PERSPECTIVE

Transcriptional regulation by histone ubiquitination and deubiquitination

Yi Zhang[1]

Department of Biochemistry and Biophysics, Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, North Carolina 27599, USA

Ubiquitin (Ub) is a 76-amino acid protein that is ubiquitously distributed and highly conserved throughout eukaryotic organisms. Whereas the extreme C-terminal four amino acids are in a random coil, its N-terminal 72 amino acids have a tightly folded globular structure (Vijay-Kumar et al. 1987; Fig. 1A). Since its discovery ~28 years ago (Goldknopf et al. 1975), a variety of cellular processes including protein degradation, stress response, cell-cycle regulation, protein trafficking, endocytosis signaling, and transcriptional regulation have been linked to this molecule (Pickart 2001). Ubiquitylation is proposed to serve as a signaling module, and the information transmitted by this tag may depend on the nature of the modification, such as mono or poly-Ub, or the lysine residues on which the Ub attaches (Di Fiore et al. 2003).

Ub is covalently attached to a target protein through an isopeptide bond between its C-terminal glycine and the ε-amino group of a lysine residue on the acceptor protein. At least four lysine residues (11, 29, 48, and 63) on Ub can serve as attachment sites for further additions of Ub to generate a poly-Ub chain (Pickart 2001). Although mono- and Lys 63-linked diubiquitination have been implicated in endocytosis and DNA repair, the best studied is Lys 48-linked polyubiquitination, which usually targets a tagged protein to the 26S proteasome for degradation (Pickart 2001). Attachment of a Ub molecule to the side chain of a lysine residue in the acceptor protein is a complex process involving multi-enzyme-catalyzed steps including E1 activating, E2 conjugating and E3 ligase enzymes. It is believed that E3 is responsible for specific recognition and ligation of Ub to its substrates. At least four units of linked ubiquitin are required for efficient proteasome recognition and degradation (Pickart 2001).

Recent studies indicate that covalent modifications of histone tails including acetylation, methylation, and phosphorylation play a vital role in regulating chromatin dynamics and gene expression (Strahl and Allis 2000; Jenuwein and Allis 2001). Although identified 28 years ago (Goldknopf et al. 1975), histone ubiquitination remains one of the least understood histone modifications. The fact that histone ubiquitination occurs in the largely monoubiquitinated form and is not linked to degradation, in combination with the lack of information regarding the responsible enzymes, prevented us from understanding the functional significance of this modification. Recent identification of the E2 and E3 proteins involved in H2B ubiquitination (Robzyk et al. 2000; Hwang et al. 2003; Wood et al. 2003a) and the discovery of cross-talk between histone methylation and ubiquitination (Dover et al. 2002; Sun and Allis 2002) have set the stage for functional analysis of histone ubiquitination. In a timely paper published in the previous issue of *Genes & Development*, Shelley Berger and colleagues (Henry et al. 2003) report that Ubp8, a component of the SAGA (Spt–Ada–Gcn5–Acetyltransferase) complex (Grant et al. 1997), is a histone H2B ubiquitin protease. They demonstrate that, unlike other reversible histone modifications in which addition or removal of a group from a histone molecule results in opposing transcriptional effects, sequential ubiquitination and deubiquitination are both involved in transcriptional activation. They provide evidence suggesting that the effect of ubiquitination and deubiquitination signals is likely mediated through histone methylation. These findings establish that sequential ubiquitination and deubiquitination of histones as well as cooperation among different histone modifications play an important role in transcriptional regulation.

Histone ubiquitination

As integral components of chromatin, histones are rich in covalent modifications. In addition to the well-known modifications, such as acetylation (Roth et al. 2001), methylation (Zhang and Reinberg 2001; Lachner and Jenuwein 2002), and phosphorylation (Cheung et al. 2000), histones can also be modified through ubiquitination (Jason et al. 2002). Histone H2A was the first protein identified to be ubiquitinated (Goldknopf et al. 1975). The ubiquitination site has been mapped to the highly conserved residue, Lys 119. (Nickel and Davie 1989). Although ~5%–15% of total H2A has been reported to be ubiquitinated in a variety of higher eukaryotic organisms, ubiquitinated H2A (uH2A) has not been reported in the budding yeast *Saccharomyces cerevisiae* (Robzyk et al. 2000). The majority of uH2A is in monoubiquitinated form; however, polyubiquitinated H2A has also

[1]**Correspondence.**
E-MAIL yi_zhang@med.unc.edu; FAX (919) 966-9673.
Article and publication are at http://www.genesdev.org/cgi/doi/10.1101/gad.1156403.

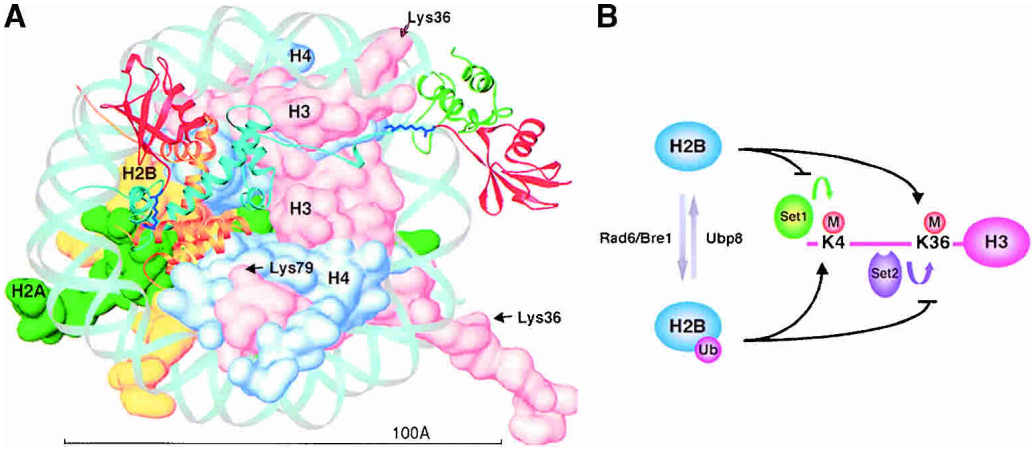

Figure 1. (*A*) Proposed nucleosome structure with ubiquitinated histones. Modeled ubiquitin molecules (red) covalently attached to Lys 119 of histone H2A (cyan) and Lys 120 of histone H2B (gold) are shown as ribbon diagrams. For clarity, only one histone H2A and one H2B are shown in detail. The other H2A (green) and H2B (yellow) are shown in a surface representation together with H3 (pink) and H4 (light blue). The modeled isopeptide bond between the acceptor lysines and Gly 76 of ubiquitin are shown in a stick model (blue). The globular domain of histone H1 is shown in a green ribbon diagram. Location of Lys 36 and Lys 79 of H3 is indicated. The structure is modeled based on previous publications (Vijay-Kumar et al. 1987; Cerf et al. 1994; Goytisolo et al. 1996; Luger et al. 1997). Courtesy of Dr. Rui-Ming Xu. (*B*) Diagram depicting the differential effects of H2B ubiquitination and deubiquitination on H3-K4 and H3-K36 methylation. Enzymes (Rad6/Bre1, Ubp8, Set1, and Set2) that participate in this interplay are indicated.

been detected in many tissues and cell types (Nickel et al. 1989).

