

PRC2 crystal clear

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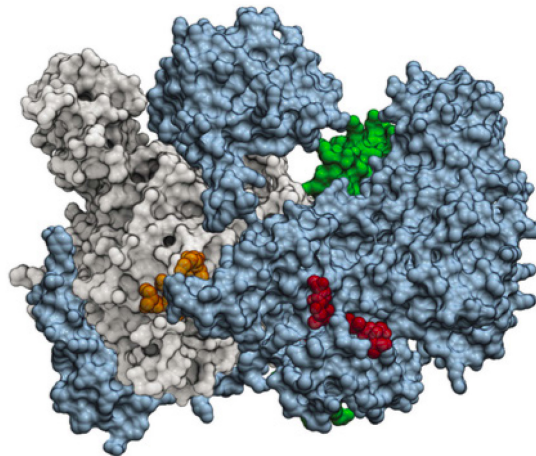
Epigenetic mechanisms control the combination of genes that are switched on and off in any given cell. In turn, this combination, called the transcriptional program, determines the identity and the fate of cells, which are deregulated in diseases such as cancer, inflammation or neurological disorders. Chemical modifications (such as methylation or acetylation) of chromatin – an ensemble of nuclear factors, especially histone proteins around which DNA is wrapped – act as epigenetic signals. Enzymes that write or erase these chemical “marks” and proteins that bind and interpret them represent an important target class for tomorrow’s drugs¹. Two epigenetic target at the center of attention are EZH2, an enzyme that methylates lysine 27 of histone 3 (H3K27) to turn down gene expression, and BRD4, a protein that binds acetylated lysines to turn on genes. Compounds against both proteins are currently in multiple clinical trials for cancer therapy. Hyperactivating mutations of EZH2 drive 22% of diffuse large B-cell lymphoma, a form of blood cancer, which is sensitive to EZH2 inhibitors²⁻⁶; solid tumors carrying pre-defined genetic or epigenetic signatures are also vulnerable to EZH2 inhibition⁷⁻⁹. While rich structural data has supported BRD4 drug discovery, the structural characterization of EZH2 has so far remained largely elusive. This is no longer the case, thanks to the structures presented on page xxx of this issue by Jiao and Liu.

High-resolution crystal structures of many protein methyltransferases – enzymes that transfer a methyl group from the cofactor S-adenosyl-methionine onto side-chains of substrate proteins – have been solved (including that of 39 out of 68 human enzymes). Given its prominence, considerable effort has been invested into solving the EZH2 structure. What makes this enterprise particularly challenging is that EZH2 is active only when it is part of a larger, multi-protein complex called polycomb repressive complex 2 (PRC2). Other components of the PRC2 complex include EED, SUZ12 and RBBP4. In particular, EED and a domain called VEFS of SUZ12 are absolutely necessary for the catalytic activity

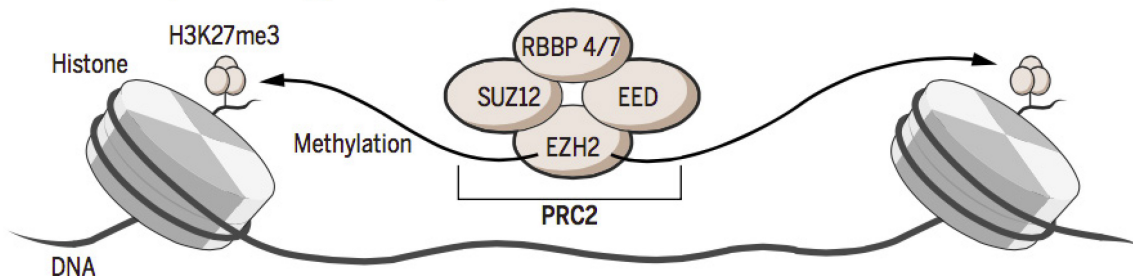
of EZH2. Crystal structures of human EZH2 isolated from the PRC2 complex were previously solved^{10, 11}, but both substrate and cofactor binding sites were impaired in this incomplete structure. A low-resolution structure of the human PRC2 complex was also solved by electron-microscopy, and provided information on the relative arrangement of the different components of the complex¹². However, the resolution was too low for detailed mechanistic analysis of the PRC2 machinery, interpretation of structural mechanisms underlying disease-associated mutations, or the exploration of novel opportunities for drug discovery. Now, Jiao and Liu report the high-resolution (2.3 Å) crystal structure of an active form of the yeast *C. thermophilum* PRC2 complex composed of full-length EZH2, EED, the VEFS domain of SUZ12, a stimulatory tri-methylated H3K27 (H3K27me3) peptide, and an inhibitory peptide from mutant histone H3 that drives pediatric brain cancer. They also present a PRC2 structure in the basal state, in the absence of stimulatory peptide.

Prc2 structure (active)

- Ezh2
- Eed
- Suz12
- H3K27me3 stimulatory peptide
- H3K27M inhibitory peptide



PRC2 represses gene expression



Long-sought structure. Structure of an active Prc2 complex (from yeast) provides insights into the propagation of the epigenetic mark H3K27me3 and the mechanism of disease-associated mutations. It should also lead to the structure of human PRC2 and thereby accelerate drug discovery efforts.

