the aromatic cage to residues flanking H3K27 contributed to enhancing the methyltrasnferase activity of PRC2. The methyltrasnferase activity of the reconstituted PRC2 complex on recombinant nucleosomes substrates was enhanced by the addition of K27me2 and K27me3 peptides in trans. However, a mutant PRC2 complex containing EED-Phe97A or -Try365A was not enhanced by the addition of these peptides (Margueron et al., 2009). As these EED amino acids interact with an H3 residue flanking K27 (-2), the data suggest that the binding of EED to the flanking residue enhances PRC2 enzymatic activity. Importantly, the EED aromatic cage is necessary for binding but not sufficient for enhancement

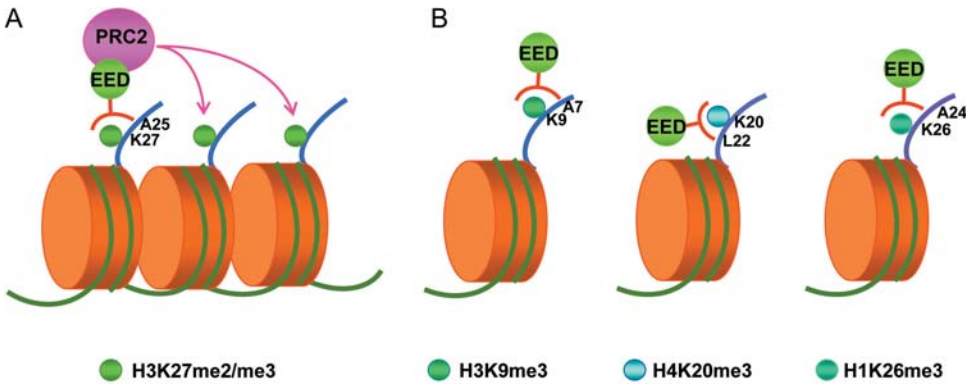

Figure 1 (**A**) Model of the propagation of silencing by PRC2. The interaction of EED with silencing methyl marks, including H3K27me2/me3, enhances the methyltransferase activity of PRC2 to bring about methylation of adjacent nucleosomes. (**B**) Recognition of different silencing methyl marks by EED. EED distinguishes silencing methyl marks from activating methyl marks through additional interactions with amino acids in the +2 or −2 position. Recognition of H3K9me3 includes interaction with A7, H4K20me3 recognition involves interactions with L22, and H1K26me3 recognition includes interaction with A24. PRC2 complex recognizes K27me3 through additional interactions with A25 (**A**).

of PRC2 activity. K27me3 peptides containing R26A (a flanking amino acid that does not interact with the cage) did not enhance the methyltransferase activity. Thus, a specific structure of the histone tail containing methyl K27 may be required for enhancing PRC2 activity.

Since the *in vitro* data suggested that PRC2 might propagate the K27me3 mark through EED, the authors sought to test this possibility *in vivo*. The Polycomb response element (PRE), the UBX promoter and the UBS upstream enhancer region were analyzed in *Drosophila*. In the wild type flies, this long region was enriched in K27me3, however, K27me3 levels on this region was lost in the *esc* mutant (the *Drosophila* homologue of EED mutated with the residues corresponding to EED-Phe97 or EED-Try356). Other subunits of PRC2 are likely to contribute to the binding of PRC2 to chromosomal regions; however, EED could provide specific recognition of silenced regions. Thus, if PRC2 is recruited to a chromosome location that already contains repressive histone modifications, EED can enhance the methyltransferase activity of PRC2 reinforcing and propagating the repressive marks. It is not yet clear how the PRC2 methyltrasnferase activity is enhanced, or how the initial repressive marks (K27me2 and K27me3) to activated PRC2 were previously generated.

Interestingly, PRC2 contains another WD40 repeat protein, p55, which binds H4, amino acids 31–41 through the side of its beta-propeller. This is quite a distinct mode of binding from that of EED binding the H3K27 through the center of its beta-propeller. The human homologue of p55, Rbbp48, is complexed with HAT1 and mutation of its H4-binding pocket reduces the global levels of H3 acetylation by HAT1. However, p55 is also part of PRC2 and its H4 binding pocket is actually required for its assembly into the PRC2 complex (Wang et al., 2001). It may be that in PRC2 the histone binding domain of p55 functions to associate it with the PRC2 complex and EED is responsible for nucleosome recognition in this complex. However, it is intriguing that there are two WD40 repeat proteins with distinct binding specificities in the PRC2 complex and it is easy to speculate that their activities are in some way related and important in PRC2 function (Song et al., 2008).

PcG silencing involving H3K27 methylation is broadly spread from the UBX promoter, to the *cis*-acting PRE (−23 kb), and onto the PBX enhancer element (−30 kb) (Talbert and Henikoff, 2006; Margueron et al., 2009). Margueron et al. proposed that the binding of EED to H3K27 flanking residue facilitated di- and tri-methylation of H3K27 by PRC2. Interestingly, EED can bind to other methylated lysines associated with silencing such as H3K9me3 and H4K20me3 (Figure 1). Thus the function of EED may have additional signals for activating PRC2 if the methylation of H3K9/H4K20 overlap with K27 in regions of silenced chromatin (Margueron et al., 2009). In this case, it becomes important to consider whether complexes containing distinct methyltransferases are functionally related.

Spreading of silencing mediated by H3K9 methylation is partially dependent on the siRNAs pathway (Martin and Zhang, 2005; Li et al., 2009). H3K27 methylation is not directly coupled with generation of siRNAs, however, H3K27 methyl marks require the RNAi pathway in *Tetrahymena* (Liu et al., 2007). The human EZH2 K27 methyltrasnferase of PRC2 is recruited to the inactive X chromosome, and it is coincident with the methylation of H3K27 and the ubiquitination of H2A during X inactivation (Fang et al., 2004). Interestingly, EED is required for primary and secondary trophoblast giant cell development in female embryos. *eed* deletion mice have one additional active X chromosome, indicating that EED is required for X inactivation (Wang et al., 2001). It is still unknown whether EED functions through the same mechanisms for homeotic gene silencing and X inactivation (Wang et al., 2001). Further studies of cross talk between complexes although comparing different silenced regions will help to reveal whether individual silencing mechanisms have common roles for establishing or spreading silencing.

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