The language of covalent histone modifications

Brian D. Strahl & C. David Allis

Department of Biochemistry and Molecular Genetics, University of Virginia Health Science Center, Charlottesville, Virginia 22908, USA

Histone proteins and the nucleosomes they form with DNA are the fundamental building blocks of eukaryotic chromatin. A diverse array of post-translational modifications that often occur on tail domains of these proteins has been well documented. Although the function of these highly conserved modifications has remained elusive, converging biochemical and genetic evidence suggests functions in several chromatin-based processes. We propose that distinct histone modifications, on one or more tails, act sequentially or in combination to form a 'histone code' that is, read by other proteins to bring about distinct downstream events.

How eukaryotic genomes are manipulated within a chromatin environment is a fundamental issue in biology. At the heart of chromatin structure are highly conserved histone proteins (H3, H4, H2A, H2B and H1) that function as building blocks to package eukaryotic DNA into repeating nucleosomal units that are folded into higher-order chromatin fibres[1,2] (Fig. 1). Once thought of as static, non-participating structural elements, it is now clear that histones are integral and dynamic components of the machinery responsible for regulating gene transcription. The same is probably true for other DNA-templated processes such as replication, repair, recombination and chromosome segregation.

An extensive literature documents an elaborate collection of post-translational modifications including acetylation, phosphorylation, methylation, ubiquitination and ADP-ribosylation[3] that take place on the 'tail' domains of histones. These tails, which protrude from the surface of the chromatin polymer and are protease sensitive, comprise ~25–30% of the mass of individual histones[3,4], thus providing an exposed surface for potential interactions with other proteins (for example, Sir3/4 and Tup1 proteins in yeast[5,6]). Because of the inherent disordered nature of histone tails, their precise location in higher-order fibres and the atomic details of their structure are not known[1,7].

Long-standing models have suggested that histone modifications may alter chromatin structure by influencing histone–DNA and histone–histone contacts[4,8]. However, growing awareness of the remarkable diversity and biological specificity associated with distinct patterns of covalent histone marks has caused us and others[9–13] to favour the view that a histone 'language' may be encoded on these tail domains that is read by other proteins or protein modules. We refer to this language as the 'histone code' and present evidence supporting the existence of this language and discuss some potential ramifications. To illustrate the potential complexity of covalent marks decorating a single histone tail, we have chosen to focus most of our discussion on a short stretch of core histone H3. However, many of the concepts presented here are likely to apply to all of the histone termini and, in particular, to that of histone H4[10,14].

Lysine acetylation sets the stage

Of the modifications listed above, histone acetylation has been the most studied and appreciated[14]. Fuelled, in part, by the discovery of enzymes responsible for bringing about the steady-state balance of this modification—histone acetyltransferases (HATs) and histone deacetylase (HDACs)—compelling evidence has recently been provided that acetylation of specific lysine residues in the amino termini of the core histones plays a fundamental role in transcriptional regulation[2,15].

In H3 from most species, the main acetylation sites include lysines 9, 14, 18 and 23 (refs 3, 16), and, as is the case with the functionally redundant tail of its nucleosomal partner, H4[14], selected lysines become acetylated during specific cellular processes (Figs 1b and 2). Transcription-linked acetylation, catalysed by the GCN5 family of HATs, shows a preference for lysine 14 of H3 *in vitro*[17] although an expanded set of lysine residues is likely to be used *in vivo*[18,19]. How is this acetylation site specificity in H3 brought about?

Solution and crystal structure data of various members of the GCN5 HAT family, including co-crystals of the enzyme with H3 tail peptides[20], have begun to yield important insights into the enzymatic mechanisms underlying the site specificity of these HATs[21–25]. One important concept to emerge from these studies is that residues outside the preferred lysine 14 acetylation site in H3 are important for histone-binding specificity. For example, glycine 13 and proline 16 have a critical role in leading to a restricted GCN5–H3 peptide recognition site, G-K14*-X-P (ref. 20). Thus, as is the case with protein kinases and phosphatases, short preferred consensus motifs are likely to exist for individual HATs and HDACs[26] which help to establish the final histone code.

Acetylation of specific lysine residues in H3 is also associated with biological processes apart from transcription (Fig. 2). During DNA replication, for example, new histones are rapidly synthesized and assembled onto the replicated DNA. H3 and H4 are brought to replicating chromatin in a pre-acetylated state that becomes erased after replication is completed and the newly assembled chromatin matures[27,28]. Whereas the sites of deposition-related H4 acetylation are highly conserved[29,30] (for example, lysines 5 and 12; see Fig. 2), the situation with H3 is less clear. However, lysine 9 in H3 appears to have a more dominant role in histone deposition and chromatin assembly in some organisms[17,27,30]. The finding that a chromatin assembly complex in *Drosophila*, called RCAF (for replication-coupling assembly factor), contains H4 specifically acetylated at lysines 5 and 12 suggests that these acetylation sites play an important role in chromatin assembly[31]. Does this acetylation pattern represent a code that has been deciphered by a component of a histone chaperone complex?