In addition to H2A, H2B is also ubiquitinated (West and Bonner 1980). Although uH2B is less abundant (1%–2%) when compared with that of uH2A (5%–15%), it appears to be widely distributed throughout eukaryotic organisms from budding yeast to humans. Like H2A, the ubiquitinated site has also been mapped to lysine residues located at the C terminus of H2B, namely, Lys 120 in human H2B and Lys 123 in yeast H2B, respectively (Thorne et al. 1987). Thus far, only monoubiquitinated H2B has been reported. In addition to H2A and H2B, ubiquitination on H3 and H1 have also been reported (Chen et al. 1998; Pham and Sauer 2000). However, ubiquitination of these two proteins is not as prevalent as that of H2A and H2B. Thus far, the ubiquitination site on H3 or H1 has not been determined.

Enzymes involved in histone ubiquitination and deubiquitination

Histone ubiquitination is a reversible modification, like that of acetylation and phosphorylation. Therefore, the steady-state histone ubiquitination levels are determined by the availability of free ubiquitin and enzymatic activities involved in adding or removing the ubiquitin moiety from histones. Addition of a ubiquitin moiety to a protein involves the sequential action of E1, E2, and E3 enzymes. Removing the ubiquitin moiety, on the other hand, is achieved through the action of enzymes called isopeptidases (Wilkinson 2000). Although ubiquitination of all proteins uses the same E1, different E2s, and especially E3s, appear to be required for ubiquitination of different proteins (Pickart 2001). Two budding yeast proteins, Rad6 and Cdc34, have been shown to be capable of ubiquitinating H2B in vitro without the presence of E3 (Jentsch et al. 1987; Goebl et al. 1988). However, only Rad6 is indispensable for H2B ubiquitination in vivo (Robzyk et al. 2000). Recent studies suggest that a Rad6-associated RING finger protein Bre1 is likely to be the E3 ligase involved in H2B ubiquitination, because mutation in the RING domain of Bre1 abolished H2B ubiquitination in vivo (Hwang et al. 2003; Wood et al. 2003a). Both Rad6 and Bre1 are conserved during evolution. HR6A and HR6B are the two mammalian homologs of yeast Rad6. They are capable of partially complementing mutations of yeast Rad6 in vivo and also can ubiquitinate histones in vitro (Koken et al. 1991). Two putative Bre1 homologs have also been identified in humans (Hwang et al. 2003).

In contrast to the situation concerning H2B ubiquitination, the physiological E2 and E3 enzymes involved in H2A ubiquitination have not been identified. In vitro studies indicate that purified Rad6 can ubiquitinate both H2B and H2A with similar kinetics (Haas et al. 1991); however, it is not clear whether H2A and H2B share the same E2 in vivo. The lack of detectable levels of H2A ubiquitination in yeast prevented the identification of the relevant E2 and E3 enzymes for H2A in this organism. Analysis of uH2A during mouse spermatogenesis revealed a strong correlation between HR6B and uH2A levels (Baarends et al. 1999). However, a defect in the overall uH2A pattern during mouse spermatogenesis was not detected in the HR6B knockout mice (Baarends et al. 1999). This result argues against a major role of HR6B in H2A ubiquitination in vivo, although it could be explained by functional redundancy between HR6A and HR6B. Given that there are also two putative Bre1 homologs in mammals, a conclusive demonstration of either of the two proteins as the E3 ligase for H2A in vivo

will not be simple (Hwang et al. 2003). A systematic biochemical approach coupled with genetic studies in a genetically tractable organism that contains uH2A should shed light on this.

Recent studies revealed that $TAF_{II}250$, a component of the general transcription factor TFIID, possess ubiquitin-activating/conjugating activity for H1 in vitro (Pham and Sauer 2000). This finding is intriguing, as it is the first report that E1 and E2 activities are contained within the same protein. H1 may represent a bona fide in vivo target for $TAF_{II}250$, as point mutations on $TAF_{II}250$ that cripple the H1 ubiquitination activity in vitro also lead to decreases in the level of ubiquitinated H1 in the *Drosophila* embryo (Pham and Sauer 2000). It is interesting to note that the same $TAF_{II}250$ mutations do not cause a general transcriptional defect, suggesting that H1 ubiquitination may participate in transcriptional regulation of a subset of genes, for example, those controlled by the transcriptional factor Dorsal (Pham and Sauer 2000).

The ubiquitin moiety can be removed through hydrolysis of the peptide bond at Gly 76 of the ubiquitin molecule. There are at least 19 proteins in yeast that are members of three families that are able to catalyze this process (D'Andrea and Pellman 1998). The deubiquitinating enzymes (DUB) consist of the ubiquitin C-terminal hydrolases (UCH) and the ubiquitin-specific processing proteases (UBP). In general, the UCH isozymes are papain-like thiol proteases with a 230 amino acid core catalytic domain, whereas the core catalytic domain of UBPs is ~350 amino acids (D'Andrea and Pellman 1998). Budding yeast has 16 UBPs with molecular weight ranging from 50 to 250 kD. The differences among these UBPs reside in the variable N-terminal extensions believed to contribute to substrate specificity (D'Andrea and Pellman 1998). The first UBP to be genetically and biochemically characterized was Ubp4. Mutations in this protein result in a defect in the degradation of the yeast mating factor MATα2 (Swaminathan et al. 1999). Ubp4 mutant cells accumulate polyubiquitin with peptide remnants still attached, indicating that the protein is responsible for removing the polyubiquitin chain from the substrate. In addition, Ubp3 has been shown to associate with Sir4, a protein involved in heterochromatin silencing, although the substrate of this ubiquitin protease is not known (Moazed and Johnson 1996).

