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population decline only few males and females would have to be released to actually recall the transgenes and revert the mosquito population to wild type (drive out, reversion).

So far these systems have only been described in the non-pest insect model organism *Drosophila* [8], and it will take its time until similar strategies will have been successfully developed for human disease vectors. In addition, it will take enormous efforts by international regulators in collaboration with molecular entomologists, ecologists, and operational pest managers to develop clear regulatory frameworks for the safe release of such beneficial transgenic insects. Nevertheless, the principal concepts to establish transgenic refractoriness to malaria or dengue transmission in mosquitoes as well as to control a locally refined spread and to recall the transgenes are established, which nurtures the long-standing hope that insect transgenesis can indeed be employed for novel strategies to fight human vector-borne diseases.

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Chromatin: A Tail of Repression

Genetic evidence on the role of specific histone amino acids or their posttranslational modifications in metazoan development has been lacking. A recent study reports that fruit flies carrying histone H3 lysine 27 (H3K27) mutations have the same homeotic gene expression and developmental defects as mutations in the enzyme that trimethylates H3K27.

Danesh Moazed

The four canonical histones, H2A, H2B, H3, and H4, are among the most highly conserved eukaryotic proteins. Between cow and pea, all but 2 of the 103 amino acids in histone H4 are the same, and there are only 8 amino acid differences between the yeast and human H4 proteins. Histones package DNA into chromatin and in keeping with their extraordinary conservation play important roles in nearly all DNA transactions. That the post-translational modifications of histones play a central role in the regulation of chromatin structure and transcription is a basic tenant of current models of gene regulation. It may come as a surprise to many that in multicellular eukaryotes a direct demonstration of a role for a specific histone amino acid, or its modification, in gene activation or silencing was lacking until recently. In a paper published in *Science* last month [1], Muller, Herzig and colleagues now remedy this situation by demonstrating that a point mutation in lysine 27 of histone H3 (H3K27) fails to silence genes that are targeted by the Polycomb Repressive Complex 2 (PRC2), the methyltransferase that modifies H3K27.

The basic unit of chromatin is the nucleosome, which contains 147 base pairs of DNA wrapped twice around an octamer composed of four histones [2]. Histones contain a variety of posttranslational modifications, which are mostly but not exclusively concentrated on their amino termini. These modifications provide binding sites for proteins that mediate downstream functions, ranging from activation and repression of transcription to coordination of DNA damage repair. In addition, they affect the interaction of the positively charged histone tails with DNA, thereby regulating nucleosome stability [3]. In *Drosophila*, mammals, and many other multicellular organisms, the stable silencing of developmental regulators, such as the homeotic master regulators, outside of their proper

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domains of expression requires the Polycomb group of genes [4,5]. The members of this gene family encode subunits for several complexes that either modify histones or bind to them or both. The Polycomb Repressive Complex 1 (PRC1) contains the Polycomb protein, which binds to nucleosomes that are trimethylated on lysine 27 of histone H3 (H3K27), a modification that is produced by E(z), the catalytic subunit of Polycomb Repressive Complex 2 (PRC2) [6,7]. This, together with the observation that flies carrying mutations in either Polycomb or E(z) have identical homeotic transformations, suggested that the critical role of E(z) in silencing involved the methylation of H3K27 [8,9]. However, histone-modifying enzymes also have non-histone substrates and the possibility that (E)z modifies a non-histone substrate critical for silencing could not be ruled out based on the above evidence.

There is a wealth of histone mutational data in yeast that supports a role for posttranslational modification of specific histone amino acids in gene silencing (see below). In contrast to yeast, the genomes of multicellular organisms contain 10 to 400 copies of the genes that code for canonical histones. These genes are often dispersed among several chromosomes, making histone mutagenesis in these organisms particularly challenging [10]. However, in *Drosophila*, the histone genes are located in a single cluster on chromosome II containing 23 gene units, each containing genes for histone H1 and the four canonical core histones [10]. Flies that are homozygous for a deletion of this cluster die as embryos after exhaustion of the supply of maternally deposited histones, but this lethality can be fully rescued by insertion of 12 histone gene units on chromosome III [11]. This transgenic system made it possible to perform mutagenesis of canonical histone genes in a metazoan organism for the first time.