Finally, we note that the spacing between acetylatable lysines (Fig. 1b) is strikingly regular in the amino termini of many histones (for example, lysines at 9, 14, 18 and 23 in H3; and 5, 8, 12 and 16 in H4), and, curiously, this spacing periodicity is reminiscent of that of an α-helix (that is, 3.6 residues). To our knowledge, no group has systematically attempted to expand or contract the characteristic three-to-four residue spacing between many known acetylation sites. Along this line, the alternating, and seemingly, invariant pattern of deposition-related acetylation wherein lysines 5 and 12, but not lysines at 8 and 16, are acetylated in newly synthesized H4 is particularly intriguing[29,30] (Fig. 2).

Beyond histone acetylation

Phosphorylation, particularly that of histones H1 and H3, has long been implicated in chromosome condensation during mitosis[32,33]. However, converging evidence suggests that H3 phosphorylation

(specifically serine 10; see Fig. 1b) is also directly correlated with the induction of immediate-early genes such as c-*jun*, c-*fos* and *c-myc*[34–36]. Mutations in Rsk-2, recently shown to be an H3 kinase *in vitro*, are associated with Coffin–Lowry syndrome in humans and result in a loss of epidermal growth factor-stimulated H3 phosphorylation *in vivo*[9,37]. Transcriptional activation in response to mitogenic and other stimuli are altered in Coffin–Lowry cells[38], suggesting a potential direct role for H3 phosphorylation in regulating gene transcription through a remodelling step that is most consistent with chromatin decondensation, a result seemingly at odds with the use of this mark in chromosome condensation.

The potential importance of the serine 10 phosphorylation mark in H3 is strengthened by the finding that MSK1, a kinase activated by growth factor and stress stimuli, also phosphorylates H3 *in vitro*[39]. Interestingly, another H3 kinase has recently been identified that is associated with dosage compensation in flies[40]. In *Drosophila*, equalization of transcription from the sex chromosomes is achieved by a twofold upregulation of transcription from the male X chromosome[41] that is associated with acetylation of H4 at lysine 16[42]. Thus, H4 acetylation on lysine 16, possibly in concert with H3 phosphorylation at serine 10, may establish a combinatorial mark that leads to enhanced transcription from the male X chromosome.

What about other histone modifications?

Just as histone acetylation and phosphorylation have become topics of renewed interest, we expect other known histone modifications will soon be back in the limelight. Methylation of lysine and/or arginine residues, for example, are among the least understood post-translational modifications affecting histones. This is partly because the responsible enzymes are not known, immunological reagents selective to detect histone methylation do not exist, and, unlike histone acetylation and phosphorylation, the charge on the individual lysine and arginine residues is not greatly affected, making electrophoretic resolution of methylated histones difficult. H3 and H4 are the predominant histones modified by methylation, and sequencing studies from many organisms indicate that multiple lysines in H3 (4, 9 and 27) are the preferred sites of methylation[3,43,44]. Moreover, lysine residues can be mono- di- or trimethylated, adding yet another layer of complexity to this histone mark.

The recent discovery of a nuclear receptor co-activator-interacting protein, called CARM1, which possesses arginine-specific, histone H3-selective methyltransferase (HMT) activity[45] provides evidence to support the notion that histone methylation contributes to transcriptional activation. CARM1 HMT activity is required for ligand-dependent transcriptional activation, and the finding that CARM1 functions through association with co-activators containing HAT activity is particularly intriguing given that Rsk-2 interacts with the transcriptional co-activator CREB-binding protein (CBP)[46]. These data suggest that large multisubunit enzyme complexes containing multiple histone- and non-histone-modifying activities[9,47] work in concert with other chromatin remodelling machines[48,49] to regulate gene transcription (Fig. 3, left panel).

The 'histone code' hypothesis

Considering only the electrostatic requirements for folding the chromatin polymer[4,8,50], histone acetylation, through the neutralization of positive charge, and histone phosphorylation, through the addition of negative charge, would probably cause decondensation of the chromatin fibre[51]. Thus, the use of multiple marks on histone tails (that is, combining acetylation and phosphorylation) could serve to amplify the readout of upstream signalling pathways causing greater changes in the overall charge density of tails that

