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Supplementary Materials

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A Histone Mutant Reproduces the Phenotype Caused by Loss of Histone-Modifying Factor Polycomb

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Although many metazoan enzymes that add or remove specific modifications on histone proteins are essential transcriptional regulators, the functional significance of posttranslational modifications on histone proteins is not well understood. Here, we show in *Drosophila* that a point mutation in lysine 27 of histone H3 (H3-K27) fails to repress transcription of genes that are normally repressed by Polycomb repressive complex 2 (PRC2), the methyltransferase that modifies H3-K27. Moreover, differentiated H3-K27 mutant cells show homeotic transformations like those seen in PRC2 mutant cells. Taken together, these analyses demonstrate that H3-K27 is the crucial physiological substrate that PRC2 modifies for Polycomb repression.

Posttranslational modifications on histone proteins are thought to be important for regulating gene transcription in eukaryotes (*1*). In metazoans, this view is mainly based on the fact that many transcriptional regulators that modify histones are essential for organism viability. However, in animals and plants, it is not known whether mutation of a modified histone residue reproduces the phenotype caused by mutation of the modifying enzyme. Moreover, many of these enzymes modify not only histones but also other proteins, which raises the possibility that some of these may be the physiologically relevant substrates (*2*). A key transcriptional regulator in animals and plants is Polycomb repressive complex 2 (PRC2), a histone methyltransferase that specifically methylates H3 at lysine 27 (H3-K27) (*3*–*6*). PRC2 represses transcription of specific target genes and trimethylates H3-K27 (H3-K27me3) in their chromatin (*3*–*6*). To investigate the physiological role of H3-K27 modification, we examined the phenotype of developing *Drosophila* larvae in which wild-type nucleosomes were replaced by $H3^{K27R}$ mutant nucleosomes.

In *Drosophila*, the canonical histone genes are located in a single cluster (*HisC*) that comprises 23 repeats of the histone gene unit (His-GU) containing the four canonical core histones and linker histone H1. Animals that are homozygous for a chromosomal deletion that removes the histone gene cluster (Δ*HisC*) die at the blastoderm stage, after exhaustion of the supply of maternally deposited histones (*7*). A transgene cassette providing a total of 12 copies of the wild-type His-GU (12×His-GU) rescues Δ*HisC* homozygotes into viable adults (*7*). To study the phenotype of cells that contain $H3^{K27R}$ instead of wild-type H3, we used an analogous transgene cassette with a $H3^{K27R}$ point mutation (12×His-$GU^{H3\text{-}K27R}$). Δ*HisC* homozygotes that carry the 12×His-$GU^{H3\text{-}K27R}$ cassette die toward the end of embryogenesis. Their cells contain a mixture of H3 and $H3^{K27R}$ histones, because maternally supplied wild-type H3 incorporated into chromatin before the blastoderm stage is only partially replaced by $H3^{K27R}$ during the few cell divisions that take place before the end of embryogenesis. To analyze cells with a more complete replacement of H3 by $H3^{K27R}$, we generated clones of Δ*HisC* homozygous cells in imaginal discs of Δ*HisC* heterozygotes carrying 12×His-$GU^{H3\text{-}K27R}$ (fig. S1). Δ*HisC* homozygous cells stop proliferation in animals lacking a rescue transgene cassette (Fig. 1A) but are rescued into normally proliferating cells in animals carrying the wild-type *12×His-GU* or the *12×His-*$GU^{H3\text{-}K27R}$ cassette (Fig. 1A). H3-K27me3 is undetectable in clones of $H3^{K27R}$ cells, which suggests that most wild-type H3 has been replaced by $H3^{K27R}$ and that the H3.3 histone variant expressed in those cells is largely absent in PRC2 substrate chromatin (Fig. 1A). We investigated whether repression of PRC2 target genes is affected in $H3^{K27R}$ mutant cells. Like clones lacking E(z), the catalytic subunit of PRC2, $H3^{K27R}$ mutant clones also show misexpression of the Polycomb group target genes *Ultrabithorax* (*Ubx*), *Abdominal-B* (*Abd-B*), *Sex combs reduced* (*Scr*), and *engrailed* (*en*) in

