Goldilocks meets Polycomb

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The Polycomb system modulates chromatin structure to maintain gene repression during cell differentiation. Polycomb repression involves methylation of histone H3K27 (H3K27me3) by Polycomb repressive complex 2 (PRC2), monoubiquitylation of H2A (H2Aub1) by noncanonical PRC1 (ncPRC1), and chromatin compaction by canonical PRC1 (cPRC1), which is independent of its enzymatic activity. Puzzlingly, Polycomb repression also requires deubiquitylation of H2Aub1 by Polycomb repressive deubiquitinase (PR-DUB). In this issue of *Genes & Development*, Bonnet and colleagues (pp. 1046–1061) resolve this paradox by showing that high levels of H2Aub1 in *Drosophila* lacking PR-DUB activity promotes open chromatin and gene expression in spite of normal H3K27me3 levels and PRC binding. Pertinently, gene repression is restored by concomitant loss of PRC1 E3 ubiquitin ligase activity but depends on its chromatin compaction activity. These findings suggest that PR-DUB ensures just-right levels of H2Aub1 to allow chromatin compaction by cPRC1.

In the children's story "The Three Bears," a little girl named Goldilocks wanders into the house of three bears and finds three different bowls of porridge that are cooling down. She rejects the porridge that is either too hot or too cold but eats the porridge that has just the right temperature. She next goes to sleep in the bed that is neither too hard nor too soft but just right. In this issue of *Genes & Development*, Bonnet et al. (2022) show that the opposing activities of ncPRC1 and PR-DUB generate the just-right level of H2Aub1 for gene repression by the Polycomb system.

Originally identified in *Drosophila melanogaster*, Polycomb proteins modulate chromatin structure at multiple scales to maintain repression of lineage-specific genes, thus ensuring cellular identity during development (Loubiere et al. 2019; Piunti and Shilatifard 2021). Most Polycomb proteins function as part of multisubunit Polycomb repressive complexes (PRCs). The PRC2 class of methyltransferases monomethylate, dimethylate, and trimethylate histone H3K27. The PRC1 class harbors two distinct activities: ubiquitylation of histone H2A (K118 in *Drosophila* and the corresponding K119 in mammals) by ncPRC1 and chromatin compaction by cPRC1. Chromatin compaction by cPRC1 remains poorly defined and involves long-range interactions, subnuclear clustering, and possibly changes in chromatin accessibility (Loubiere et al. 2019). Note that chromatin compaction is independent of PRC1 catalytic activity (Boyle et al. 2020). While the general requirement of H3K27me3 for Polycomb repression is firmly established (Pengelly et al. 2013; Sankar et al. 2022), the role of H2Aub1 is less clear. Based on studies in mouse embryonic stem cells (mESCs), H2Aub1 was proposed to be central to Polycomb repression (Tamburri et al. 2022). However, during mouse and *Drosophila* development, PRC1—rather than its enzymatic activity—was found to be essential (Pengelly et al. 2015; Tsuboi et al. 2018; Boyle et al. 2020). Multiple studies established that, although there is substantial cross-talk between PRC2, cPRC1, and ncPRC1, they can also function largely independently to ensure robust gene silencing during development (Loubiere et al. 2019; Piunti and Shilatifard 2021). Counterintuitively, deubiquitylation of H2Aub1 by PR-DUB also contributes to Polycomb silencing (Tamburri et al. 2022). The core of PR-DUB comprises the deubiquitinase CALYPSO (CALY; BAP1 in mammals) associated with ASX (ASXL1-3 in mammals), which stabilizes CALY/BAP1 and stimulates its enzymatic activity. This creates a conundrum: How can the opposing activities of H2Aub1 deposition (by ncPRC1) and its removal (by PR-DUB) yield the same functional outcome?

To resolve this paradox, Bonnet et al. (2022) generated *Drosophila* harboring catalytic mutants of PRC1 or PR-DUB and analyzed the effects on Polycomb repression during development. The genomic distribution of H2Aub1 turned out to be remarkably dynamic: In early embryos, H2Aub1 is enriched at PcG targets, but during late embryonic and larval stages, PR-DUB activity generates a low, even distribution of H2Aub1 across the genome (Fig. 1A). Thus, at the developmental stages when the Polycomb system actually represses transcription, H2Aub1 is no longer enriched at its canonical target loci. Moreover, although H2Aub1 accelerates the buildup of H3K27me3 domains at *Hox* and other developmental genes in early embryos, these domains are ultimately