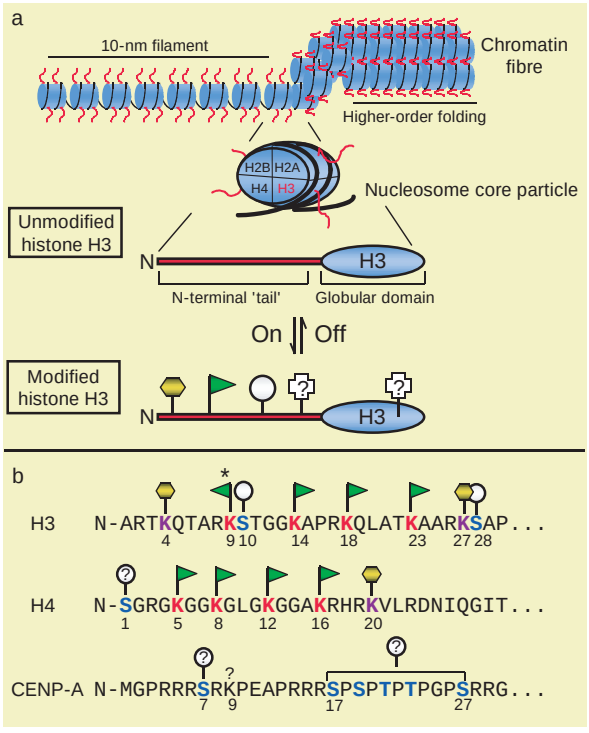

Figure 1 Chromatin organization and the tail of histone H3. **a**, General chromatin organization. Like other histone 'tails', the N terminus of H3 (red) represents a highly conserved domain that is likely to be exposed or extend outwards from the chromatin fibre. A number of distinct post-translational modifications are known to occur at the N terminus of H3 including acetylation (green flag), phosphorylation (grey circle) and methylation (yellow hexagon). Other modifications are known and may also occur in the globular domain. **b**, The N terminus of human H3 is shown in single-letter amino-acid code. For comparison, the N termini of human CENP-A, a centromere-specific H3 variant, and human H4, the nucleosomal partner to H3, are shown. Note the regular spacing of acetylatable lysines (red), and potential phosphorylation (blue) and methylation (purple) sites. The asterisk indicates the lysine residue in H3 that is known to be targeted for acetylation as well as for methylation; lysine 9 in CENP-A (bold) may also be chemically modified (see text). The above depictions of chromatin structure and H3 are schematic; no attempt has been made to accurately portray these structures.

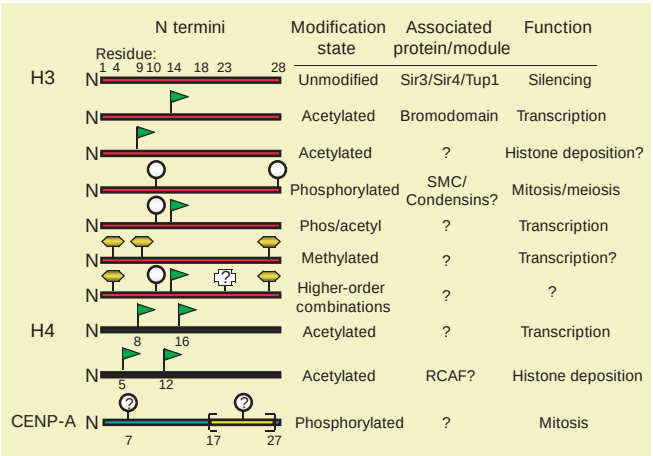

Figure 2 The 'histone code' hypothesis. Histone modifications occur at selected residues and some of the patterns shown have been closely linked to a biological event (for example, acetylation and transcription). Emerging evidence suggests that distinct H3 (red) and H4 (black) tail modifications act sequentially or in combination to regulate unique biological outcomes. How this hierarchy of multiple modifications extends (depicted as 'higher-order combinations') or how distinct combinatorial sets are established or maintained in localized regions of the chromatin fibre is not known. Relevant proteins or protein domains that are known to interact or associate with distinct modifications are indicated. The CENP-A tail domain (blue) might also be subjected to mitosis-related marks such as phosphorylation; the yellow bracket depicts a motif in which serines and threonines alternate with proline residues.

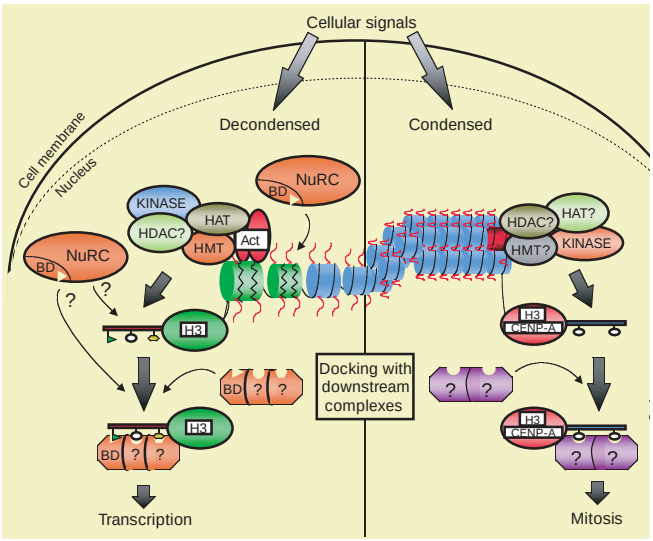

Figure 3 Coordinated recruitment of histone-modifying activities. Recent discoveries suggest that distinct histone-modifying activities interact to form multisubunit complexes that probably work in concert with nucleosome remodelling complexes (NuRCs; for example, Swi/Snf, RSC, NURF) to remodel chromatin. Interactions demonstrated thus far include CARM1, a histone methyltransferase (HMT), with histone acetyltransferase (HAT)-containing coactivators that interact with nuclear receptors (Act)[45] and Rsk kinase with the CBP/p300 HAT[46]. Not depicted is the possibility of non-histone substrates being *bona fide* targets of these activities. In addition to remodelling nucleosomes (indicated by zigzag DNA), NuRCs may chemically modify and/or bind histone tails. Binding of a NURC or HAT complex (depicted as single ovals) to histone tails may be mediated by the bromodomain (BD). Although many of the complexes identified to date are implicated in events leading to transcription (left panel; green nucleosomes), we speculate that similar, but unique, complexes may exist that modify and direct chromatin condensation (right panel; red nucleosome). This 'code' of modifications may dictate the biological outcome through changes caused in higher-order chromatin structure or may direct the downstream biological effect by recruiting and interacting with docking proteins or complexes that remain to be identified. Although H3 and CENP-A are the only histones depicted here, it is likely that all other histones are subjected to this type of regulation.