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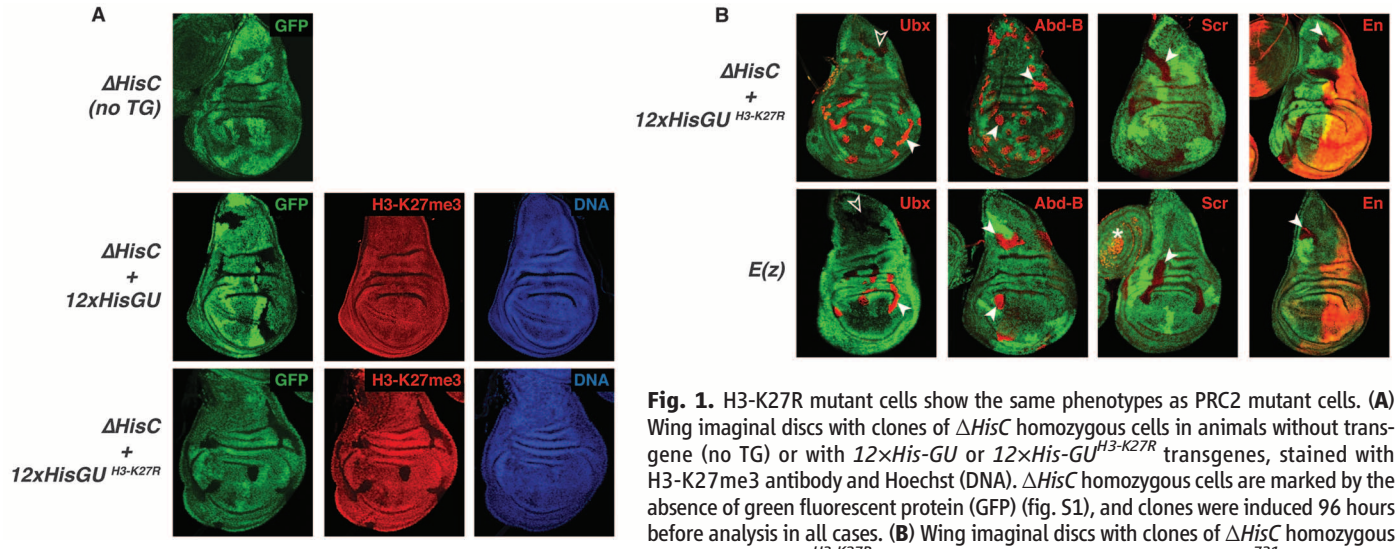

Fig. 1. H3-K27R mutant cells show the same phenotypes as PRC2 mutant cells. (**A**) Wing imaginal discs with clones of Δ*HisC* homozygous cells in animals without transgene (no TG) or with *12×His-GU* or *12×His-GU*$^{H3\text{-}K27R}$ transgenes, stained with H3-K27me3 antibody and Hoechst (DNA). Δ*HisC* homozygous cells are marked by the absence of green fluorescent protein (GFP) (fig. S1), and clones were induced 96 hours before analysis in all cases. (**B**) Wing imaginal discs with clones of Δ*HisC* homozygous cells in *12×His-GU*$^{H3\text{-}K27R}$ transgenics (top row) or with clones of *E(z)*^{731} homozygous cells (bottom row), stained with antibodies against indicated proteins. Clone marking and induction as in (A). Ubx and Abd-B are misexpressed at high levels (arrowheads), Scr and En at lower levels (arrowheads); in both genotypes, not all mutant clones show misexpression (open arrowheads). Asterisk marks Scr expression in peripodial membrane cells in third leg disc.