In the previous issue of *Genes & Development*, Berger and colleagues (Henry et al. 2003) demonstrate that mutation of Ubp8, a component of the SAGA complex, results in the accumulation of uH2B. Furthermore, SAGA complex purified from wild-type strain, but not from Ubp8 deletion strain, can deubiquitinate H2B in vitro. The effect of Ubp8 on uH2B is specific, as similar effects were not observed in Ubp3 mutants. Importantly, the study established that H2B ubiquitination is a reversible process and removal of ubiquitin is catalyzed by Ubp8 in budding yeast. Equally intriguing is the finding that two histone modification activities (deubiquitination and acetylation) are found in the SAGA complex. Potential Ubp8 homologs exist in a variety of organisms from plants to humans. Whether Ubp8 homologs are involved in H2A deubiquitination remains to be determined.

Role of histone ubiquitination in transcriptional regulation

Accumulating evidence indicates that ubiquitin plays an important role in regulating transcription either through proteasome-dependent destruction of transcription factors or proteasome-independent mechanisms (Conaway et al. 2002). Because transcription occurs in the context of nucleosomes, the effect of histone ubiquitination on transcription had been investigated. Several lines of evidence from early studies suggest that histone ubiquitination may participate in gene activation. For example, it has been reported that nucleosomes of transcriptionally poised hsp 70 genes contain up to 50% uH2A, whereas nucleosomes of nontranscribed satellite DNA contain only one uH2A per 25 nucleosomes (Levinger and Varshavsky 1982). In addition, diubiquitinated H2A was found to be preferentially enriched around the first exon of the actively transcribing mouse dihydrofolate reductase (DHFR) gene (Barsoum and Varshavsky 1985). Furthermore, based on differences in solubility of actively transcribing and silenced chromatin domains, both uH2A and uH2B were found to be enriched around transcriptionally active sequences in bovine thymus, chicken erythrocyte, and *Tetrahymena* macronuclei (Nickel et al. 1989). Finally, transcriptional inhibition experiments have demonstrated that inhibition of rRNA synthesis does not significantly change histone ubiquitination levels, whereas inhibition of hnRNA synthesis almost completely abolished uH2B (Ericsson et al. 1986; Davie and Murphy 1990), suggesting that maintenance of uH2B is dependent on ongoing transcription, especially that of hnRNA.

Although most studies suggest a positive correlation between transcription and histone ubiquitination, different results have also been reported. For example, the active immunoglobulin κ chain gene was found to be packaged with nonubiquitinated histones (Huang et al. 1986). Fractionation of micrococcal nuclease-digested myotube nuclei revealed that uH2A was not enriched in transcriptionally active or inactive chromatin, but simply in nuclease-sensitive fractions (Parlow et al. 1990). Random distribution of uH2A in chromatin fractions was also reported in another study using DNase I sensitivity to differentiate active and inactive transcription regions (Dawson et al. 1991). In addition, ubiquitinated histones have also been found in transcriptionally inactive compartments, such as the *Tetrahymena* micronuclei (Nickel et al. 1989) or the sex body of mouse spermatids (Baarends et al. 1999). Thus, histone ubiquitination most likely regulates gene transcription in a positive and negative fashion, depending on its genomic and gene location (see below).

Identification of Rad6 as an E2 for H2B ubiquitination has opened another avenue for addressing the role of H2B ubiquitination in transcription. Several studies suggest that Rad6-mediated H2B ubiquitination is linked to gene

silencing. Deletion of *RAD6* in budding yeast resulted in defects in telomeric and HML silencing. Importantly, mutant Rad6, containing alterations of the active cysteines that cripple its ubiquitin-conjugating activity, failed to complement the silencing defect, indicating that the ubiquitin-conjugating activity of Rad6 is critical for telomeric and HML silencing (Huang et al. 1997). Recent studies indicate silencing of a euchromatic gene ARG1 also requires a functional Rad6 capable of H2B ubiquitination (Turner et al. 2002). The silencing function of Rad6 appears to be conserved as its fission yeast homolog is also required for mating-type silencing (Naresh et al. 2003). Consistent with a role of H2B ubiquitination in telomeric silencing, methylation of H3-K4 and H3-K79, which are required for telomeric silencing, have been shown to be dependent on Rad6-mediated H2B ubiquitination (Briggs et al. 2002; Dover et al. 2002; Ng et al. 2002; Sun and Allis 2002).

Despite studies linking Rad6-mediated H2B ubiquitination to gene silencing, two recent studies have provided strong evidence suggesting that H2B ubiquitination also play an important role in activation of specific genes. For example, activation of the SAGA regulated genes *GAL1*, *SUG2*, and *PHO5* is at least partially dependent on H2B-K123 ubiquitination (Henry et al. 2003; M.A. Osley, pers. comm.). Using a chromatin double immunoprecipitation (ChDIP) assay, it was demonstrated that activation of *GAL1* gene is accompanied by dynamic changes in H2B ubiquitination levels. The ubiquitinated H2B level at the *GAL1* promoter increases early during activation, and then decreases as mRNA accumulates. This dynamic change in ubiquitinated H2B levels can be explained by the differential binding of Rad6 and Ubp8, enzymes involved in ubiquitination and deubiquitination of H2B, respectively, to the *GAL1* promoter. Binding of Rad6 at the activated *GAL1* promoter appears to be transient, with maximal occupancy at 30–60 min after galactose induction (M.A. Osley, pers. comm.). Binding coincides with the level of ubiquitinated H2B as revealed by ChDIP assay (Henry et al. 2003). In contrast, SAGA binds to the promoter during the whole course of induction (Bhaumik and Green 2001; Larschan and Winston 2001; Bryant and Ptashne 2003). Hence, upon dissociation of Rad6 from the promoter, Ubp8 removes the ubiquitin moiety from H2B. Although Rad6 and Ubp8 are involved in opposing enzymatic reactions, they are both required for optimal activation of the *GAL1* gene. How can two opposing events have the same effect on transcription?