lead to greater changes in the chromatin structure of target genes. Indeed, evidence supports synergism between histone acetylation and phosphorylation in the induction of immediate-early genes after mitogenic stimulation[52], and it seems likely that these signalling pathways may converge at other loci as well[35].

H3 phosphorylation at serine 10, possibly in conjunction with phosphorylation at serine 28 (Figs 1b and 2; and see below), is also required for proper segregation and condensation of chromosomes during mitosis and meiosis[53,54]. If the function of H3 phosphorylation is to 'open' chromatin, how then can H3 phosphorylation at the same site also be involved in chromosome condensation? This question seems to beg a simple answer: perhaps a single histone modification does not function alone. We will refer to the hypothesis—that multiple histone modifications, acting in a combinatorial or sequential fashion on one or multiple histone tails, specify unique downstream functions—as the histone code hypothesis. What is the evidence in support of this hypothesis?

Serine 28 in H3 is embedded in surrounding sequences similar to serine 10 (that is, both are R-K-S*), and data has shown that serine 28 is phosphorylated during chromosome condensation in mammalian cells[55] (Fig. 1b). Whether serine 28 is phosphorylated during interphase or during immediate-early gene induction is not yet known. Thus, the formal possibility remains that multiple phosphorylation events on the same histone tail, or on several tails, may be required for efficient chromosome condensation during mitosis and meiosis (see below).

Even if the H3 tail is doubly marked by serine 10 and serine 28 phosphorylation during mitosis, it seems likely that this is not the complete story regarding chromosome condensation. H3 phosphorylation at serine 10 initiates in the pericentric heterochromatin, an A/T-rich region of satellite DNA closely associated with centromeric DNA[53]. Centromeric DNA itself is packaged with specialized proteins, one of which is a specialized H3 'variant' found in both yeast and humans, CENP-A[56]. CENP-A differs from H3 primarily in its unique N-terminal tail (Fig. 1b). Apart from lysine 9, arginine residues comprise all other positively charged side chains. Thus, the role of acetylation at lysine 9 (if it occurs) probably differs from that of acetylation of canonical H3 in association with transcription. Moreover, the CENP-A tail contains many serine and threonine residues, which raises the possibility that this specialized H3, like the main H3, becomes phosphorylated during mitosis. An alternating S/T/G-P motif repeats five times in this tail generating an 11-amino-acid stretch flanked on either side by arginine residues (Fig. 1b). This motif suggests that CENP-A may be phosphorylated during mitosis, and it will be interesting to determine whether the CENP-A and H3 proteins are substrates for the same or unique sets of kinases and phosphatases. It would also be of interest to determine whether the SMC/condensing proteins, which have a central role in mitotic chromosome condensation, bind to these tails and contain histone-modifying activities[57] (Fig. 2).

The enzymology of multiple histone modifications

The existence of multiple modifications within a short stretch of the same histone tail (Fig. 1b), begs the question: how is a complex, multimark code established and maintained in the first place? One attractive hypothesis is that covalent modification of a histone tail by one enzyme influences the rate or efficiency with which a second enzyme follows using the now-modified histone tail as substrate. Does site-specific phosphorylation or methylation influence the ability of a HAT to recognize and bind to the tail? Alternatively, does a histone kinase or methylase care whether a histone tail is acetylated at a specific lysine residue? To that end, we point out that many modifications are close enough to each other on the histone tail (Fig. 1b) to influence, positively or negatively, the ability of enzymes to further modify these residues. Along this line, modifications on one histone tail might influence the outcome of other enzymatic activities acting on other histone tails.

The explosion of recent discoveries of histone-modifying enzymes, many available in recombinant form, paves the way for future experimental tests of some of these questions. Structural studies with modified histone substrates will be necessary to determine which residues, if any, are used to stabilize or promote interactions with modified substrates. Site-directed mutagenesis of these residues, followed by *in vivo* and *in vitro* assays, will help to

dissect meaningful functional relationships.

How is the histone code read?

