Histone modifications in transcriptional regulation
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Covalent modifications of the amino termini of the core histones in nucleosomes have important roles in gene regulation. Research in the past two years reveals these modifications to consist of phosphorylation, methylation and ubiquitination, in addition to the better-characterized acetylation. This multiplicity of modifications, and their occurrence in patterns and dependent sequences, argues persuasively for the existence of a histone code.

Introduction
It is now difficult to recall that a scant five years ago many scientists working in the field of gene regulation believed chromatin was not a central player. Key observations that changed this view were that promoter-associated coactivators and corepressors possessed histone acetylation and deacetylation activity, respectively [1]. Since then, the amount of related research has been staggering, including investigations into identities of histone acetyltransferases (HATs), recognition of other histone modifications, and explorations of mechanisms (e.g. see [2,3] for review). Moreover, understanding HAT/histone deacetylase (HDAC) function has become a useful paradigm for other modifications that are just now being discovered.

A unifying concept in this field is that of a histone code, which posits that the totality of modifications, both in kind and number, dictate a particular biological outcome [4,5]. Strongly supporting the histone code hypothesis is evidence for several different covariant modifications, including acetylation, phosphorylation, methylation and ubiquitination (Figure 1a), all involved in gene-specific regulation. In addition, documentation of patterns and order of modification events points to a code (Figure 1b). These ideas culminate in the recognition of ‘off’ and ‘on’ states, characterized by alternative histone modifications. Although beyond the scope of this review, there is an emerging correlation with modifications, such as acetylation, of DNA-binding transcription factors themselves, which causes increased association with HATs for histone acetylation, indicating the existence of modification cascades [6,7]. It is also important to point out that the transcriptional HATs also have an apparent non-transcriptional role in establishing the global genomic balance of acetylation [8–11].

As the outcome of histone modifications has been examined, two non-exclusive models have emerged. One is that histone modifications affect chromatin structure directly. The second model is that modifications present a special surface for interaction with other proteins. Either model can be reconciled with the histone code hypothesis, both may operate simultaneously, and both have great explanatory power regarding the relationship between histone modification and gene control.

Histone acetylation
While the identities of histone methyltransferases (HMTs), HKs and HUs are still being worked out (see below), many HATs are now known. The most consistent functional characteristic of the HATs is that they are transcriptional coactivators (i.e. they do not bind directly to DNA but rather with DNA-binding activators). The fact that HATs are coactivators rather than DNA-binding moieties underscores the need for flexibility, regulation and alternative strategies in regulating chromatin and the basal transcriptional machinery.

Many of the HATs are components of large multisubunit complexes, recruited to promoters by interaction with DNA-bound activator proteins [12]. Two well-studied examples are yeast SAGA/human PCAF/Gcn5 and the yeast NuA4/human Tip60 complexes, which both have transcriptional roles [13,14]. Previous observations have indicated that there are several interaction surfaces within the complexes for association with activators. New data indicates that the Tra1/TRRAP protein is most likely the predominant direct target of activators [15,16]. The ‘Tra1 role is logical, as it is the only conserved subunit between the SAGA and NuA4 complexes, which both interact with activators. The subunit composition of the NuA4-related Tip60 complex in humans indicates that it may have an additional direct role in DNA repair/apoptosis [17].

Before their rebirth as histone acetylation ‘delivery’ vehicles, there was a wealth of evidence that many of the proteins later identified in HAT complexes possessed ‘classic’ coactivator activity, that is, they promote ‘TATA-binding protein (TPB) or other general transcription factor association with the basal promoter [18]. Harking back to these models are new experiments showing that SAGA has non-chromatin-dependent coactivator activity — that is, at some promoters that do not require Gcn5’s acetylation.
activity, other components of the SAGA complex are required for full transcription by way of TBP recruitment [19**,20**]. Thus, the complexes are modular, wherein chromatin modification and TBP recruitment are distinct, non-overlapping and independent functions [21].