Mechanism of transcriptional regulation by histone ubiquitination and deubiquitination

There are at least three possible explanations of how histone ubiquitination affects transcription. First, histone ubiquitination may affect higher-order chromatin folding, thereby resulting in greater access of the underlying DNA to the transcription machinery. Second, ubiquitination may function as a signal for the recruitment of regulatory molecules that, in turn, affect transcription, in the way that H3-K9 methylation signals the recruitment of the HP1 protein (Bannister et al. 2001; Lachner et al. 2001) The third possibility is that histone ubiquitination affects transcription through its impact on other histone modifications. As discussed below, although the first two possibilities have not been ruled out, recent studies have given the most support for the third possibility.

Because the ubiquitin molecule is about half the size of core histones, it was believed that incorporation of ubiquitinated histones into the nucleosome would impact nucleosome structure and hamper chromatin folding, thus affecting transcription. This notion is compatible with the reported changes of uH2A and uH2B levels during the cell cycle. For example, both uH2A and uH2B have been reported to disappear during the G_2 to M-phase transition when chromatin becomes condensed, but reappear as chromatin decondenses at the M to G_1 transition (Wu et al. 1981). However, biochemical studies reported so far have failed to demonstrate a major role for histone ubiquitination in nucleosome core particle assembly and nucleosome folding (Moore et al. 2002). Earlier in vitro experiments of nucleosome core particle reconstitution indicate that replacement of one or both copies of H2A with uH2A does not affect the rate or pattern of DNase I digestion (Martinson et al. 1979; Kleinschmidt and Martinson 1981). Subsequent studies indicated that core nucleosome particles with a normal structure can be reconstituted when both H2A and H2B are replaced with their ubiquitinated counterparts (Davies and Lindsey 1994). More recent studies indicate that the lack of structural effects of histone ubiquitination apply not only to the mononucleosome, but to oligonucleosome arrays as well (Jason et al. 2001). Incorporation of uH2A into a 12-mer nucleosomal array does not affect nucleosome folding, as judged by quantitative agarose gel electrophoresis and analytical centrifugation. However, uH2A arrays were found to oligomerize at lower $MgCl_2$ concentration than control nucleosomal arrays, suggesting that histone ubiquitination may affect chromatin folding at the chromatin fiber level (Jason et al. 2001). Because K119 of H2A is in close proximity to the linker histone H1 (Fig. 1A), and H1 can be cross-linked to uH1A (Bonner and Stedman 1979), it is possible that histone ubiquitination may affect higher-order chromatin folding through affecting linker histone binding. If the effect of histone ubiquitination on chromatin structure is mostly at the level of the chromatin fiber, the lack of an effect using in vitro reconstituted mono or oligonucleosomes is not surprising, as these nucleosomes do not adopt a higher-order chromatin structure (Hayes and Hansen 2001). The availability of Rad6 and H2B-K123R mutant strains coupled with in vivo footprinting on genes known to be regulated by Rad6 should allow evaluation of the structural role of H2B ubiquitination in vivo.

Whereas the role of histone ubiquitination in chromatin structure is still elusive, recent studies indicate that its role in transcription may be achieved through affecting other histone modifications, such as acetylation and methylation. It was reported that the murine histone deacetylase 6 (mHDAC6) associates with two proteins

implicated in ubiquitination, and that a zinc-finger domain located at the C-terminal of HDAC6 can directly bind to ubiquitin (Seigneurin-Berny et al. 2001), suggesting a potential link between histone ubiquitination and histone acetylation. However, substantial evidence indicates that histone ubiquitination is functionally linked to histone methylation (Fig. 1B). In a seminal study, both the Allis and Shilatifard groups discovered, independently, that Set1-mediated H3-K4 methylation requires functional Rad6 and intact H3-K123 (Dover et al. 2002; Sun and Allis 2002). However, deletion of the *SET1* gene does not affect H2B-K123 ubiquitination, suggesting a unidirectional regulatory pathway in which H2B ubiquitination is upstream of H3-K4 methylation (Sun and Allis 2002). Subsequent studies indicated that H3-K79 methylation is also dependent on Rad6-mediated H2B-K123 ubiquitination. Interestingly, H3-K36 methylation does not appear to have such a requirement (Briggs et al. 2002; Ng et al. 2002).

As discussed above, both positive and negative effects of histone ubiquitination on transcription have been reported. Both can be explained by an impact on histone methylation. Most of the negative effects of histone ubiquitination on transcription was observed in heterochromatic regions such as telomeres, mating type, and rDNA loci. Gene silencing in these regions involves the Sir proteins. Because histone ubiquitination facilitates H3-K4 and H3-K79 methylation, which are preferentially localized in euchromatin regions (Ng et al. 2003a), it has been suggested that H3-K4 and H3-K79 methylation prevents Sir proteins from association with active euchromatic regions, thereby restricting Sir proteins to heterochromatic regions to mediate silencing (van Leeuwen et al. 2002). Hence, in mutant cells deficient in H2B ubiquitination with drastically reduced H3-K4 and H3-K79 methylation, the Sir complex at silenced loci may be destabilized, leading to impaired silencing (Krogan et al. 2003a; Ng et al. 2003b; Wood et al. 2003b).

For the genes located in euchromatic region, H2B ubiquitination appears to result in gene activation. Because the Set1 methylase associates with RNA polymerase II through interaction with the Paf1 elongation complex (Krogan et al. 2003a; Ng et al. 2003c), H2B ubiquitination may participate in transcriptional activation by facilitating H3-K4 methylation and transcriptional elongation.

How, then, is deubiquitination also important for transcription? Berger and colleagues (Henry et al. 2003) proposed an explanation based on the observation that H2B ubiquitination has an opposite effect on H3-K4 and H3-K36 methylation. Whereas H2B ubiquitination facilitates H3-K4 methylation (Dover et al. 2002; Sun and Allis 2002), it down-regulates H3-K36 methylation (Henry et al. 2003). Conversely, whereas H2B deubiquitination reduces H3-K4 methylation levels, it dramatically increases H3-K36 methylation levels (Henry et al. 2003; Fig. 1B). Although both the Set1 and Set2 methylation complexes participate in transcriptional elongation by association with the Paf1 elongation complex (Li et al. 2002; Krogan et al. 2003a,b; Li et al. 2003; Ng et al. 2003c; Xiao et al. 2003), they appear to function at different stages in the transcription elongation cycle. Specifically, Set1-mediated H3-K4 methylation occurs during the early part of the elongation cycle, whereas Set2-mediated H3-K36 methylation occurs during later stages. Thus, it is likely that Set1 and Set2 are both required for proper gene expression. It should also be emphasized that genetic evidence supporting a role of Set1 in transcriptional elongation is still missing. Given the inverse relationship of H2B ubiquitination and deubiquitination on H3-K4 and H3-K36 methylation, it is likely that the transient levels of H2B ubiquitination are essential for the establishment of proper Set1 and Set2 levels on genes.