Could histone modifications exist simply to regulate chromatin structure? Core histone acetylation alone has been shown to relax higher-order chromatin structure *in vitro*, and to promote factor-binding to cognate DNA elements[4,8]. Alternatively, histone modifications could also act as specific 'receptors' to recruit unique biological complexes that mediate downstream function (Figs 2 and 3). Phosphorylation of H3 at serine 10 is closely associated with both chromosome condensation during mitosis and immediate-early gene induction following mitogenic stimulation. One possible explanation for this discrepancy is shown in Fig. 3. Here we envisage that a phosphorylation mark alone, or in combination with other marks (such as phosphorylation at serine 28), may recruit a binding factor that, in turn, has a role in mediating chromosome condensation and segregation. In contrast, a distinct mark or set of marks (for example, phosphorylation and acetylation at residues 10 and 14, respectively) may provide a unique binding surface to recruit factors promoting decondensation and transcription (for example, Swi/Snf; see below). Assuming that phosphorylation of CENP-A occurs during mitosis, this marked tail may provide an attractive binding surface for a kinase that carries out general H3 phosphorylation at serine 10 and/or serine 28. In the specific case of the CENP-A tail (Fig. 1b), clusters of arginine residues are in some cases interrupted by serine residues (for example, R-R-R-S*-R-K) where the marked serine is serine 7. Phosphorylation at these positions may possibly serve to modify interactions with proteins that recognize this basic patch in the CENP-A tail.

One appealing feature of the histone code hypothesis is that it offers a possible explanation for 'exceptions' to the general rule that histone acetylation correlates positively with gene activation, whereas histone deacetylation acts to create repressive chromatin. For example, recent studies on the mouse mammary tumour virus (MMTV) promoter suggest that histone acetylation is actually involved in transcriptional repression (T. K. Archer and C. L. Smith, personal communication). Similarly, mutations in the HDAC homologue RPD3 cause enhancement of position effect variegation in flies, not suppression as would first be expected[58]. Along these lines, pericentric heterochromatin in flies[42] and silent loci in yeast[59] are marked by acetylation of lysine 12 of H4.

The disparity of having histone acetylation linked to both gene activation and repression is reminiscent of the situation with histone H3 phosphorylation being linked to both chromosome condensation and immediate-gene induction. Part of the solution to this paradox may be in having unique histone codes read by distinct sets of proteins that then bring about different downstream responses. If correct, it may be that mitosis-specific HATs, HDACs and HMTs act during chromosome condensation and that distinct sets of histone-modifying enzymes mark chromatin for decondensation during gene activation (Fig. 3).

Who reads the code?

Direct evidence that H3 and H4 tails can act as specific 'receptors' has been provided for Sir3 and Sir4[5] and Tup1/Ssn6 (ref. 6) proteins involved in transcriptional silencing and repression in yeast. Moreover, Tup1 binding is influenced by the acetylation state of the H3 and H4 N-terminal tails: unacetylated or monoacetylated H3 and H4 are more strongly bound by Tup1 compared with hyperacetylated H3 or H4, suggesting that alterations in tail structure and/or charge due to acetylation can modulate non-histone protein/tail binding interactions. Whether or not other histone marks (such as phosphorylation, methylation) regulate these interactions further remains an important issue for future studies.

Recent evidence shows that the bromodomain of human PCAF (P300/CBP-associated factor), a domain of little known function which is shared between many, but not all HATs, binds acetylated lysine in the context of H3 and H4 tail sequences[60]. This result suggests that protein motifs may have evolved to recognize histone modifications[61] (Fig. 3). Precedence for this type of receptor–ligand interaction already exists in nature. Phosphorylated tyrosine, for example, is known to be read in specific contexts by SH2-containing modules that, in turn, have an impact on downstream biological events[62]. Have similar mechanisms evolved for lysine acetylation, as well as other covalent histone modifications[61]?

Relevant to this discussion may be the observation that the spacing of acetylatable lysines in the N termini of many histones is regular. Is this spacing part of the histone recognition motif? To that end, we note that many bromodomain-containing proteins have two adjacent bromodomain modules (for example, $TAF_{II}250$). Whether in these proteins each bromodomain functions independently or synergistically to bind acetylated lysines, in one or more histone tails, is not known, but remains an intriguing possibility.

If bromodomains bind acetylated histones, then this suggests that other chromatin-associated polypeptides containing this domain may also function through recognition and binding to specific acetylation patterns on histones (Fig. 3). For example, components of the Swi/Snf and RSC (for remodels the structure of chromatin) family of ATP-dependent chromatin remodelling enzymes contain bromodomains[61]. Is it possible that these remodelling complexes function through a combination of factor recruitment[48] and recognition of distinct acetylation patterns on the histone tails at promoters regulated by these complexes[49]? Some support for this is provided by genetic evidence that suggests that Swi/Snf function is partially redundant with the functions of Gcn5-containing HAT complexes at specific promoters[63–65] and by studies that show that ATP-dependent remodelling complexes like NURF (nucleosome remodelling factor) require histone tails to function properly[66].

Additionally, could the interdependency of Swi/Snf remodelling complexes with HATs be due, in part, to the fact that each is capable of leaving different covalent marks on the chromatin fibre as part of its remodelling function? In so doing, are signals laid down on the histone tails that recruit the next remodelling complex? It is known, for example, that Gcn5-containing HAT complexes are recruited to specific promoters in yeast after the recruitment of Swi/Snf[48,49]. Could this recruitment partly be caused by the ability of Swi/Snf to leave a mark for HATs to see? It will be of interest to determine whether any nucleosome remodelling complexes contain enzymatic activities that leave covalent marks on histones.

