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Crosstalk among Histone Modifications

Tamaki Suganuma[1] and Jerry L. Workman[1,*]
[1]Stowers Institute for Medical Research, Kansas City, MO 64110, USA
*Correspondence: jlw@stowers-institute.org
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Histone modifications play a complex role in the regulation of transcription. Recent studies (Duncan et al., 2008; Lee et al., 2007; Li et al., 2008) reveal that regulation of histone modifications can be functionally linked to reinforce the activation or repression of gene expression.

Chromosomal processes such as transcription are influenced by a variety of posttranslational modifications to histones, including acetylation, phosphorylation, methylation, and ubiquitination. These modifications may act alone or in concert in a context-dependent manner to facilitate or repress chromatin-mediated processes (Berger, 2007). Some histone modifications are thought to influence nucleosome stability, but an exciting emerging theme is that histone modifications can influence one another such that one modification recruits or activates chromatin-modifying complexes to generate a different histone modification. There appear to be three main crossregulatory mechanisms that allow reinforcement of the actions of histone modifications in regulating gene expression. One mechanism is mediated by an initial histone modification that triggers increased activity in a histone-modifying enzyme. A second mechanism involves the coordination of different histone-modifying enzymes present in the same protein complex. The third mechanism connects histone modifications to the cleavage of the N-terminal tail of histone H3, providing a new way for a histone modification-activated enzymatic activity to achieve an irreversible modification.

Histone Modification Crosstalk

The earliest examples of histone modification crosstalk—one histone modification promoting the generation of another—were observed for modifications on the same histone tail. In the budding yeast *Saccharomyces cerevisiae*, phosphorylation of serine 10 (S10) on histone H3 by the Snf1 kinase promotes the acetylation of H3 lysine 14 (K14) by the Gcn5 acetyltransferase, enhancing H3's interaction with the 14-3-3 proteins Bmh1 and Bmh2 during gene activation (Walter et al., 2008). Acetylation of mammalian H3 on lysine 18 (K18) and lysine 23 (K23) promotes the methylation of arginine 17 (R17) by the CARM1 methyltransferase, resulting in activation of estrogen-responsive genes (Daujat et al., 2002). Histone modification crosstalk can also direct the loss of particular modifications. For example, in budding yeast, the RNA polymerase II-associated Set2 methyltransferase methylates H3 lysine 36 (K36), creating a mark that targets nucleosomes for H3 and H4 deacetylation by the Rpd3S deacetylase complex after the passage of RNA polymerase (Lee and Shilatifard, 2007).

Some of the most interesting examples of histone modification crosstalk involve *trans*-histone effects, where one histone and its modifications affect the modification of a different histone. Of particular interest is the role of H2B monoubiquitination, a modification that modulates multiple methylation events on histone H3 in *S. cerevisiae*. H2B ubiquitination by the Rad6 enzyme is necessary to trigger H3 lysine 4 (H3K4) methylation by the Set1 methyltransferase subunit of the COMPASS complex and H3 lysine 79 (H3K79) methylation by the Dot1 methyltransferase (Weake and Workman, 2008). Lee et al. (2007) provide insight into the mechanism governing this crosstalk. The COMPASS methyltransferase complex requires the Cps35 subunit to methylate H3K4. However, Cps35 will only associate with COMPASS when it is bound to chromatin containing ubiquitinated H2B (UbH2B). Lee and colleagues find that Cps35 binds to chromatin containing UbH2B before associating with the COMPASS complex and activating it for H3K4 methylation. Methylation of H3K79 by the Dot1 methyltransferase also requires ubiquitination of H2B (Weake and Workman, 2008). In the budding yeast, H3K79 methylation is also dependent on Cps35, suggesting a crosstalk mechanism similar to that observed with COMPASS (Lee et al., 2007). However, recent in vitro experiments show that chemically ubiquitinated H2B, when incorporated into nucleosomes, could directly stimulate the catalytic activity of recombinant human Dot1, suggesting an additional mechanism for direct UbH2B and H3K79 interaction and crosstalk (McGinty et al., 2008).