How would H2B ubiquitin levels control the steady-state levels of H3-K4 and H3-K36 methylation? Recent evidence shows that Set1 and Set2 are recruited to Pol II, at least in part, via the Paf1 elongation complex. Berger and colleagues (Henry et al. 2003) propose that H2B ubiquitin levels might preferentially facilitate the association of Set1 or Set2 to Paf1 complex. How H2B ubiquitination would control the outcome of Set1 and Set2 occupancy at genes is unknown. Perhaps the deubiquitination event is required to trigger the release of Set1 from transcribing genes. Because ubiquitin will not be removed from H2B in *UBP8* strain, Set1 may associate for a longer period with active genes, leading to hypermethylation. On the other hand, it is clear that Set1 recruitment to the 5′ end of active ORF in RAD6 strains does not appear to be affected by the loss of ubiquitinated H2B (Ng et al. 2003c). Assuming that the binding capacity of Set1 or Set2 to the Paf1 complex is limited, more associated Set1 (in the case of the *UBP8* strain) might prevent Set2 from interacting with Paf1 (and vice versa in the case of a H2B K123R mutant strain).

The finding that active deubiquitination occurs during gene activation can also provide an explanation as to why methylated histones are much more abundant than ubiquitinated histones. Transient levels of H2B ubiquitination are important for the establishment of normal Set1 and Set2 levels for histone methylation, through the control of Set1/Set2 association with Pol II. As methylation of histones is considered to be a stable modification, the overall levels of H2B ubiquitination appear very low.

Based on these and other previous studies (Bhaumik and Green 2001; Larschan and Winston 2001; Li et al. 2002; Bryant and Ptashne 2003; Henry et al. 2003; Krogan et al. 2003a,b; Li et al. 2003; Ng et al. 2003c; Xiao et al. 2003; M.A. Osley, pers. comm.), a model for how histone ubiquitination and methylation affect *GAL1* gene activation can be proposed (Fig. 2). Under noninducing condition, the activator protein Gal4 binds to its cognate recognition site within the upstream activating sequence (UAS). Upon induction by galactose, SAGA and Rad6 are recruited by Gal4. Because H2B ubiquitination peaks during the early period of induction (30–60 min), it is likely that Rad6 activity predominates. The catalytic activity of Ubp8 may also be inactive. Binding of Rad6 at the *GAL1* promoter is transient, unlike that of SAGA (M.A. Osley, pers. comm.). Dissociation of Rad6 may also activate Ubp8 deubiquitination activity. Persistence of SAGA (and Ubp8) at the active promoter leads to the

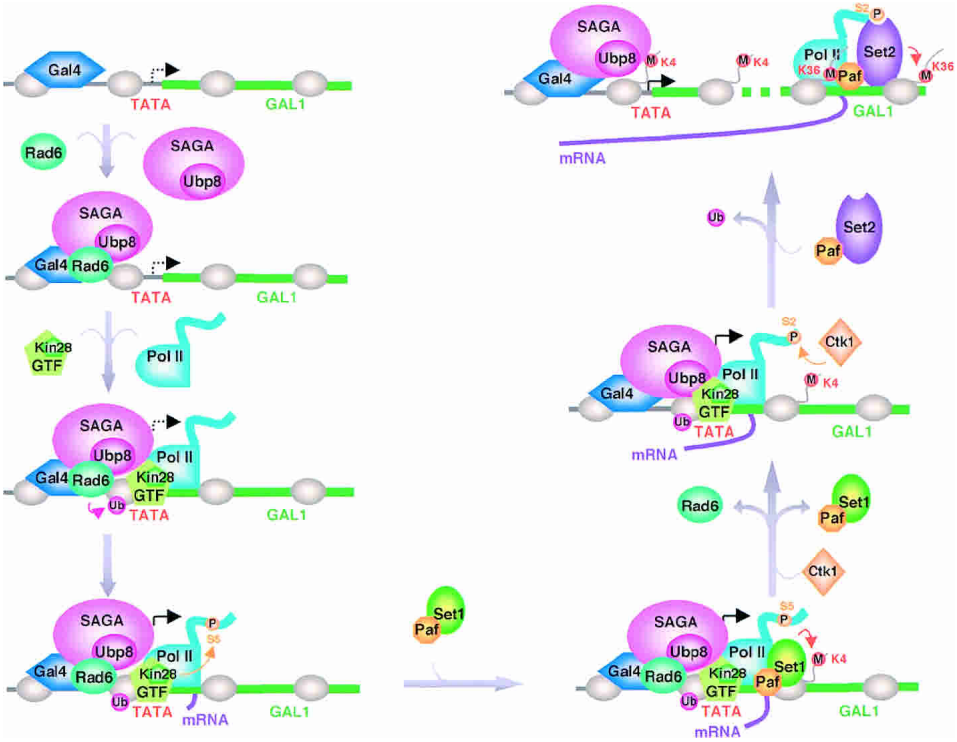

Figure 2. A proposed model depicting the molecular events during transcription of *GAL1* gene. Binding of the transcription factor Gal4 to the GAL1 promoter directly or indirectly recruits Rad6 and the SAGA complex. Then, the SAGA complex recruits RNAPII and the general transcription factors. Phosphorylation of the CTD on Ser 5 by Kin28 subunit of the TFIIH triggers initiation and elongation of transcription concomitant with the recruitment of the Set1 complex through the Paf1 elongation complex. Set1 complex methylates H3-K4 of the nucleosomes in the promoter and 5′ region of the coding region during initiation and early phase of transcriptional elongation. Phosphorylation of the CTD on Ser 2 by Ctk1 may trigger release of Rad6 and Set1 complex from the elongating RNAPII. Ser 2-phosphorylated CTD serves as a docking site for the recruitment of the Set2/Paf complex that travels with the elongating RNAPII resulting in H3-K36 methylation in the coding region. Phosphorylation of Ser 2 may also serves as a signal for deubiquitination of H2B. It is important to note that the exact time for some of the events, such as release of Rad6 from the promoter, deubiquitination of H2B and recruitment of Set2, is not known.