Parallels in nature and conclusions

Like chromatin, microtubules are polymers composed of highly conserved subunits, (α- and β-tubulin) that heterodimerize to form the repeating unit of this cytoskeletal fibre. Tubulins also have 'tail' domains that are decorated by a diverse array of post-translational modifications, some of which are in common with histones (acetylation and phosphorylation)[67,68]. Like histone tails, the microtubule tails, which are located in the C terminus, also lack a defined structure at atomic resolution[69] but are known to be recognized by microtubule-associated proteins (MAPs)—polypeptides thought to impart dynamic features to the polymer. We wonder whether unique combinations of post-translational modifications exist on tubulins that modulate their function as in chromatin. The apparent parallels between these two types of cellular polymers are intriguing and suggest that a general theme is used by nature to regulate the dynamics of large polymers. In both cases, the complexity and potential redundant nature of these covalent marks may underlie the general difficulty in obtaining clear phenotypes in mutational analyses of known modification sites.

In summary, the large network of post-translational modifications that decorates histone tails appears to represent a mechanism for differential regulation of chromatin activity in several distinct biological settings. The histone code described here is by no means deciphered, and we have begun to consider the staggering possi-

bility that every amino acid in histone tails has specific meaning and is part of the vocabulary of the overall code. The realization that histone variants, such as the centromere-associated protein CENP-A, exist in special chromosomal locations adds yet another level of variation to the chromatin fibre and the histone code. The recent track record suggests the continued need to: (1) identify and map the sites for the complete dictionary of covalent histone modifications in all histones; (2) mutate and identify phenotypes associated with each of the modifications, singly and in combination; (3) identify and characterize the enzymes systems that add or subtract these modifications; (4) determine how complexes containing these activities are recruited to key genomic targets; and (5) learn how these covalent marks specify interactions with downstream partners or modulate higher-order structures. After a long incubation period, interest in covalent modifications of histones is at an all-time high. Understanding the rules and the consequences of this histone code is likely to impact on many, if not all, DNA-templated process with far-reaching implications for human biology and disease. □