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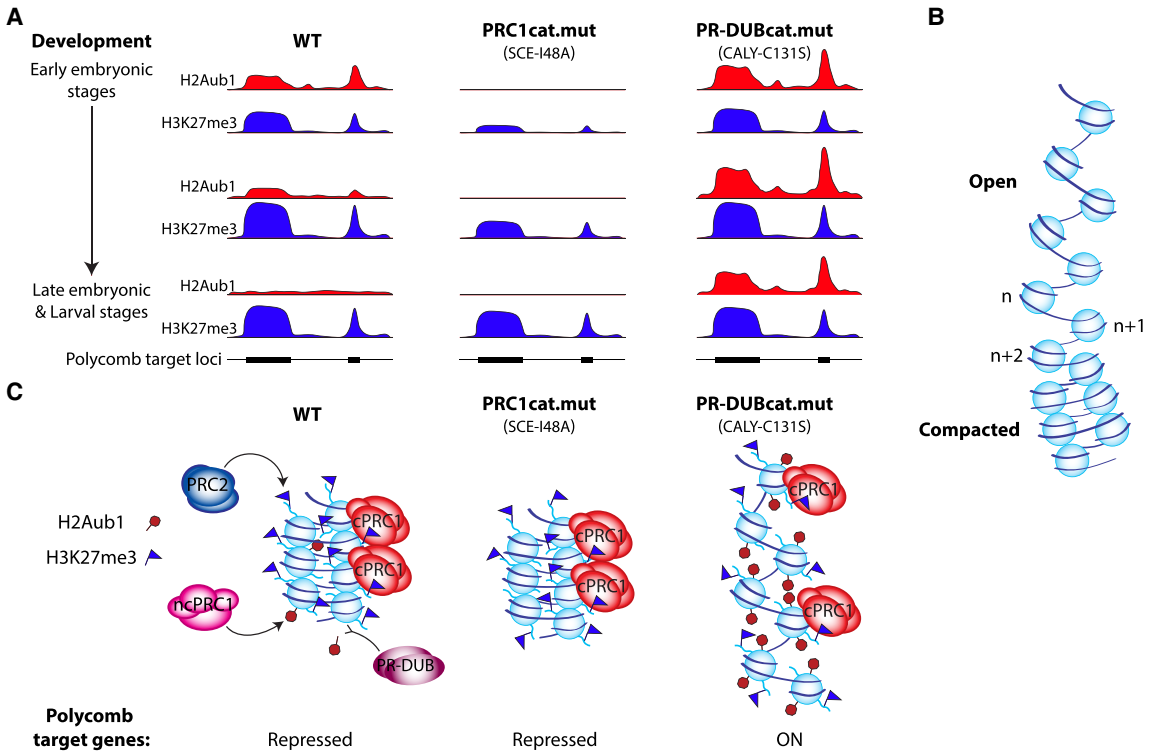

Figure 1. Developmental dynamics of H2Aub1 and its role in Polycomb repression in *Drosophila*. (*A*) Simplified, schematic representation of the genomic distribution of H2Aub1 (red) and H3K27me3 (blue) during *Drosophila* development. H2Aub1, deposited by ncPRC1, is enriched at Polycomb target loci during early embryogenesis, but at later embryonic and larval stages, deubiquitylation by PR-DUB generates an even distribution of low-level H2Aub1 across the genome. In contrast, H3K27me3 domains persist at Polycomb-regulated loci. Thus, H2Aub1 is no longer enriched at genes when they are repressed by Polycomb. In animals expressing a catalytically defective mutant PRC1 (PRC1cat.mut) harboring an I48A substitution in its enzymatic subunit (SCE; RING1A/B in mammals), there is no H2Aub1. Although delayed, H3K27me3 domains are established in ESC-I48A animals, and canonical Polycomb targets are repressed normally. In *Drosophila* expressing a PR-DUB catalytic mutant (CALY-C131S), there is persistent accumulation of H2Aub1 at Polycomb target loci, while the H3K27me3 domains remain largely normal. Misexpression of *Hox* genes causes homeotic transformations in CALY-C131S animals. (*B*) Chromatin compaction involves stacking contacts between the exposed histone surfaces of *n* and *n+2* nucleosomes. (*C*) Chromatin structure at Polycomb target loci in WT, SCE-I48A, and CALY-C131S *Drosophila*. In WT animals, Polycomb-repressed loci are characterized by high levels of H3K27me3, low levels of H2Aub1, and chromatin compaction by cPRC1. In SCE-I48A animals, there is no H2Aub1, but chromatin at Polycomb loci is high in H3K27me3, compacted, and bound by cPRC1. Repression of canonical Polycomb targets is unaffected by the absence of H2Aub1. In CALY-C131S animals, the failure to remove ubiquitin leads to very high levels of H2Aub1 at Polycomb target loci. The high local density of H2Aub1 blocks the association between *n* and *n+2* nucleosomes, thereby disrupting stacking and folding of the chromatin fiber. Consequently, in spite of normal H3K27me3 domains and PRC binding, a subset of Polycomb targets is misexpressed, causing homeotic transformations.