Deubiquitination of H2B also affects H3 methylation. H2B is deubiquitinated by the Ubp8 ubiquitin protease, a component of the SAGA acetyltransferase complex in budding yeast, flies, and mammals (Weake and Workman, 2008). In budding yeast, deubiquitination of H2B by Ubp8 allows Ctk1 kinase to associate with nucleosomes at the 5′ end of genes. This permits Ctk1 to phosphorylate serine 2 on the RNA polymerase II C-terminal repeats. The Set2 methyltransferase then associates with serine 2 phosphorylated RNA polymerase II and travels with the polymerase to methylate H3 at lysine 36 in the open reading frames of genes (Wyce et al., 2007). Thus, H2B ubiquitination and deubiquitination act as master switches, regulating multiple methylation events on histone H3. Histone modifications, and even modification of RNA polymerase II, are involved in crosstalk at many steps during transcription and may serve as checkpoints for the correct assembly of the machinery required to accurately load and launch RNA polymerase during gene expression.

Multifunctional Histone Modification Complexes

An increasing number of chromatin-modifying complexes are found to contain more than one distinct histone-modifying enzyme. These enzymes may act independently, but, in some

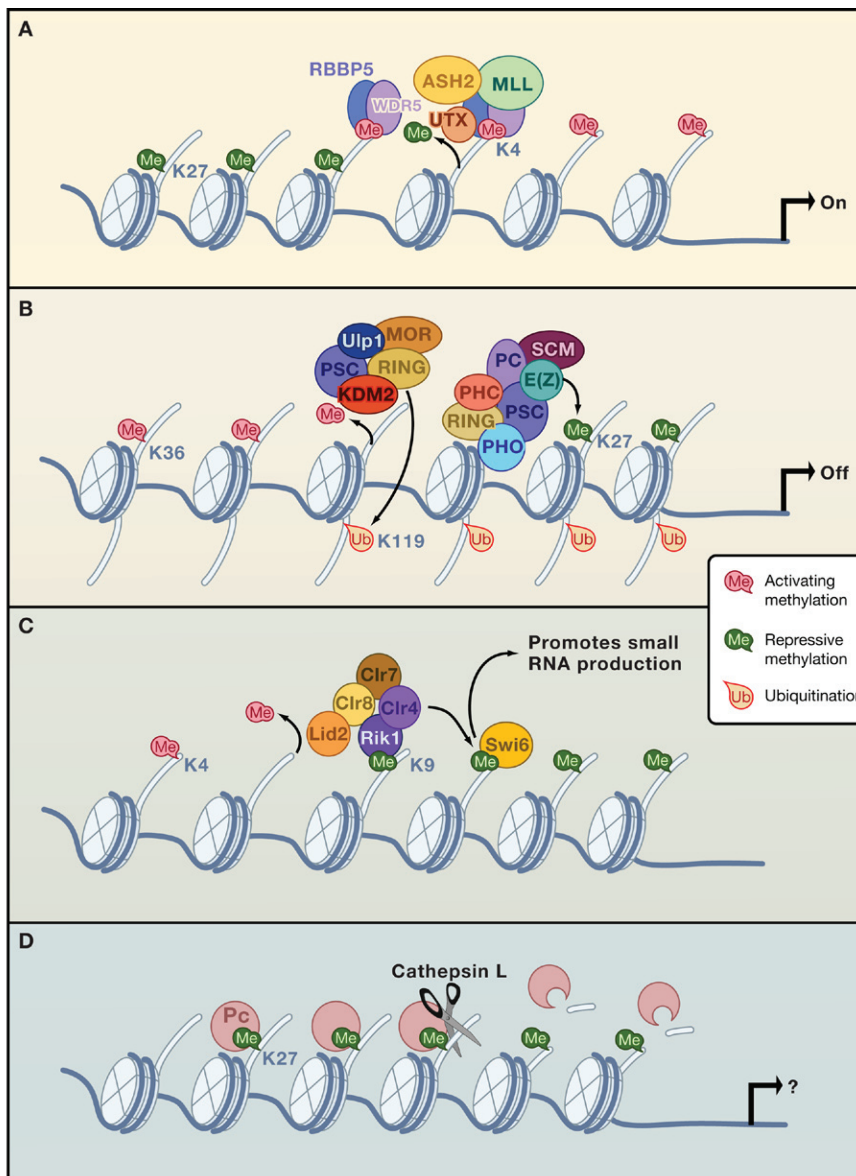

Figure 1. Crossregulation among Histone Modifications

(A) Shown is a complex containing demethylase and methyltransferase enzymes that coordinates the removal of a repressive mark—methylation of histone H3 on lysine 27 (H3K27)—with the formation of an activating mark—methylation of histone H3 on lysine 4 (H3K4). The MLL3/4 Set1-H3K4 methyltransferase complex is recruited by WD40 domain proteins (WDR2 and RBBP2) that recognize H3K4 at active regions of the Hox genes. The UTX subunit of the complex demethylates di- and trimethylated H3K27.