1. Luger, K. & Richmond, T. J. The histone tails of the nucleosome. *Curr. Opin. Genet. Dev.* **8,** 140–146 (1998).
2. Kornberg, R. D. & Lorch, Y. Twenty-five years of the nucleosome, fundamental particle of the eukaryotic chromosome. *Cell* **98,** 285–294 (1999).
3. van Holde, K. E. in *Chromatin* (ed. Rich, A.) 111–148 (Springer, New York, 1988).
4. Wolffe, A. P. & Hayes, J. J. Chromatin disruption and modification. *Nucleic Acids Res.* **27,** 711–720 (1999).
5. Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S. M. & Grunstein, M. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. *Cell* **80,** 583–592 (1995).
6. Edmondson, D. G., Smith, M. M. & Roth, S. Y. Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. *Genes Dev.* **10,** 1247–1259 (1996).
7. Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* **389,** 251–260 (1997).
8. Hansen, J. C., Tse, C. & Wolffe, A. P. Structure and function of the core histone N-termini: more than meets the eye. *Biochemistry* **37,** 17637–17641 (1998).
9. Mizzen, C. *et al.* Signaling to chromatin through histone modifications: how clear is the signal? *Cold Spring Harb. Symp. Quant. Biol.* **63,** 469–481 (1998).
10. Turner, B. M. Decoding the nucleosome. *Cell* **75,** 5–8 (1993).
11. Lopez-Rodas, G. *et al.* Histone deacetylase. A key enzyme for the binding of regulatory proteins to chromatin. *FEBS Lett.* **317,** 175–180 (1993).
12. Loidl, P. Histone acetylation: facts and questions. *Chromosoma* **103,** 441–449 (1994).
13. Tordera, V., Sendra, R. & Perez-Ortin, J. E. The role of histones and their modifications in the informative content of chromatin. *Experientia* **49,** 780–788 (1993).
14. Grunstein, M. Histone acetylation in chromatin structure and transcription. *Nature* **389,** 349–352 (1997).
15. Struhl, K. Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.* **12,** 599–606 (1998).
16. Thorne, A. W., Kmiciek, D., Mitchelson, K., Sautiere, P. & Crane-Robinson, C. Patterns of histone acetylation. *Eur. J. Biochem.* **193,** 701–713 (1990).
17. Kuo, M. H. *et al.* Transcription-linked acetylation by Gcn5p of histones H3 and H4 at specific lysines. *Nature* **383,** 269–272 (1996).
18. Grant, P. A. *et al.* Expanded lysine acetylation specificity of Gcn5 in native complexes. *J. Biol. Chem.* **274,** 5895–5900 (1999).
19. Zhang, W., Bone, J. R., Edmondson, D. G., Turner, B. M. & Roth, S. Y. Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase. *EMBO J.* **17,** 3155–3167 (1998).
20. Rojas, J. R. *et al.* Structure of Tetrahymena GCN5 bound to coenzyme A and a histone H3 peptide. *Nature* **401,** 93–98 (1999).
21. Tanner, K. G. *et al.* Catalytic mechanism and function of invariant glutamic acid 173 from the histone acetyltransferase GCN5 transcriptional coactivator. *J. Biol. Chem.* **274,** 18157–18160 (1999).
22. Trievel, R. C. *et al.* Crystal structure and mechanism of histone acetylation of the yeast GCN5 transcriptional coactivator. *Proc. Natl Acad. Sci. USA* **96,** 8931–8936 (1999).
23. Clements, A. *et al.* Crystal structure of the histone acetyltransferase domain of the human PCAF transcriptional regulator bound to coenzyme A. *EMBO J.* **18,** 3521–3532 (1999).
24. Lin, Y., Fletcher, C. M., Zhou, J., Allis, C. D. & Wagner, G. Solution structure of the catalytic domain of GCN5 histone acetyltransferase bound to coenzyme A. *Nature* **400,** 86–89 (1999).
25. Sternglanz, R. & Schindelin, H. Structure and mechanism of action of the histone acetyltransferase gcn5 and similarity to other *N*-acetyltransferases. *Proc. Natl Acad. Sci. USA* **96,** 8807–8808 (1999).
26. Kimura, A. & Horikoshi, M. How do histone acetyltransferases select lysine residues in core histones? *FEBS Lett.* **431,** 131–133 (1998).
27. Turner, B. M. & O'Neill, L. P. Histone acetylation in chromatin and chromosomes. *Semin. Cell Biol.* **6,** 229–236 (1995).
28. Annunziato, A. T. in *The Nucleus* (ed. Wolffe, A. P.) 31–56 (JAI, Greenwich, Connecticut, 1995).
29. Allis, C. D., Chicoine, L. G., Richman, R. & Schulman, I. G. Deposition-related histone acetylation in micronuclei of conjugating Tetrahymena. *Proc. Natl Acad. Sci. USA* **82,** 8048–8052 (1985).
30. Sobel, R. E., Cook, R. G., Perry, C. A., Annunziato, A. T. & Allis, C. D. Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc. Natl Acad. Sci. USA* **92,** 1237–1241 (1995).
31. Tyler, J. K. *et al.* The RCAF complex mediates chromatin assembly during DNA replication and repair. *Nature* **402,** 555–560 (1999).
32. Bradbury, E. M. Reversible histone modifications and the chromosome cell cycle. *Bioessays* **14,** 9–16 (1992).
33. Koshland, D. & Strunnikov, A. Mitotic chromosome condensation. *Annu. Rev. Cell Dev. Biol.* **12,** 305–333 (1996).
34. Mahadevan, L. C., Willis, A. C. & Barratt, M. J. Rapid histone H3 phosphorylation in response to growth factors, phorbol esters, okadaic acid, and protein synthesis inhibitors. *Cell* **65,** 775–783 (1991).
35. Thomson, S., Mahadevan, L. C. & Clayton, A. L. MAP kinase-mediated signalling to nucleosomes and immediate-early gene induction. *Semin. Cell Dev. Biol.* **10,** 205–214 (1999).
36. Chadee, D. N. *et al.* Increased Ser-10 phosphorylation of histone H3 in mitogen-stimulated and oncogene-transformed mouse fibroblasts. *J. Biol. Chem.* **274,** 24914–24920 (1999).
37. Sassone-Corsi, P. *et al.* Requirement of Rsk-2 for epidermal growth factor-activated phosphorylation of histone H3. *Science* **285,** 886–891 (1999).
38. De Cesare, D., Jacquot, S., Hanauer, A. & Sassone-Corsi, P. Rsk-2 activity is necessary for epidermal growth factor-induced phosphorylation of CREB protein and transcription of c-fos gene. *Proc. Natl Acad. Sci. USA* **95,** 12202–12207 (1998).