Pengelly *et al.* [1] constructed transgenic fly strains that were heterozygous for either wild-type or H3K27 to arginine (H3K27R) histone transgene units and homozygous for the deletion of the endogenous histone gene locus. PRC2 mutant homozygous embryos die at the end of embryogenesis with striking anterior to posterior homeotic transformations,

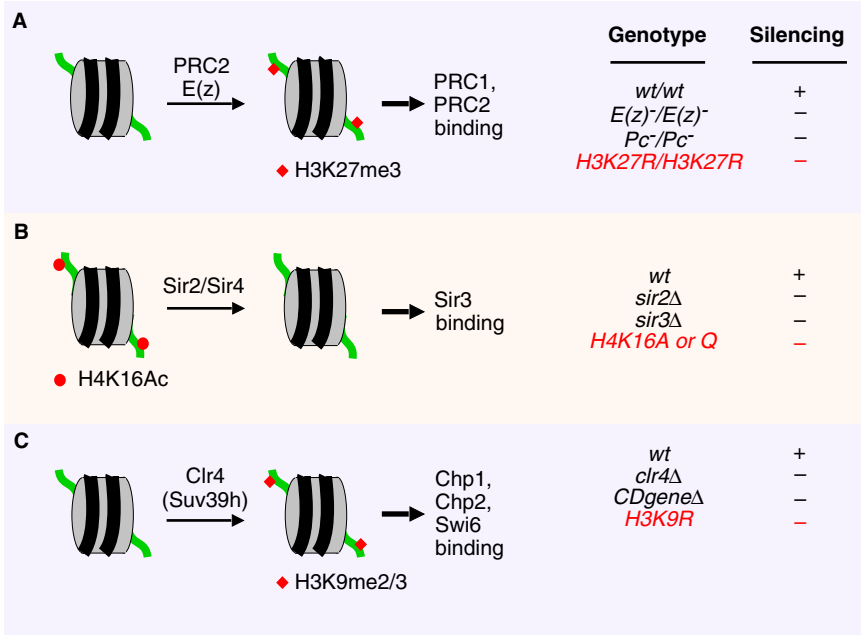

Figure 1. The relationship between histone-modifying enzymes and their substrates in *Drosophila*, *S. cerevisiae*, and *S. pombe*.

(A) In *Drosophila*, the E(z) subunit of the PRC2 complex methylates histone H3K27, creating a binding site for the Polycomb (Pc) subunit of PRC1. Silencing of target genes is lost in flies or cells homozygous for mutations of either E(z), Pc, or H3K27 to arginine. (B) In the budding yeast *S. cerevisiae*, the Sir2 subunit of the SIR complex deacetylaes histone H4K16, creating a binding site for Sir3. Silencing is lost in yeast cells carrying mutations in either Sir2, Sir3, H4K16 to alanine, or H4K16 to glutamine. (C) In the fission yeast *S. pombe*, Clr4 (homolog of *Drosophila* Su(var)3-9 and human Suv39h), methylates histone H3K9, creating binding sites for chromodomain proteins Chp1, Chp2, and Swi6. Silencing is lost in cells carrying mutations in either Clr4, any of the three chromodomain proteins, or histone H3K9 to R. Chp1 is only required for silencing at pericentromeric heterochromatin.

which result from derepression of the homeotic genes. However, embryos homozygous for H3K27R mutation lacked homeotic transformations because their chromatin is packaged with a mixture of mutant and maternally deposited wild-type histone H3. The authors therefore had to resort to another strategy involving the generation of Flip recombinase-induced mitotic clones at later stages of development. They used this strategy to generate mutant clones in imaginal disks, sacks of highly programmed cells, which differentiate to form adult structures such as wing, leg, or abdominal segments. Strikingly, they observed that in H3K27R homozygous clones the targets of PRC2 such as Ultrabithorax and Engrailed were derepressed. Moreover, this derepression was accompanied by transformation of the tissue formed by H3K27R clones in anterior segments to more posterior phenotypes, resembling loss of E(z).

These results clearly indicate that the identity of the amino acid at position 27 of histone H3 is critical for proper silencing of PRC2 target genes. Since arginine cannot be acetylated, the results further strongly suggest that H3K27 methylation, rather than H3K27 deacetylation, is critical for the silencing function of this residue (Figure 1A). Although not surprising, this pleasing outcome is important because it provides unequivocal genetic evidence that the methylation of H3K27 is critical for maintenance of epigenetic gene expression patterns.

