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Cellular Memory and the Histone Code

Review

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The histone tails on the nucleosome surface are subject to enzyme-catalyzed modifications that may, singly or in combination, form a code specifying patterns of gene expression. Recent papers provide insights into how a combinatorial code might be set and read. They show how modification of one residue can influence that of another, even when they are located on different histones, and how modifications at specific genomic locations might be perpetuated on newly assembled chromatin.

It is an obvious but easily forgotten truth that cells must have a mechanism for remembering who they are. A cell's identity is defined by its characteristic pattern of gene expression and silencing, so remembering who it is consists of maintaining that pattern of gene expression through the traumas of DNA replication, chromatin assembly, and the radical DNA repackaging that accompanies mitosis. The mechanisms by which around 2 m of DNA is packaged into the cell nucleus while remaining functional border on the miraculous and are still poorly understood. However, we do know more about the first stage in this packaging process, the nucleosome core particle. This structure comprises an octamer of core histones (two each of H2A, H2B, H3, and H4), around which are wrapped 146 base pairs of DNA in 1 3/4 superhelical turns (Luger et al., 1997). The reduction in DNA length produced by this histone-induced supercoiling is modest, but is an essential first step in the formation of higher-order chromatin structures. In recent years it has become clear that the nucleosome has an additional role, perhaps equally important and conserved, namely regulation of gene expression. Particularly exciting is the growing probability that the nucleosome can transmit epigenetic information from one cell generation to the next and has the potential to act, in effect, as the cell's memory bank.

This information storage function resides primarily in the amino-terminal tails of the four core histones. The tails are exposed on the nucleosome surface and are subject to a variety of enzyme-catalyzed, posttranslational modifications of selected amino acids, including lysine acetylation, lysine and arginine methylation, serine phosphorylation, and attachment of the small peptide ubiquitin (Spotswood and Turner, 2002). Potential sites of posttranslational modification on nucleosomal histones are shown in Figure 1. These modifications are not just a means of reorganizing nucleosome structure, but provide a rich source of epigenetic information. It has been suggested that specific tail modifications, or combinations thereof, constitute a code that defines actual or potential transcriptional states (Jenuwein and Allis, 2001; Richards and Elgin, 2002; Spotswood and Turner, 2002). The code is set by histone modifying enzymes of defined specificity and read by nonhistone proteins that bind in a modification-sensitive manner. In order to realize its full information carrying potential, the code must use *combinations* of modifications. This requires not only proteins that can read such combined modifications, but mechanisms by which they can be put in place and maintained. Recent papers have provided new insights into how specific combinations of tail modification might be generated and revealed mechanisms by which the modification of one residue can determine that of another.

Interplay of Modifications in *cis*

Selected lysines and arginines can be methylated in the N-terminal tail domains of H3 and H4 (Figure 1). The enzymes responsible, the histone methyl transferases (HMT), are either lysine or arginine specific and several have now been identified and characterized (Zhang and Reinberg, 2001). In higher eukaryotes, two H3 lysines, K4 and K9, are commonly methylated. They seem to have complementary functions, with methyl K4 being enriched in transcriptionally active regions and methyl K9 in silent regions, particularly heterochromatin (see Zhang and Reinberg, 2001; Richards and Elgin, 2002, for references). In vitro peptide binding assays and immunolocalization have provided strong evidence that heterochromatin formation is mediated by the preferential binding of the heterochromatin protein HP1 to chromatin in which H3 tails are methylated at K9 (Nielsen et al., 2002; Jacobs and Khorasanizadeh, 2002, and references therein). Methylation of H3 K9 in mammals is carried out by the enzyme SUV39H (Rea et al., 2000) and in fission yeast by the homologous enzyme Clr4. Reinberg and colleagues (Nishioka et al., 2002a) have isolated from HeLa cells an enzyme designated Set9, which catalyzes the methylation of H3 specifically at lysine 4. It is homologous to the budding yeast enzyme Set1. The various histone (lysine) methyl transferases featured in this review are listed in Table 1.