It is evident that histone modification and ATP-dependent chromatin remodeling are functionally connected for gene regulation as their activities are required at the same promoters in *Saccharomyces cerevisiae*. However, an important issue is whether there is an actual mechanistic interrelationship between them. One indication of this is that the same activators interact with both SAGA and the ATP-dependent Swi/Snf family of chromatin remodeling enzyme complexes [22], providing coordinated promoter recruitment. Recent investigations have also examined the relative timing of recruitment of each class of enzymes. A priori, it is not predictable whether alteration of chromatin structure would precede or follow chemical modification of histone tails, and interestingly, both paradigms seem to operate. In *S. cerevisiae*, for genes that are transcribed during mitosis when the genome is highly condensed, it appears that Swi/Snf-dependent remodeling occurs before Gcn5-dependent histone acetylation, and is required for acetylation to occur [22,23,24**], suggesting that the HAT complexes cannot penetrate closed chromatin without initial remodeling (Figure 2a). However, for other, highly inducible cell cycle independent genes, histone acetylation appears to occur in advance of ATP-dependent remodeling (Figure 2b). These include the IFN-β and hormone receptor dependent genes in mammals [25*,26*], and the PHO8 gene in *S. cerevisiae* [27*]. *In vitro*, acetylated templates provide more stable Swi/Snf complex binding [28*]. The order of events has been particularly well-characterized at the IFN-β promoter: binding of activators to the promoter initiates recruitment of the Gcn5 complex and histone acetylation, which stimulates CREB-binding protein (CBP)/RNA polymerase recruitment, followed by CBP-dependent recruitment of Swi/Snf complex, leading to ATP-dependent sliding of a nucleosome near the TATA box, culminating in TBP binding [25*]. Finally, it is interesting that *in vitro*, VDJ recombination is stimulated in a concerted fashion by acetylation and Swi/Snf remodeling [29], and thus these activities most likely act together during genomic processes other than transcription.

Exemplifying the maturing study of HATs are reports on regulation of HAT activity. A novel broad-spectrum HAT–inhibitor dependent protein complex was isolated from human cells [30]. Two related studies show an unexpected role of DNA-binding factors in modulating the HAT activity of CBP and PCAF, in addition to simple recruitment [31,32]. Although the identity and mechanism of action has dominated the field of chromatin modification in the past five years, the next stage of discovery will be the biological role of these enzymes in higher eukaryotes, including connection to disease. Mouse nulls have been constructed for
the homologous HATs, Gcn5 and PCAF. Whereas the PCAF null is normal, Gcn5 has an essential role in early embryonic development that is different from that of the p300 HAT, suggesting separate functions [33•,34]. An interesting connection was made between the Alzheimer’s protein APP and the Tip60 MYST family HAT, in that a cleaved portion of APP and the HAT form a complex to activate as yet unknown target genes [35••]. A second neurodegenerative disease in humans, manifest in the Drosophila melanogaster model system, is caused by polyglutamine repeat expansion in certain proteins such the Huntington’s disease-related abnormal protein Htt. These polyglutamine repeats inhibit HAT activity to manifest the mutant phenotype, which can be ameliorated with HDAC inhibitor treatment [36••]. It is certain that in the near future a great many human diseases will be linked to HATs and other histone modifications.

Histone phosphorylation
Histone phosphorylation involving Ser-10 of histone H3 has also emerged as an important modification, both in transcriptional activation and in chromosome condensation during mitosis [37]. As chromosome condensation and transcription are expected to involve opposing physical alterations of chromatin (i.e. closing of chromatin during mitosis and opening during transcription), the finding that the same modification is involved in both processes is circumstantial support for the modifications-as-binding surfaces rather than direct alteration of chromatin (see below).

Initial studies showed that histone phosphorylation has a role in transcriptional induction of immediate early genes in mammalian cells, such as the c-Fos gene [38]. The Rsk/Msk families of kinases may cause the phosphorylation directly [39,40]. A transcriptionally-linked histone kinase has been identified in S. cerevisiae as the previously known Snf1 kinase [41••]. The identity of these HKs as previously known transcription-associated factors suggests that they may be recruited to specific promoters as coactivators, much like the HATs and Swi/Snf complexes discussed above. Finally, heat-shock gene induction in Drosophila is accompanied by dramatic increases in histone H3 Ser-10 phosphorylation [42•].

Histone methylation
There are two types of histone methylation, targeting either arginine or lysine residues. Histone arginine methylation is involved in gene activation and, again, methylases are recruited to promoters as coactivators. These are the CARM1/PRMT1 family of HMTs, and they predominantly target either H3 or H4, respectively [43,44•]. The role of the SET domain family of lysine HMTs in heterochromatic gene silencing is very exciting. The heterochromatic Suvar3-9 enzyme in mammalian cells was the first to be shown to have this activity in vitro, to methylate Lys-9 of histone H3 [45•], and the modification targets a well-known silencing protein, called HP1, to heterochromatin [46,47,48•]. Two additional advances are conceptual leaps forward. First, the Suvar39 mechanism is used for gene-specific repression, where it is recruited by corepressors, such as by the tumor suppressor and corepressor Rb (retinoblastoma protein; see Kouzarides, this issue Figure 2).
Histone ubiquitination