(B) Crossregulation of histone H3 lysine 27 (H3K27) methylation, histone H3 lysine 36 (H3K36) demethylation, and H2A ubiquitination during transcriptional silencing. The *Drosophila* dRAF (dRING-associated factors) complex contains RING, PSC, Ulp1, MOR, and the KDM2 H3K36 demethylase. dRAF demethylates H3K36, an activating mark, and ubiquitinates H2A lysine 119 (H2AK119), a repressive mark, through the actions of dRING/PSC proteins in cooperation with the PcG complex. Association of PcG complexes results in the methylation of histone H3 on lysine 27 (H3K27), a repressive mark, by the E(Z) methyltransferase. dRAF is a separate PcG complex that cooperates with PRC1 in transcriptional silencing.

(C) Lid2, a histone H3 lysine 4 (H3K4) demethylase, coordinates H3K4 demethylation and H3K9 methylation with the RNA interference pathway. In the fission yeast *S. pombe*, H3K9 methylation in heterochromatin is catalyzed by Clr4 in association with Rik. H3K9 methylation is linked to small interfering RNA (siRNA) production by Clr4- and Clr8-mediated association with the RITS (RNA-induced initiation of transcriptional gene silencing) complex. H3K9 methylation also creates the binding site for Swi6, resulting in transcriptional repression. Lid2 is recruited to the Clr4-Clr7-Clr8 complex through an interaction with Clr8. The H3K4 demethylase activity of Lid2 is required for H3K9 methylation by the complex and is also needed for the production of small RNAs and the loading of RITS onto heterochromatin.

(D) Histone modifications and H3 cleavage. The N-terminal tail of the histone H3.2 is cleaved between amino acids 21 and 22 by cathepsin L during differentiation of mouse embryonic stem cells. This cleavage is enhanced by methylation of histone H3 lysine 27 (H3K27). Binding of the mouse CBX7 Polycomb protein to methylated H3K27 is disrupted by cathespin L cleavage of H3.

cases, they work together to coordinate histone modifications. For example, the mammalian MLL3/4 Set1-H3K4 methyltransferase complex coordinates the removal of a repressive methyl mark with the formation of an activating methyl mark on histone H3. This protein complex contains H3K4 methylation activity (an activating mark), as well as a subunit called UTX that removes the transcriptionally repressive methyl mark from H3K27 (Figure 1A) (Cho et al., 2007).

Other examples of two histone-modifying enzymes in the same macromolecular protein complex can be found in the Polycomb group (PcG) of transcriptional silencing complexes. The PcG silencing complexes consist of three separate protein complexes (PRC1, PRC2, and PhoRC) that assemble on chromatin and coordinate H2A lysine 119 (H2AK119) ubiquitination and H3K27 methylation (Schwartz and Pirrotta, 2007). Polycomb repressive complex 1 (PRC1) was initially purified from the fruit fly *Drosophila melanogaster* and contains the proteins Polycomb (PC), Posterior sex combs (PSC), polyhomeotic (PH), dRING, and Sex comb on midleg (SCM) (Figure 1). The mammalian PRC1 complex purified from cultured HeLa cells is similar to that of *Drosophila*. The dRING subunit of PRC1 and its mammalian homologs, RING1A and B, function as E3 ubiquitin ligases and monoubiquitinate H2AK119. Polycomb repressive complex 2 (PRC2), or the E(Z)/ESC complex, contains the E(Z) H3K27 methyltransferase. The PhoRC complex, identified in *Drosophila*, contains the pleiohomeotic/pleiohomeotic-like (PHO/PHOL) proteins (homologs of mammalian Yin-Yang1) and binds to DNA (Schwartz and Pirrotta, 2007). The recruitment of PcG silencing components in *Drosophila* is hierarchical (Martin and Zhang, 2005): PHO and PHOL are first bound to a Polycomb-specific site (PRE) in the DNA. The E(Z) complex is then recruited to this PRE site through interactions with PHO and

PHOL. The E(Z) complex methylates H3K27, forming a binding site for PC, the protein required to recruit PRC1. Once bound to the PRE, PRC1 mediates ubiquitination of H2AK119, possibly indirectly through the dRAF complex (Figure 1B). Thus, the Polycomb group complexes coordinate and establish two repressive marks on chromatin.