What are the lessons learned from the PRC2 structure? First, and foremost, a fundamental understanding at the atomic level of the mechanism behind the propagation of tri-methylated H3K27 (H3K27me3): it has been known for some time that, the reaction product of EZH2, H3K27me3, can bind EED, which in turn stimulates EZH2 activity. This positive feedback loop is believed to play a central role in the efficient propagation of H3K27me3 along chromatin¹³. At sites devoid of H3K27me3, PRC2 can recruit and tri-methylate JARID2, a chromatin-targeting protein that, once methylated, mimics H3K27me3 in stimulating PRC2, and initiates H3K27 methylation¹⁴. The PRC2 structure reveals that the N-terminal half of EZH2 wraps like a belt around EED (clearly showing that EZH2 requires EED to fold properly). Of particular interest, a previously unknown stimulation responsive motif (SRM) docks onto a surface composed of EED and the stimulatory peptide H3K27me3, but is disordered in the absence of peptide. Proper folding of the SRM propagates to the catalytic site, and facilitates the methylation of a distinct H3K27 peptide, revealing how H3K27me3 stimulates methylation of neighboring H3K27 substrates.

Mutation of H3K27, into a methionine (H3K27M) is the genetic cause behind 80% of diffuse intrinsic pontine gliomas, and 22% of non-brain stem gliomas, two forms of brain cancer found in children¹⁵; EZH2 binds the mutant histones and remains trapped at H3K27M sites due to its high affinity of the mutant sequence, resulting in global decrease in H3K27 methylation¹⁶. Given that both lysine and methionine have long, linear side-chains, the expectation would be for the H3K27M residue to occupy the substrate H3K27 binding site in EZH2, while the binding pose of flanking residues would remain unchanged. Surprisingly, in the PRC2 complex structure, it is the arginine immediately upstream of residue 27

(H3R26) that occupies the substrate-binding cavity, while H3K27M projects away from the catalytic site, and is partially unfolded. It will be interesting to see whether this unexpected arrangement, supported here by mutational analysis, holds in the human PRC2 complex.

While the present structure is that of a PRC2 ortholog from yeast, it is in agreement with the low-resolution human PRC2 structure and the high resolution but incomplete human EZH2 structure. This tour-de-force in crystallizing a catalytically active PRC2 complex constitutes a major breakthrough on the road to a human PRC2 structure. This will undoubtedly bring light on the elusive binding mode of existing EZH2 inhibitors in clinical trials. The structural mechanism underlying EZH2 mutations that confer resistance to anti-cancer drugs will also gain in clarity from a human PRC2 complex¹⁷, and rational design strategies to overcome resistance may follow. In-depth analysis of this and future PRC2 structures may reveal novel binding sites, for instance at the EZH2-EED interface, that could be targeted by next generation drug candidates to allosterically inhibit both wild-type and mutant PRC2.

References

1. Arrowsmith, C.H., Bountra, C., Fish, P.V., Lee, K. & Schapira, M. Epigenetic protein families: a new frontier for drug discovery. *Nat Rev Drug Discov* **11**, 384-400 (2012).
2. Knutson, S.K. et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. *Nat Chem Biol* **8**, 890-6 (2012).
3. McCabe, M.T. et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature* **492**, 108-12 (2012).
4. Morin, R.D. et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. *Nat Genet* **42**, 181-5 (2010).
5. Sneeringer, C.J. et al. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. *Proc Natl Acad Sci U S A* **107**, 20980-5 (2010).
6. Yap, D.B. et al. Somatic mutations at EZH2 Y641 act dominantly through a mechanism of selectively altered PRC2 catalytic activity, to increase H3K27 trimethylation. *Blood* **117**, 2451-9 (2011).

7. Bitler, B.G. et al. Synthetic lethality by targeting EZH2 methyltransferase activity in ARID1A-mutated cancers. *Nat Med* **21**, 231-8 (2015).
8. Mack, S.C. et al. Epigenomic alterations define lethal CIMP-positive ependymomas of infancy. *Nature* **506**, 445-50 (2014).
9. Knutson, S.K. et al. Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. *Proc Natl Acad Sci USA* **110**, 7922-7 (2013).
10. Antonysamy, S. et al. Structural context of disease-associated mutations and putative mechanism of autoinhibition revealed by X-ray crystallographic analysis of the EZH2-SET domain. *PLoS One* **8**, e84147 (2013).
11. Wu, H. et al. Structure of the catalytic domain of EZH2 reveals conformational plasticity in cofactor and substrate binding sites and explains oncogenic mutations. *PLoS One* **8**, e83737 (2013).
12. Ciferri, C. et al. Molecular architecture of human polycomb repressive complex 2. *Elife* **1**, e00005 (2012).
13. Margueron, R. et al. Role of the polycomb protein EED in the propagation of repressive histone marks. *Nature* **461**, 762-7 (2009).
14. Sanulli, S. et al. Jarid2 Methylation via the PRC2 Complex Regulates H3K27me3 Deposition during Cell Differentiation. *Mol Cell* **57**, 769-83 (2015).
15. Wu, G. et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. *Nat Genet* **44**, 251-3 (2012).
16. Lewis, P.W. et al. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. *Science* **340**, 857-61 (2013).
17. Gibaja, V. et al. Development of secondary mutations in wild-type and mutant EZH2 alleles cooperates to confer resistance to EZH2 inhibitors. *Oncogene* (2015).