erasure of ubiquitin mark. Based on kinetic studies, it is apparent that SAGA recruitment precedes general transcription factors and RNA pol II binding at the TATA region of the core promoter (Bhaumik and Green 2001; Larschan and Winston 2001; Bryant and Ptashne 2003). Assembly of the basal transcription machinery and the RNA pol II complex followed by phosphorylation at Ser 5 of the RNA pol II C-terminal domain (CTD) by Kin28, a component of the basal transcription factor TFIIH, triggers transcriptional initiation. This event is important for the recruitment of Set1/Paf1 to the early elongating polymerase (Krogan et al. 2003a; Ng et al. 2003c). However, unlike Paf1 and RNA pol II complex, Set1 does not localize to the whole body of the gene. Preferential localization of Set1 at the 5′ end of the gene also creates a localized H3-K4 hypermethylation domain. On the other hand, Set2 is localized throughout the transcribing ORF, and recruitment is dependent on Paf1 as well as Ctk1, an RNA pol II CTD Ser 2 kinase (Li et al. 2002, 2003; Krogan et al. 2003b; Xiao et al. 2003). Interaction between Set2 and phospho-Ser 2 CTD peptide in vitro suggests that Set2 associates with actively transcribed coding regions through association with the elongating RNA pol II. Interestingly, genetic data also suggests that the elongation process may potentially feed back to histone ubiquitination as Rtf1 mutant cells are deficient in this modification (Ng et al. 2003b; Wood et al. 2003b). Although the basic molecular events leading to GAL1 activation are beginning to be revealed, many questions remain to be addressed. For example, why is Ubp8 not functional at early stages of recruitment? When is Rad6 released from the promoter and what are the signals that trigger its release? When is Set2 recruited, and does deubiquitination of H2B trigger this event? Does Paf1 form distinct complexes with Set1 and Set2 independent of RNA pol II, or does Paf1 always binds to elongating RNA pol II and Set1 and Set2 come and go? How does the HAT activity and deubiquitiation activity of SAGA coordinate with each other during transcription? Given the pace of discoveries and functional dissection of novel histone modifying enzymes, it is likely that answers will be uncovered soon.

Acknowledgments

I thank Brain Strahl and Huck Hui Ng for critical reading of the manuscript and Rui-Ming Xu and Qin Feng for help in gener-

ating the figures. I apologize for not being able to quote all references due to limitations in space. Research in my laboratory is supported by NIH and ACS.

References

Baarends, W.M., Hoogerbrugge, J.W., Roest, H.P., Ooms, M., Vreeburg, J., Hoeijmakers, J.H., and Grootegoed, J.A. 1999. Histone ubiquitination and chromatin remodeling in mouse spermatogenesis. *Dev. Biol.* **207:** 322–333.

Bannister, A.J., Zegerman, P., Partridge, J.F., Miska, E.A., Thomas, J.O., Allshire, R.C., and Kouzarides, T. 2001. Selective recognition of methylated lysine 9 on histone H3 by the HP1 chromo domain. *Nature* **410:** 120–124.

Barsoum, J. and Varshavsky, A. 1985. Preferential localization of variant nucleosomes near the 5′-end of the mouse dihydrofolate reductase gene. *J. Biol. Chem.* **260:** 7688–7697.

Bhaumik, S.R. and Green, M.R. 2001. SAGA is an essential in vivo target of the yeast acidic activator Gal4p. *Genes & Dev.* **15:** 1935–1945.

Bonner, W.M. and Stedman, J.D. 1979. Histone 1 is proximal to histone 2A and to A24. *Proc. Natl. Acad. Sci.* **76:** 2190–2194.

Briggs, S.D., Xiao, T., Sun, Z.W., Caldwell, J.A., Shabanowitz, J., Hunt, D.F., Allis, C.D., and Strahl, B.D. 2002. Gene silencing: Trans-histone regulatory pathway in chromatin. *Nature* **418:** 498.

Bryant, G.O. and Ptashne, M. 2003. Independent recruitment in vivo by Gal4 of two complexes required for transcription. *Mol. Cell* **11:** 1301–1309.

Cerf, C., Lippens, G., Ramakrishnan, V., Muyldermans, S., Segers, A., Wyns, L., Wodak, S.J., and Hallenga, K. 1994. Homo- and heteronuclear two-dimensional NMR studies of the globular domain of histone H1: Full assignment, tertiary structure, and comparison with the globular domain of histone H5. *Biochemistry* **33:** 11079–11086.

Chen, H.Y., Sun, J.M., Zhang, Y., Davie, J.R., and Meistrich, M.L. 1998. Ubiquitination of histone H3 in elongating spermatids of rat testes. *J. Biol. Chem.* **273:** 13165–13169.

Cheung, P., Allis, C.D., and Sassone-Corsi, P. 2000. Signaling to chromatin through histone modifications. *Cell* **103:** 263–271.

Conaway, R.C., Brower, C.S., and Conaway, J.W. 2002. Emerging roles of ubiquitin in transcription regulation. *Science* **296:** 1254–1258.

D'Andrea, A. and Pellman, D. 1998. Deubiquitinating enzymes: A new class of biological regulators. *Crit. Rev. Biochem. Mol. Biol.* **33:** 337–352.

Davie, J.R. and Murphy, L.C. 1990. Level of ubiquitinated histone H2B in chromatin is coupled to ongoing transcription. *Biochemistry* **29:** 4752–4757.

Davies, N. and Lindsey, G.G. 1994. Histone H2B (and H2A) ubiquitination allows normal histone octamer and core particle reconstitution. *Biochim. Biophys. Acta* **1218:** 187–193.

Dawson, B.A., Herman, T., Haas, A.L., and Lough, J. 1991. Affinity isolation of active murine erythroleukemia cell chromatin: Uniform distribution of ubiquitinated histone H2A between active and inactive fractions. *J. Cell. Biochem.* **46:** 166–173.

Di Fiore, P.P., Polo, S., and Hofmann, K. 2003. When ubiquitin meets ubiquitin receptors: A signalling connection. *Nat. Rev. Mol. Cell. Biol.* **4:** 491–497.