A recent study shows that in *Drosophila*, the PRC1 subunits dRING and PSC also form another Polycomb complex called dRAF (dRING-associated factors), which is distinct from PRC1 (Lagarou et al., 2008). Interestingly, dRAF contains the dKDM2 protein that removes dimethyl groups from K36 (Figure 1B). dKDM2 enhances homeotic gene silencing by PcG complexes, suggesting that the dRAF and PRC1 complexes cooperate in vivo. dKDM2 also stimulates H2A ubiquitination by dRING/PSC in a manner independent of dKDM2 catalytic activity (Lagarou et al., 2008). Thus, the dRAF complex can coordinate the removal of an active mark, H3K36me2, with addition of a repressive mark, H2AK119 ubiquitination, although neither of these two activities appears to depend on the other (Figure 1B).

A recent study in *Cell* by Li et al. (2008) provides another example of histone modification coordination by a protein complex in the fission yeast *Schizosaccharomyces pombe*, but in this case, the modification activities may be interdependent. Heterochromatin in *S. pombe* is found at centromeres, telomeres, and the mating-type region. These heterochromatic regions are enriched in H3K9 methylation catalyzed by Clr4, the homolog of the mammalian histone methyltransferase SUV39H1. The catalytic activity of Clr4 is dependent on its association with Rik1, a WD40-repeat protein that is the homolog of the human DNA damage binding protein (DDB1). These two proteins form a complex with Clr7 and Clr8 chromatin binding factors and the Cul4 ubiquitin ligase to promote H3K9 methylation by Clr4. Li et al. demonstrated that the JmjC domain protein Lid2, a H3K4-specific demethylase, binds to this protein complex through an interaction with Clr8. Lid2 promotes heterochromatin formation by removing the activating H3K4 methyl marks. Surprisingly, the H3K4 methylation activity of Lid2 in the Clr4/7/8-Rik-Cul4 complex facilitates the H3K9 methylation activity of this complex, thereby functionally linking H3K9 methylation with H3K4 demethylation in the formation of heterochromatin (Figure 1C). Li and colleagues report that Lid2 may also play a role in euchromatin, coordinating the Set1 methyltransferase and the Lsd1 demethylase to regulate H3K4 and H3K9 methylation levels, although this function is less clear and may not depend on Lid2 enzymatic activity.

Histone methylation and demethylation are also intimately connected with the RNA interference (RNAi) pathway of gene silencing. In *S. pombe*, deletion of Clr4 (the H3K9 methyltransferase) abolishes the generation of small interfering RNAs (siRNAs), revealing a self-enforcing loop and interdependence of histone methylation and the RNAi pathway (Buhler et al., 2006). The binding of the RNA-induced initiation of transcriptional gene silencing (RITS) complex to centromeres requires H3K9 methylation and the presence of the chromatin factors Swi6 and HP1 (Buhler et al., 2006). Li et al. (2008) now show a further link by demonstrating that demethylation of H3K4 by Lid2 is also required for the loading of RITS onto centromeres (Figure 1C). A similar connection between methylation and RNA is observed in the ciliated protozoan *Tetrahymena*. Although H3K27 methylation is not directly coupled with generation of siRNAs, generation of methyl marks by the enzyme *EZL1* requires the RNAi pathway (Liu et al., 2007). RNAi-dependent H3K27 methylation is in turn required for heterochromatin formation and for H3K9 methylation.

The RNAi pathway has also been linked to histone methylation and transcriptional gene silencing in *Drosophila* and mammals. For example, components of the RNAi machinery are involved in PcG-mediated transcriptional repression at the PREs of the Fab-7 regulatory element in *Drosophila*. In mammals, microRNAs and RNAi pathway components also have been shown to participate in the repression of target promoters, the recruitment of PcG components, and the methylation of H3K27 (e.g., Kim et al., 2008). Clearly, the RNAi pathway plays important roles in mediating transcriptional gene silencing in cooperation with multifunctional histone modification enzymes such as PcG complexes.