The results of the above *Drosophila* H3 mutagenesis experiments are reminiscent of histone mutagenesis experiments performed in yeast many years ago. The first evidence in support of a role for histones in gene regulation came from pioneering studies of Grunstein and colleagues in the budding yeast *Saccharomyces cerevisiae* [12]. Yeast has fewer copies

of histone genes than metazoans (two for each canonical histone). So extensive genetic analysis including every histone side chain has been carried out over the past few years. Early analysis of histone tail deletions revealed that a conserved basic region spanning amino acids 15 to 19 in the amino terminus of histone H4 was specifically required for silencing of the heterochromatic mating-type genes but not viability [12]. Subsequent studies showed that within this region the acetylation state of H4 lysine 16 (H4K16) played a critical role. For example, an H4K16 to glutamine substitution, which mimics acetylated lysine, disrupts silencing, while an H4K16 to arginine substitution, which mimics deacetylated lysine, is tolerated. Furthermore, biochemical and genetic studies showed that the H4 amino terminus serves as a binding site for the Sir3 silencing protein (for example, see [13,14]). This binding is regulated by the acetylation state of H4K16, which is deacetylated by Sir2, a highly conserved NAD-dependent deacetylase. These results provided a coherent picture, supported by genetic and biochemical studies, for how the posttranslational modification of a specific histone lysine controls the binding of a silencing protein to chromatin (Figure 1B).

Genetic evidence in support of the importance of specific histone amino acids in silencing also comes from studies in the fission yeast *Schizosaccharomyces pombe*. Jenuwein and colleagues discovered that SET domain proteins encoded by the *Drosophila* gene *Su(var)3-9* and the mammalian gene *Suv39h* contained a methyltransferase activity that targeted histone H3 lysine 9 (H3K9) [15]. This methylation creates a binding pocket for HP1 proteins and occurs in heterochromatic DNA domains in most eukaryotes [16,17]. In fission yeast, the substitution of H3K9 with either alanine or arginine, as well as deletion of the *Suv39h* homolog, *Clr4*, results in loss of heterochromatin [18]. Furthermore, the methylation of H3K9 creates a binding site for the fission yeast chromodomain proteins Chp1, Chp2, and Swi6, all of which are required for heterochromatic gene silencing (Figure 1C).

Numerous examples of histone modifications that regulate the binding of specific proteins to chromatin have been reported in other model organisms. In addition to the repressive systems describe above, histone modifications also recruit effectors that promote transcriptional activation. However, with regard to activating marks, the genetic evidence has been harder to interpret, in particular with regard to histone H3 methylation on lysines 4 and 36 (H3K4 and H3K36). One reason for this difficulty is that the primary function of these marks in yeast may involve their role in repression of cryptic promoters within open reading frames (reviewed in [19]). For example, H3K4me3 and H3K36me3 recruit different histone deacetylase complexes to the 5' and 3' regions of open reading frames that repress cryptic transcription.

In *Drosophila*, the same histone transgene system used for the analysis of H3K27 was used by Hodl and Basler [20] to examine the consequences of mutating H3K4 to alanine or arginine. Unlike the case with H3K27 [1], imaginal disk clones containing H3K4R mutations do not display changes in homeotic gene expression, even though both sources of potential H3K4 methylation, H3.2 and H3.3, were simultaneously mutated. In this case, H3K4 trimethylation levels were reduced to a level below the immunoflourescence detection limit in the homozygous disk clones. It remains possible that the quantitative requirement of H3K4 methylation for active transcription could be less than H3K27 methylation for gene repression. Alternatively, Trithorax may act on non-H3K4 targets in disks, or the role of H3K4 methylation in transcriptional activation may be redundant with other modifications. To address these possibilities, it would be interesting to test whether disk clones homozygous for mutations in Trithorax, the *Drosophila* H3K4 methyltransferase required for maintenance of homeotic gene expression, have defects in homeotic gene expression.

We can now look forward to a more complete genetic dissection of the role of histones in gene regulation in *Drosophila*. More importantly, positive genetic verification as reported for the H3K27 mutation highlights the importance of ongoing mechanistic studies on the role of histones and their posttranslational modifications in regulation of chromatin structure and gene expression.

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Cell Biology: DUBing CP110 Controls Centrosome Numbers

Accurate control of centrosome number is critical for the maintenance of genomic integrity. A recent study reveals that the deubiquitinating enzyme USP33 regulates centrosome biogenesis by stabilizing the centriolar protein CP110.