Dover, J., Schneider, J., Tawiah-Boateng, M.A., Wood, A., Dean, K., Johnston, M., and Shilatifard, A. 2002. Methylation of histone H3 by COMPASS requires ubiquitination of histone H2B by Rad6. *J. Biol. Chem.* **277:** 28368–28371.

Ericsson, C., Goldknopf, I.L., and Daneholt, B. 1986. Inhibition of transcription does not affect the total amount of ubiquitinated histone 2A in chromatin. *Exp. Cell. Res.* **167:** 127–134.

Goebl, M.G., Yochem, J., Jentsch, S., McGrath, J.P., Varshavsky, A., and Byers, B. 1988. The yeast cell cycle gene CDC34 encodes a ubiquitin-conjugating enzyme. *Science* **241:** 1331–1335.

Goldknopf, I.L., Taylor, C.W., Baum, R.M., Yeoman, L.C., Olson, M.O., Prestayko, A.W., and Busch, H. 1975. Isolation and characterization of protein A24, a "histone-like" nonhistone chromosomal protein. *J. Biol. Chem.* **250:** 7182–7187.

Goytisolo, F.A., Gerchman, S.E., Yu, X., Rees, C., Graziano, V., Ramakrishnan, V., and Thomas, J.O. 1996. Identification of two DNA-binding sites on the globular domain of histone H5. *EMBO J.* **15:** 3421–3429.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., et al. 1997. Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: Characterization of an Ada complex and the SAGA (Spt/Ada) complex. *Genes & Dev.* **11:** 1640–1650.

Haas, A.L., Reback, P.B., and Chau, V. 1991. Ubiquitin conjugation by the yeast RAD6 and CDC34 gene products. Comparison to their putative rabbit homologs, E2(20K) AND E2(32K). *J. Biol. Chem.* **266:** 5104–5112.

Hayes, J.J. and Hansen, J.C. 2001. Nucleosomes and the chromatin fiber. *Curr. Opin. Genet. Dev.* **11:** 124–129.

Henry, K.W., Wyce, A., Lo, W.-S., Duggan, L.J., Emre, N.C.T., Kao, C.-F., Pillus, L., Shilatifard, A., Osley, M.A., and Berger, S.L. 2003. Transcriptional activation via sequential histone H2B ubiquitylation and deubiquitylation, mediated by SAGA-associated Ubp8. *Genes & Dev.* **17:** 2648-2663.

Huang, H., Kahana, A., Gottschling, D.E., Prakash, L., and Liebman, S.W. 1997. The ubiquitin-conjugating enzyme Rad6 (Ubc2) is required for silencing in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **17:** 6693–6699.

Huang, S.Y., Barnard, M.B., Xu, M., Matsui, S., Rose, S.M., and Garrard, W.T. 1986. The active immunoglobulin κ chain gene is packaged by non-ubiquitin-conjugated nucleosomes. *Proc. Natl. Acad. Sci.* **83:** 3738–3742.

Hwang, W.W., Venkatasubrahmanyam, S., Ianculescu, A.G., Tong, A., Boone, C., and Madhani, H.D. 2003. A conserved RING finger protein required for histone H2B monoubiquitination and cell size control. *Mol. Cell* **11:** 261–266.

Jason, L.J., Moore, S.C., Ausio, J., and Lindsey, G. 2001. Magnesium-dependent association and folding of oligonucleosomes reconstituted with ubiquitinated H2A. *J. Biol. Chem.* **276:** 14597–14601.

Jason, L.J., Moore, S.C., Lewis, J.D., Lindsey, G., and Ausio, J. 2002. Histone ubiquitination: A tagging tail unfolds? *BioEssays* **24:** 166–174.

Jentsch, S., McGrath, J.P., and Varshavsky, A. 1987. The yeast DNA repair gene RAD6 encodes a ubiquitin-conjugating enzyme. *Nature* **329:** 131–134.

Jenuwein, T. and Allis, C.D. 2001. Translating the histone code. *Science* **293:** 1074–1080.

Kleinschmidt, A.M. and Martinson, H.G. 1981. Structure of nucleosome core particles containing uH2A (A24). *Nucleic Acids Res.* **9:** 2423–2431.

Koken, M.H., Reynolds, P., Jaspers-Dekker, I., Prakash, L., Prakash, S., Bootsma, D., and Hoeijmakers, J.H. 1991. Structural and functional conservation of two human homologs of the yeast DNA repair gene RAD6. *Proc. Natl. Acad. Sci.* **88:** 8865–8869.

Krogan, N.J., Dover, J., Wood, A., Schneider, J., Heidt, J., Boateng, M.A., Dean, K., Ryan, O.W., Golshani, A., Johnston, M., et al, 2003a. The Paf1 complex is required for his-

tone H3 methylation by COMPASS and Dot1p: Linking transcriptional elongation to histone methylation. *Mol. Cell* **11:** 721–729.

Krogan, N.J., Kim, M., Tong, A., Golshani, A., Cagney, G., Canadien, V., Richards, D.P., Beattie, B.K., Emili, A., Boone, C., et al. 2003b. Methylation of histone H3 by Set2 in *Saccharomyces cerevisiae* is linked to transcriptional elongation by RNA polymerase II. *Mol. Cell. Biol.* **23:** 4207–4218.

Lachner, M. and Jenuwein, T. 2002. The many faces of histone lysine methylation. *Curr. Opin. Cell. Biol.* **14:** 286–298.

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., and Jenuwein, T. 2001. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature* **410:** 116–120.

Larschan, E. and Winston, F. 2001. The *S. cerevisiae* SAGA complex functions in vivo as a coactivator for transcriptional activation by Gal4. *Genes & Dev.* **15:** 1946–1956.

Levinger, L. and Varshavsky, A. 1982. Selective arrangement of ubiquitinated and D1 protein-containing nucleosomes within the *Drosophila* genome. *Cell* **28:** 375–385.

Li, B., Howe, L., Anderson, S., Yates III, J.R., and Workman, J.L. 2003. The Set2 histone methyltransferase functions through the phosphorylated carboxyl-terminal domain of RNA polymerase II. *J. Biol. Chem.* **278:** 8897–8903.

Li, J., Moazed, D., and Gygi, S.P. 2002. Association of the histone methyltransferase Set2 with RNA polymerase II plays a role in transcription elongation. *J. Biol. Chem.* **277:** 49383–49388.

Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F., and Richmond, T.J. 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389:** 251–260.