Two recent developments indicate that histone ubiquitylation is joining the ranks of important modifications. First, in *S. cerevisiae*, Lys-123 within the H2B carboxy-terminal tail is a substrate for the Rad6 ubiquitin ligase [53]. This modification is critical to mitotic and meiotic growth, although it is not yet clear whether it is involved in transcription. Second, TafII250 in the TBP-associated complex TFIID has been shown to possess histone H1 ubiquitylation activity [54], adding to its long list of enzymatic activities (kinase and HAT activities), which may be involved in transcription.

Patterns, codes and models

Thus, specific modifications correlate with specific transcriptional states. In particular, histone H3 appears to be critical: known marks occur at Lys-4 (methylation), Lys-9 (methylation), Ser-10 (phosphorylation), Lys-14 (acetylation) and Arg-17 (methylation). In fact, around K9/S10/K14 in histone H3 there appear to be specific patterns for inactivity and activity (Figure 1b). An inactive state is characterized by histone deacetylation at Lys-14, which precedes methylation at Lys-9. The enzymes that carry out these modifications are genetically linked in *Schizosaccharomyces pombe*, and deacetylation at Lys-14 precedes methylation at Lys-9 [51••]. In contrast, acetylation at Lys-14 is preceded by, and dependent upon, phosphorylation at Ser-10. This has been shown in *vitro* for the Gcn5 acetyltransferase [55,56], and in *vivo* the Snf1 histone kinase and Gcn5 are a linked pair of enzymes that operate in this sequence [56]. In addition, another transcriptional ‘on’ state within histone H4 consists of methylation at Arg-3 preceding and promoting p300-mediated acetylation at Lys-8 and Lys-12 [44•]. Thus, specific and interlinked modifications may dictate specific genomic states, such as gene activation, repression, DNA repair, recombination, chromosome segregation, and so on.

The function of the histone code may be to cause specific changes in activity at the affected loci. These changes could be direct physical alteration of the chromatin, either on a single nucleosome scale to alter histone–DNA contacts, or within a higher order of structure. There is recent genetic evidence for direct electrostatic effects of modifications in *Tetrahymena*, where the essential function provided by acetylation of amino-terminal tails of the variant H2A histone (H2AZ) is caused by direct charge alterations, rather than the presence of the actual modification [57•]. Other evidence for direct changes are that the amino-terminal tails appear to be the main regulators of transcription factor access to DNA [58], and that acetylation itself alters the accessibility of DNA for protein binding [59•].

A second possibility is that the modifications create altered surfaces on nucleosomes for interaction with effector proteins that are the actual agents of altered activity. Evidence supporting this model is that certain modifications generate stronger interactions in *vitro* with domains of chromatin-associated proteins and correlate in *vivo* with recruitment of these proteins (Figure 1a). Examples include the bromodomain, present in HATs, which has been shown to specifically interact with acetylated lysine. The interaction of Gcn5 with Lys-14 [60•] likely leads to reinforcement of acetylation during gene activation, and the interaction of the double bromodomain of Taf250 with dual acetylated histone H3 or H4 [61] may assist the binding of TFIID to nucleosomes around the TATA box. In *S. cerevisiae*, substitutions in the bromodomains associated with TFIID show synthetic phenotypes with mutations in acetylable lysines in histone H4 (S. Buratowski, personal communication) arguing for a functional link. A second example is the bromodomain, present in numerous HMTs and other proteins, which in some cases binds to the Lys-9-methylated histone H3 tail. Suvar3-9 methylates Lys-9 during gene silencing, and this leads to increased association of the chromodomain-containing protein HP1, long known to be important in heterochromatic silencing. These data predict the existence of other domains for interaction with the other known, and as yet uncharacterized, histone modifications. For example, the SANT and PHD fingers are additional chromatin-associated domains whose ‘partner’ modifications are not yet identified.