39. Thomson, S. *et al.* The nucleosomal response associated with immediate-early gene induction is mediated via alternative MAP kinase cascades: MSK1 as a potential histone H3/HMG-14 kinase. *EMBO J.* **18,** 4779–4793 (1999).
40. Jin, Y. *et al.* JIL-1: a novel chromosomal tandem kinase implicated in transcriptional regulation in *Drosophila*. *Mol. Cell* **4,** 129–135 (1999).
41. Lucchesi, J. C. Dosage compensation in flies and worms: the ups and downs of X-chromosome regulation. *Curr. Opin. Genet. Dev.* **8,** 179–184 (1998).
42. Turner, B. M., Birley, A. J. & Lavender, J. Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in Drosophila polytene nuclei. *Cell* **69,** 375–384 (1992).
43. von Holt, C. *et al.* Isolation and characterization of histones. *Methods Enzymol.* **170,** 431–523 (1989).
44. Strahl, B. D., Ohba, R., Cook, R. G. & Allis, C. D. Methylation of histone H3 at lysine 4 is highly conserved and correlates with transcriptionally active nuclei in *Tetrahymena*. *Proc. Natl Acad. Sci. USA* **96,** 14967–14972 (1999).
45. Chen, D. *et al.* Regulation of transcription by a protein methyltransferase. *Science* **284,** 2174–2177 (1999).
46. Nakajima, T. *et al.* The signal-dependent coactivator CBP is a nuclear target for pp90RSK. *Cell* **86,** 465–474 (1996).
47. Berger, S. L. Gene activation by histone and factor acetyltransferases. *Curr. Opin. Cell Biol.* **11,** 336–341 (1999).
48. Cosma, M. P., Tanaka, T. & Nasmyth, K. Ordered recruitment of transcription and chromatin remodeling factors to a cell cycle- and developmentally regulated promoter. *Cell* **97,** 299–311 (1999).
49. Krebs, J. E., Kuo, M. H., Allis, C. D. & Peterson, C. L. Cell cycle-regulated histone acetylation required for expression of the yeast HO gene. *Genes Dev.* **13,** 1412–1421 (1999).
50. Clark, D. *et al.* Chromatin structure of transcriptionally active genes. *Cold Spring Harb. Symp. Quant. Biol.* **58,** 1–6 (1993).
51. Roth, S. Y. & Allis, C. D. Chromatin condensation: does histone H1 dephosphorylation play a role? *Trends Biochem. Sci.* **17,** 93–98 (1992).
52. Barratt, M. J., Hazzalin, C. A., Cano, E. & Mahadevan, L. C. Mitogen-stimulated phosphorylation of histone H3 is targeted to a small hyperacetylation-sensitive fraction. *Proc. Natl Acad. Sci. USA* **91,** 4781–4785 (1994).
53. Hendzel, M. J. Mitosis-specific phosphorylation of histone H3 initiates primarily within pericentromeric heterochromatin during G2 and spreads in an ordered fashion coincident with mitotic chromosome condensation. *Chromosoma* **106,** 348–360 (1997).
54. Wei, Y., Yu, L., Bowen, J., Gorovsky, M. A. & Allis, C. D. Phosphorylation of histone H3 is required for proper chromosome condensation and segregation. *Cell* **97,** 99–109 (1999).
55. Goto, H. *et al.* Identification of a novel phosphorylation site on histone H3 coupled with mitotic chromosome condensation. *J. Biol. Chem.* **274,** 25543–25549 (1999).
56. Sullivan, K. F., Hechenberger, M. & Masri, K. Human CENP-A contains a histone H3 related histone fold domain that is required for targeting to the centromere. *J. Cell Biol.* **127,** 581–592 (1994).
57. Hirano, T. SMC-mediated chromosome mechanics: a conserved scheme from bacteria to vertebrates? *Genes Dev.* **13,** 11–19 (1999).
58. De Rubertis, F. *et al.* The histone deacetylase RPD3 counteracts genomic silencing in *Drosophila* and yeast. *Nature* **384,** 589–591 (1996).
59. Braunstein, M., Sobel, R. E., Allis, C. D., Turner, B. M. & Broach, J. R. Efficient transcriptional silencing in *Saccharomyces cerevisiae* requires a heterochromatin histone acetylation pattern. *Mol. Cell. Biol.* **16,** 4349–4356 (1996).
60. Dhalluin, C. *et al.* Structure and ligand of a histone acetyltransferase bromodomain. *Nature* **399,** 491–496 (1999).
61. Winston, F. & Allis, C. D. The bromodomain: a chromatin-targeting module? *Nature Struct. Biol.* **6,** 601–604 (1999).
62. Pawson, T. Protein modules and signalling networks. *Nature* **373,** 573–580 (1995).
63. Roberts, S. M. & Winston, F. Essential functional interactions of SAGA, a *Saccharomyces cerevisiae* complex of Spt, Ada, and Gcn5 proteins, with the Snf/Swi and Srb/mediator complexes. *Genetics* **147,** 451–465 (1997).
64. Biggar, S. R. & Crabtree, G. R. Continuous and widespread roles for the Swi–Snf complex in transcription. *EMBO J.* **18,** 2254–2264 (1999).
65. Sudarsanam, P., Cao, Y., Wu, L., Laurent, B. C. & Winston, F. The nucleosome remodeling complex, Snf/Swi, is required for the maintenance of transcription in vivo and is partially redundant with the histone acetyltransferase, Gcn5. *EMBO J.* **18,** 3101–3106 (1999).
66. Georgel, P. T., Tsukiyama, T. & Wu, C. Role of histone tails in nucleosome remodeling by *Drosophila* NURF. *EMBO J.* **16,** 4717–4726 (1997).
67. Luduena, R. F. Multiple forms of tubulin: different gene products and covalent modifications. *Int. Rev. Cytol.* **178,** 207–275 (1998).
68. Luduena, R. F., Banerjee, A. & Khan, I. A. Tubulin structure and biochemistry. *Curr. Opin. Cell Biol.* **4,** 53–57 (1992).
69. Nogales, E., Whittaker, M., Milligan, R. A. & Downing, K. H. High-resolution model of the microtubule. *Cell* **96,** 79–88 (1999).

Acknowledgements

Research surrounding this topic in our laboratory is supported by grants from the NIH to C.D.A. and B.D.S. We wish to thank current laboratory members, especially C. A. Mizzen, for critical review of this manuscript, and T. K. Archer and C. L. Smith for kindly sharing unpublished data.

Correspondence and requests for materials should be addressed to C.D.A. (e-mail: allis@virginia.edu).