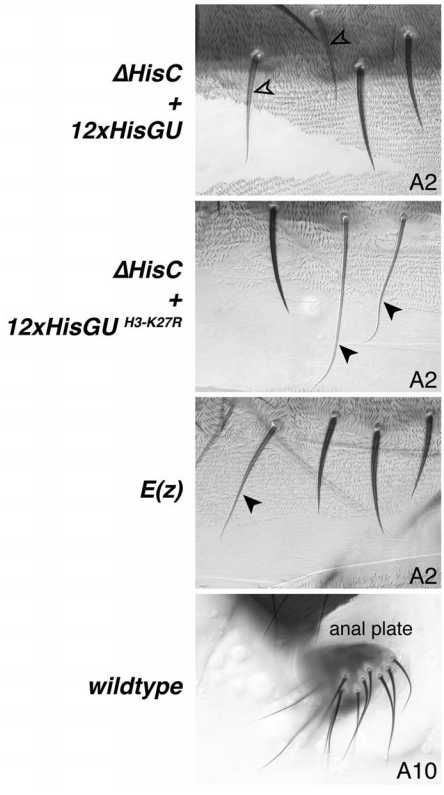

Fig. 2. Homeotic transformation of tissues formed by differentiated H3-K27R mutant cells. Top three panels show portions of abdominal segment A2 of adults with clones of Δ*HisC* homozygous cells in *12×His-GU* or *12×His-GU*$^{H3\text{-}K27R}$ transgenics or with clones of *E(z)*^{731} homozygous cells; in all cases, mutant cells are genetically marked with a *yellow* mutation (light pigmentation, fig. S1). Δ*HisC 12×His-GU*$^{H3\text{-}K27R}$ mutant clones show homeotic transformation into more posterior body segments; the mutant sensory bristles (arrowheads) are finer and more tapered than the neighboring wild-type bristles in A2 and resemble bristles characteristic of abdominal segment A10 in wild-type animals (bottom). In the case of *E(z)*, mutant bristles (arrowheads) are less extensively transformed, which may reflect the perdurance of E(z) protein in the mutant cells. Δ*HisC 12×His-GU* bristles (open arrowheads), shown as controls, are indistinguishable from neighboring wild-type bristles.

imaginal wing disc cells, where these genes are normally repressed (Fig. 1B). Both the levels of misexpression and the territories in the disc where each of these genes is misexpressed are very similar in *H3*^{K27R} and *E(z)* mutant clones (Fig. 1B). *H3*^{K27R} and *E(z)* mutant cells both differentiate to form epidermal structures in adult flies. However, these structures show homeotic transformations. This is illustrated by the changed morphology of sensory bristles generated by *H3*^{K27R} or *E(z)* mutant cells; bristles formed by mutant cells in anterior segments (e.g., in abdominal segment A2) resemble the bristles that are characteristic for and normally only present in the most posterior body segment (Fig. 2). These segmental transformations are consistent with the observation that *H3*^{K27R} and *E(z)* mutant clones in anterior body segments show misexpression of posterior *HOX* genes.

In summary, these results demonstrate that cells with H3^{K27R} nucleosomes reproduce the PRC2 mutant phenotype. This provides strong evidence that H3-K27 in target gene chromatin is the crucial substrate that PRC2 needs to methylate for Polycomb repression in *Drosophila*. Furthermore, the PRC2-mimic phenotype of H3^{K27R} mutant cells suggests that H3-K27 acetylation does not play a major role of its own but may function only to antagonize H3-K27 methylation by PRC2. The demonstration that the H3-K27 residue has an essential function in metazoans emphasizes how important it is to resolve how PRC2 establishes, maintains, and propagates H3-K27 methylation and how this modification generates repressive chromatin.

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A Histone Mutant Reproduces the Phenotype Caused by Loss of Histone-Modifying Factor Polycomb

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Genetic Epigenetics

Posttranslational modifications of histone proteins have been implicated in the regulation of gene transcription in organisms ranging from yeast to humans. However, epigenetic regulators can modify multiple proteins. By mutating specific histone sites in *Drosophila*, **Pengelly *et al.*** (p. 698) demonstrate that mutation of lysine 27 of histone H3 causes the same transcriptional defects as those observed in mutants lacking the methyltransferase PRC2 that modifies this H3 residue. These results demonstrate the functional importance of H3-K27 methylation in Polycomb repression. Furthermore, this genetic approach may be applied to investigating numerous other metazoan-specific histone modifications.