Martinson, H.G., True, R., Burch, J.B., and Kunkel, G. 1979. Semihistone protein A24 replaces H2A as an integral component of the nucleosome histone core. *Proc. Natl. Acad. Sci.* **76:** 1030–1034.

Moazed, D. and Johnson, D. 1996. A deubiquitinating enzyme interacts with SIR4 and regulates silencing in *S. cerevisiae*. *Cell* **86:** 667–677.

Moore, S.C., Jason, L., and Ausio, J. 2002. The elusive structural role of ubiquitinated histones. *Biochem. Cell. Biol.* **80:** 311–319.

Naresh, A., Saini, S., and Singh, J. 2003. Identification of Uhp1, a ubiquitinated histone-like protein, as a target/mediator of Rhp6 in mating-type silencing in fission yeast. *J. Biol. Chem.* **278:** 9185–9194.

Ng, H.H., Xu, R.M., Zhang, Y., and Struhl, K. 2002. Ubiquitination of histone H2B by Rad6 is required for efficient Dot1-mediated methylation of histone H3 lysine 79. *J. Biol. Chem.* **277:** 34655–34657.

Ng, H.H., Ciccone, D.N., Morshead, K.B., Oettinger, M.A., and Struhl, K. 2003a. Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: A potential mechanism for position-effect variegation. *Proc. Natl. Acad. Sci.* **100:** 1820–1825.

Ng, H.H., Dole, S., and Struhl, K. 2003b. The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J. Biol. Chem.* **278:** 33625–33628.

Ng, H.H., Robert, F., Young, R.A., and Struhl, K. 2003c. Targeted recruitment of Set1 histone methylase by elongating Pol II provides a localized mark and memory of recent transcriptional activity. *Mol. Cell* **11:** 709–719.

Nickel, B.E. and Davie, J.R. 1989. Structure of polyubiquitinated histone H2A. *Biochemistry* **28:** 964–968.

Nickel, B.E., Allis, C.D., and Davie, J.R. 1989. Ubiquitinated histone H2B is preferentially located in transcriptionally active chromatin. *Biochemistry* **28:** 958–963.

Parlow, M.H., Haas, A.L., and Lough, J. 1990. Enrichment of ubiquitinated histone H2A in a low salt extract of micrococcal nuclease-digested myotube nuclei. *J. Biol. Chem.* **265:** 7507–7512.

Pham, A.D. and Sauer, F. 2000. Ubiquitin-activating/conjugating activity of $TAF_{II}250$, a mediator of activation of gene expression in *Drosophila*. *Science* **289:** 2357–2360.

Pickart, C.M. 2001. Mechanisms underlying ubiquitination. *Annu. Rev. Biochem.* **70:** 503–533.

Robzyk, K., Recht, J., and Osley, M.A. 2000. Rad6-dependent ubiquitination of histone H2B in yeast. *Science* **287:** 501–504.

Roth, S.Y., Denu, J.M., and Allis, C.D. 2001. Histone acetyltransferases. *Annu. Rev. Biochem.* **70:** 81–120.

Seigneurin-Berny, D., Verdel, A., Curtet, S., Lemercier, C., Garin, J., Rousseaux, S., and Khochbin, S. 2001. Identification of components of the murine histone deacetylase 6 complex: Link between acetylation and ubiquitination signaling pathways. *Mol. Cell. Biol.* **21:** 8035–8044.

Strahl, B.D. and Allis, C.D. 2000. The language of covalent histone modifications. *Nature* **403:** 41–45.

Sun, Z.W. and Allis, C.D. 2002. Ubiquitination of histone H2B regulates H3 methylation and gene silencing in yeast. *Nature* **418:** 104–108.

Swaminathan, S., Amerik, A.Y., and Hochstrasser, M. 1999. The Doa4 deubiquitinating enzyme is required for ubiquitin homeostasis in yeast. *Mol. Biol. Cell* **10:** 2583–2594.

Thorne, A.W., Sautiere, P., Briand, G., and Crane-Robinson, C. 1987. The structure of ubiquitinated histone H2B. *EMBO J.* **6:** 1005–1010.

Turner, S.D., Ricci, A.R., Petropoulos, H., Genereaux, J., Skerjanc, I.S., and Brandl, C.J. 2002. The E2 ubiquitin conjugase Rad6 is required for the ArgR/Mcm1 repression of ARG1 transcription. *Mol. Cell. Biol.* **22:** 4011–4019.

van Leeuwen, F., Gafken, P.R., and Gottschling, D.E. 2002. Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* **109:** 745–756.

Vijay-Kumar, S., Bugg, C.E., and Cook, W.J. 1987. Structure of ubiquitin refined at 1.8 A resolution. *J. Mol. Biol.* **194:** 531–544.

West, M.H. and Bonner, W.M. 1980. Histone 2B can be modified by the attachment of ubiquitin. *Nucleic Acids Res.* **8:** 4671–4680.

Wilkinson, K.D. 2000. Ubiquitination and deubiquitination: Targeting of proteins for degradation by the proteasome. *Semin. Cell. Dev. Biol.* **11:** 141–148.

Wood, A., Krogan, N.J., Dover, J., Schneider, J., Heidt, J., Boateng, M.A., Dean, K., Golshani, A., Zhang, Y., Greenblatt, J.F., et al. 2003a. Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol. Cell* **11:** 267–274.

Wood, A., Schneider, J., Dover, J., Johnston, M., and Shilatifard, A. 2003b. The Paf1 complex is essential for histone monoubiquitination by the Rad6–Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. *J. Biol. Chem.* **278:** 34739–34742.

Wu, R.S., Kohn, K.W., and Bonner, W.M. 1981. Metabolism of ubiquitinated histones. *J. Biol. Chem.* **256:** 5916–5920.

Xiao, T., Hall, H., Kizer, K.O., Shibata, Y., Hall, M.C., Borchers, C.H., and Strahl, B.D. 2003. Phosphorylation of RNA polymerase II CTD regulates H3 methylation in yeast. *Genes & Dev.* **17:** 654–663.

Zhang, Y. and Reinberg, D. 2001. Transcription regulation by histone methylation: Interplay between different covalent modifications of the core histone tails. *Genes & Dev.* **15:** 2343–2360.

Transcriptional regulation by histone ubiquitination and deubiquitination

Yi Zhang

Genes Dev. 2003, **17:**
Access the most recent version at doi:10.1101/gad.1156403