Cleaving Histones and Their Modifications

A recent study by Duncan et al. (2008) in *Cell* suggests that histone modifications may also be linked to cleavage of the histone H3 tail. This would represent a radically different approach to irreversibly undoing a set of previous histone modifications. The unstructured N-terminal tails of the core histones are extremely sensitive to proteolysis compared to more structured regions of histone proteins. In spite of their inherent protease sensitivity, surprisingly few examples of in vivo proteolysis of histone tails have been reported. These biological examples of histone tail proteolysis also tend to occur under very specialized circumstances. For example, in *Tetrahymena*, six amino acids are proteolytically cleaved from the N-terminal tail of H3 in the chromatin of the germline micronucleus with unknown functional consequences (Allis et al., 1980). In cultured mammalian cells infected with the virus causing foot-and-mouth disease (FMDV), normal H3 is replaced by a protein called Pi that is generated by proteolytic cleavage of H3 by the viral FMDV 3C protease (Falk et al., 1990). Viral 3C protease-mediated removal of the 20 N-terminal amino acids of H3 may be a mechanism for shutting off host cell transcription (Falk et al., 1990).

These specialized examples suggest that histone tail cleavage may not play a major role in chromatin regulation. However, Duncan et al. (2008) now uncover proteolytic cleavage of histone H3 in mammalian cells, an event that may be important for early stem cell differentiation. In histone preparations from differentiating murine embryonic stem cells (ESCs), Duncan and colleagues observed the appearance of a shorter form of histone H3 lacking the N terminus. This truncated H3 associates with chromatin and appears when expression of the crucial stem cell renewal factor Oct3/4 is lost. Mass spectrometry analysis and sequencing reveal that the truncated form of H3 is derived from the H3.2 variant of histone H3 and is cleaved at several sites between amino acids 21 and 28, with the primary cleavage site between amino acids 21 and 22. Mass spectrometry analysis of a partially purified H3 cleavage activity from differentiating ESCs reveals that cathepsin L, a member of the

papain family of lysosomal proteases, is the protease responsible for the histone cleavage. Although most members of the papain family reside in lysosomes, a nuclear form of cathepsin L has been identified as a regulator of cell cycle progression through proteolytic processing of the CDP/Cux transcription factor (Goulet et al., 2004). Indeed, Duncan and coworkers observe association of cathepsin L with chromatin upon the differentiation of ESCs.

Interestingly, Duncan et al. find that the truncated H3 and the peptides cleaved from its N terminus contain both activating and repressive modification marks. As recombinant cathepsin L had similar H3 cleavage activity to the native protein, the authors used it to test whether histone modifications affect cleavage by cathepsin L. Duncan et al. show that methylation of H3 on K27 increases H3 cleavage. Intriguingly, acetylation of lysine 18 (K18) increases cleavage, whereas K23 acetylation reduces it, indicating that the location of the acetylation has distinct effects on cathepsin L activity. Cathepsin L cleavage reduces the binding nearby of the mouse Polycomb protein (CBX7) to H3 methylated at K27. This suggests that H3 tail cleavage could serve to release Polycomb proteins from K27 methylated H3, thereby derepressing genes in differentiating ESCs (Figure 1D). Thus, adjacent histone modifications may regulate the cleavage of histone H3 by cathepsin L and be regulated in turn by cathepsin L cleavage through the removal of modifications marking the H3 N terminus. It is not yet clear what role cathepsin L cleavage of H3 plays in the differentiation of ESCs. However, a potential role for cathepsin L in the epigenetic regulation of gene expression is supported by the abnormal distribution of heterochromatic and euchromatic marks and factors in cultured cells lacking cathepsin L (Bulynko et al., 2006). Thus, it is possible that cathepsin L and the histone modifications that control its activity play an important role in regulating the distribution of heterochromatic and euchromatic marks across the genome in ways yet to be determined.

The recent progress in understanding crossregulation among histone modifications suggests that there is much more to learn. There are undoubtedly more modifications and mechanisms of recognition to be discovered. Initial paradigms of known modifications are also being challenged by discoveries of new functions for histone modifications in different contexts. There is also the possibility that new combinations of histone modifications will have functions that are distinct from those of the individual modifications alone. Indeed, the histone code seems like a complex language in which we are still studying individual letters (Berger, 2007). This lack of knowledge remains the true limitation in making use of the results of epigenomic projects that map global histone modifications in multiple cell types and organisms.

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