Conclusions

The initial finding that histone acetylation is a regulatory step involved in gene activation has now expanded in many ways. First, histone phosphorylation, methylation and ubiquitylation have each been correlated with gene activation or repression. Future revelations will identify new modifications of specific residues in the tails of histones H3 and H4, and will likely indicate modifications in both the amino and extended carboxy-terminal tails of H2A and H2B. Second, there is an interconnection and interdependence of modifications in the H3 and H4 tails, suggesting specific modification ‘states’ characteristic of transcriptional activation or repression. Intriguing questions are whether dependent sequences will extend beyond two modifications, and whether modification on the tails of different histones—which may be adjacent in chromatin—may influence one another. In general, the number and patterns of modifications, and their apparent correlation with specific states of transcription support the existence of an epigenetic code on the histone terminal tails. Third, specific modifications, such as acetylation and methylation, promote the binding of specialized chromatin domains, such as the bromodomain or chromodomain. To date, it is not clear how general these observations will be: for example, does lysine acetylation invariably lead to bromodomain interaction, and does each modification have a partner chromatin domain?

Finally, it is important to emphasize that the alternative mechanistic models for the function of histone modifications
described above — either direct conformational or interaction-surfaces — are useful for experimental design but could well both be biologically relevant. Like all good models, they are intended to stimulate thought and experimental work but reality may incorporate and transcend them both.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest •• of outstanding interest

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Two mouse null lines of homologues of yeast Gcn5 have been created; one deleted for PCAF and the second for Gcn5. The results indicate that Gcn5 is critical for mesoderm lines in early development and loss causes embryonic lethality, whereas PCAF loss has no apparent phenotype. In addition, one of these knockouts, that of Gcn5, which is a physically interacting HAT. Thus acetyltransferases have distinct functional roles in mammals.


The APP protein, associated with Alzheimer’s disease, is normally membrane-associated and cleaved into extracellular — the region that accumulates into plaques — and intracellular domains. The intracellular (nuclear) domain associates into a complex with Tip60 and activates transcription, suggesting that one role of APP is to alter the chromatin of target genes.


Previous observations indicated that members of the SET domain family of proteins are histone lysine methyltransferases targeting Lys-9 of histone H3. This report extends these observations to show that Lys-9 is methylated at in the heterochromatin region of the mating type locus in S. pombe, and mutation of Clr4, the SET homologue of the Suvar3-9 histone methyltransferase, eliminat- ed the methylation and correspondingly lowered gene silencing. This is the first clear genetic demonstration of Lys-9 methylation and its importance in vivo.


This was the first report of histone lysine methylation in vitro by a specific enzyme, Suvar3-9, which was previously correlated with heterochromatin silencing. This finding opened up the idea that lysine methylation is regulatory, a notion that is of current intense interest.


This paper extends the role of histone H3 Lys-9 methylation to gene-specific repression. The authors demonstrate that the tumor suppressor and corepressor Rb associates with the promoter to target methylation of Lys-9 of histone H3, and HP1, to the promoter. This suggests that gene specific repression mechanisms may generally involve not only histone deacetylation, but also histone methylation.


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Previous observations indicate that the CARM1/PRMT1 family of protein arginine methyltransferases are transcriptional coactivators and methylate histones in vitro. In this study, histones are shown to be methylated in vivo, suggesting that arginine methylation of histones is physiologically relevant. It is shown that acetylation of Lys-8 in histone H4 into vitro promotes methyla- tion of Arg-3. The linkage of these modifications is another example of co- modification during gene activation, supporting the histone code hypothesis.

52. Litt MD, Simpson M, Gaszner M, Allis CD, Felsenfeld G: Correlation between histone lysine methylation and developmental changes at the chicken beta-globin locus. Science 2001, 293:2453-2455. This paper shows that modification of histone H3 becomes altered at the chicken β-globin locus during mouse development. Developmental changes in the large-scale pattern across the locus of K4-methylation mimics the pattern of K14-acetylation, and thus marks an ‘active’ code. In contrast, the K9-methylation pattern is in direct opposition across the locus and correlates with condensed chromatin, thus representing an ‘inactive’ code. See also annotation [51••].


57. Ren Q, Gorovsky MA: Histone H2A.Z acetylation modulates an essential charge patch. Mol Cell 2001, 7:1329-1335. This report shows that charge alteration caused by histone acetylation is itself responsible for the essential acetylation phenotype in the H2A.Z amino terminal tail. The authors alter the lysines that are acetylated in histone H2A.Z to mimic the charge manifested by acetylation, and observe the identical phenotype as caused by acetylation itself.


59. Anderson JD, Lowary PT, Widom J: Effects of histone acetylation on the equilibrium accessibility of nucleosomal DNA target sites. J Mol Biol 2001, 307:977-985. This report shows that histone acetylation alone causes increased association of proteins with their target sites in nucleosomal DNA. This indicates that the charge alteration caused by acetylation is sufficient to allow binding of proteins, supporting the hypothesis that the charge alteration by acetylation, is per se responsible for chromatin alteration.