Felix Bärenz and Ingrid Hoffmann*

The centrosome is a non-membrane-bound organelle found in most animal cells. It has several important functions, including control of cilia formation, microtubule organization and nucleation, spindle assembly and transport of organelles and vesicles. Centrosomes consist of two centrioles, which are barrel-shaped microtubule-based structures surrounded by an electron-dense matrix, the pericentriolar material (PCM). Duplication of centrioles must occur in coordination with DNA synthesis only once per cell cycle and duplication involves the assembly of a new daughter centriole (procentriole) next to the proximal end of the mother centriole [1]. In human cells, hSAS-6, STIL, CEP135, CPAP, γ-tubulin, and CP110 have been identified as essential factors required at the onset of centriole biogenesis for centriole formation [2,3]. The newly formed procentrioles then start to elongate, increase in length during S phase, and are fully assembled in G2 phase of the cell cycle. A new study by Li *et al.* [4] now reports a role for deubiquitination in the stabilization of CP110 during S/G2 phase.

The protein CP110 was first identified as a Cdk2 substrate required for centrosome overduplication in S-phase-arrested cells [5]. It is associated with the growing centriolar distal tips, forming a cap beneath which centrioles elongate through the insertion of α/β-tubulin [2]. Depletion of CP110 or overexpression of CPAP was shown to induce centriole elongation, suggesting that CPAP and CP110 play opposing roles in controlling centriole length [6–8]. CP110 in a complex with its regulator Cep97 also plays an additional role in preventing cilia formation through the inhibition of centriole to basal body conversion [9].

CP110 protein levels are tightly controlled during the cell cycle in order to prevent centriole duplication errors. CP110 physically associates with the F-box protein cyclin F on centrioles during the G2 phase of the cell cycle and is then ubiquitinated by the $SCF^{CyclinF}$ E3 ubiquitin ligase complex, leading to its degradation [10]. The new study by Li *et al.* [4] now reports that deubiquitination is also an important mechanism for the regulation of CP110 protein levels during the cell cycle. Polyubiquitinated proteins that are marked for degradation can be stabilized in response to the activity of deubiquitinating enzymes (DUBs) — proteases that remove the polyubiquitin chain. Around 100 active DUBs are found in the human genome [11] and, as a result of their ability to reverse ubiquitination, these enzymes control a broad range of key cellular processes. Li *et al.* [4] report that the DUB USP33 (ubiquitin-specific protease 33, also known as VDU1) binds to CP110 and specifically deubiquitinates CP110 in a cell-cycle-dependent manner, thus counteracting $SCF^{CyclinF}$ ubiquitin ligase activity (Figure 1).

Although several SCF ubiquitin ligase complexes have been recently shown to regulate centrosome biogenesis [12], DUBs had not been demonstrated to be involved in the regulation of this process until now. Both USP33 and the highly related USP20 (also known as VDU2) interact with CP110, but USP33 has a greater impact on CP110 levels than USP20. USP33 and USP20 are mainly localized to the cytoplasm, in particular to the endoplasmic reticulum [13]. USP33 additionally localizes to the proximal end of centrioles, whereas CP110 is a distal-end-capping protein, suggesting that only a subpopulation of CP110 interacts with USP33. USP33 localizes to centrosomes primarily during S and G2 phase of the cell cycle similar to CP110. Centriolar targeting of USP33 is at least in part dependent on CP110 because depletion of CP110 reduced USP33 localization to the centrosome. USP33 specifically deubiquitinates CP110 both *in vitro* and *in vivo*. The amino-terminal domain of CP110 interacts with the catalytic domain of USP33 and is required for USP33-mediated deubiquitination.

As CP110 is a crucial player in centriole duplication, it is intriguing to ask whether centriole duplication would also be affected in the absence of the CP110-stabilizing effects of USP33. Li *et al.* [4] found that USP33 depletion did not inhibit normal centriole duplication through loss of centrioles: instead, loss of USP33 inhibited centrosome re-duplication. In some cell lines, for example U2OS, centrosome re-duplication can be induced by the addition of hydroxyurea, which promotes cell-cycle arrest at the G1/S phase transition but does not affect centrosome duplication and thereby results in an increase in centrosome numbers. A similar effect on centrosome duplication is caused by CP110 depletion [5]. Intriguingly, co-depletion of USP33 and $SCF^{cyclinF}$ rescued the mitotic defects caused upon siRNA-mediated downregulation of $SCF^{cyclinF}$ [4]. Therefore, opposing activities of E3 ubiquitin ligases and DUBs that both target key regulators of centriole duplication may represent a general mechanism for regulating the levels of those factors during the cell cycle and centriole biogenesis.

Supernumerary centrosomes are frequently found in cancer cells. During cell division too many centrosomes

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