also established in animals lacking H2Aub1 (Fig. 1A; Pengelly et al. 2015; Bonnet et al. 2022). Likewise, in the mouse developing neocortex, H2Aub1 assists transient, reversible gene repression in neurogenic stem cells. Following fate restriction, however, PRC1 mediates persistent repression of the same genes, independent of H2Aub1 (Tsuboi et al. 2018). Collectively, these results suggest that gene repression by H2Aub1 is temporal- and context-dependent but not essential. Likewise, the dependency of H3K27me3 deposition on H2Aub1 observed in mESCs (Tamburri et al. 2022) might reflect a transient developmental stage, rather than an obligatory requirement in vivo.

Next, Bonnet et al. (2022) addressed the question of why the removal of H2Aub1 by PR-DUB promotes Polycomb repression. Surprisingly, in vitro biophysical analysis of nucleosome arrays revealed that H2Aub1 interferes with the association between the exposed surfaces of the histone octamers of the *n* and *n+2* nucleosomes. This is relevant because these contacts characterize compacted chromatin in vivo (Fig. 1B). Thus, rather than promoting chromatin compaction, high local levels of H2Aub1 interfere with stacking and folding of the chromatin fiber. In *Drosophila* lacking PR-DUB activity, the dramatic accumulation of H2Aub1 at PRC target loci is accompanied by increased local DNA accessibility, misexpression of a subset of *Hox* genes, and homeotic transformations (Fig. 1C). Importantly, the high levels of H2Aub1 did not affect either PRC binding to chromatin or formation of H3K27me3 domains. Loss of repression in the absence

of PR-DUB activity was gene-selective because different *Hox* genes responded differently and there were no global transcriptional changes. Concomitant loss of PRC1 enzymatic activity rescued the PR-DUB mutant phenotype, demonstrating that limiting local H2Aub1 accumulation is the key Polycomb function of PR-DUB. In contrast, reduction of the level of a cPRC1-specific subunit in PR-DUB mutant animals enhanced derepression of *Hox* genes and caused more severe homeotic transformations. In conclusion, the results of Bonnet et al. (2022) suggest that H2Aub1 by ncPRC1 restrains higher-order chromatin folding by cPRC1. This way, ncPRC1 can act as a negative regulator of cPRC1, possibly preventing premature lockdown of developmental genes in heterochromatin during early development. Notably, the molecular nature of chromatin compaction by Polycomb, including the relationship between chromatin topology, nucleosome stacking, nucleosome occupancy, and DNA accessibility, remains unclear.

Seemingly at odds with its function in *Drosophila*, in mESCs, BAP1 restricts H2Aub1 spreading across the genome, keeping PRCs concentrated at loci it needs to repress (Tamburri et al. 2022). However, like H2Aub1, this activity of BAP1 in mESCs might reflect an evanescent developmental function in vivo, rather than a general requirement. Genetically, *Calypso* and *Asx* can act as enhancers of both Polycomb and the opposing trithorax group activators, suggesting that removal of H2Aub1 can also assist gene activation. In support of this notion, recruitment of PR-DUB by FOXK1/2 transcription factors in mESCs prevents accumulation of H2Aub1 at selective genes, thereby enabling their expression (Kolovos et al. 2020). Thus, depending on its dosage and genomic context, H2Aub1 can either repress transcription or counter Polycomb silencing.

Genes encoding PR-DUB subunits are mutated frequently in multiple types of cancer (Tamburri et al. 2022). Strikingly, these include both loss-of-function mutations in *Bap1* and *Asxl1/2* and gain-of-function mutations in which truncated ASXL1 increases BAP1 stability. Consequently, both reduced and increased levels of H2Aub1 are associated with oncogenesis. Combined with the findings of Bonnet et al. (2022) during *Drosophila* development, these observations in human cancer emphasize the importance of maintaining the just-right dosage of H2Aub1.

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