Using in vitro peptide binding assays, Nishioka and colleagues show that methylation of H3 K4 has two potentially important functional effects. First, the preferential association of the NuRD chromatin remodeling and deacetylase complex with the H3 tail is inhibited by K4 (but not K9) methylation, a finding also reported by Zegerman et al. (2002). Second, the in vitro methylation of H3 K9 by purified SUV39H complexes is strongly inhibited if the H3 peptide substrate is methylated at K4. Thus, methylation of H3 K4 by Set9 has the ability to block both chromatin remodeling/deacetylation and methylation of H3 K9 by SUV39H, thereby preventing

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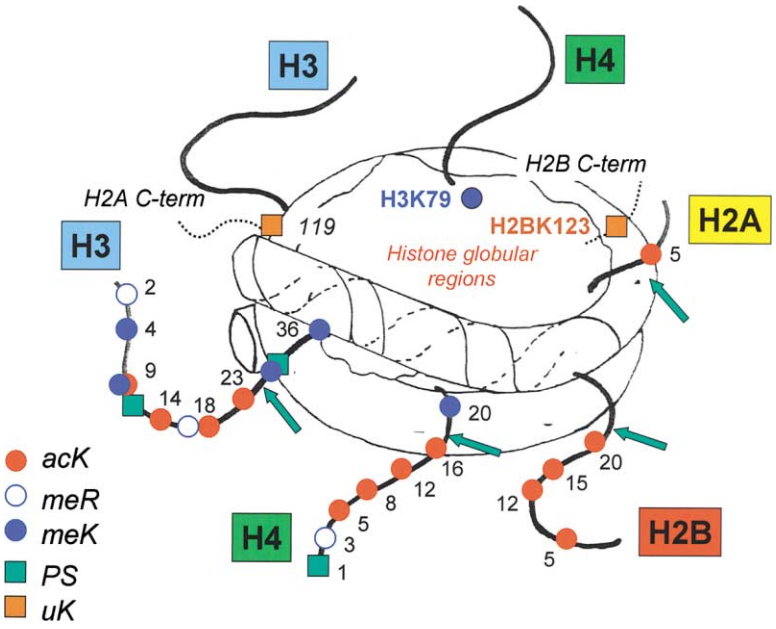

Figure 1. Histone Modifications on the Nucleosome Core Particle

The nucleosome core particle showing 6 of the 8 core histone N-terminal tail domains and 2 C-terminal tails. Sites of posttranslational modification are indicated by colored symbols that are defined in the key (lower left); acK, acetyl lysine; meR, methyl arginine; meK, methyl lysine; PS, phosphoryl serine; and uK, ubiquitinated lysine. Residue numbers are shown for each modification. Note that H3 lysine 9 can be *either* acetylated or methylated. The C-terminal tail domains of one H2A molecule and one H2B molecule are shown (dashed lines) with sites of ubiquitination at H2A lysine 119 (most common in mammals) and H2B lysine 123 (most common in yeast). Modifications are shown on only one of the two copies of histones H3 and H4 and only one tail is shown for H2A and H2B. Sites marked by green arrows are susceptible to cutting by trypsin in intact nucleosomes. Note that the cartoon is a compendium of data from various organisms, some of which may lack particular modifications (e.g., there is no H3meK9 in *S. cerevisiae*). Adapted from Spotswood and Turner (2002).

the placement of a silencing mark to be read by HP1. These interactions are summarized in Figure 2. The data complements earlier work showing that phosphorylation of H3 S10 and, not surprisingly, acetylation of H3 K9 both prevented K9 methylation by SUV39H in vitro (Rea et al., 2000; Zhang and Reinberg, 2001). In the same assays, acetylation of H3 K14 caused only a modest reduction in K9 methylation. However, fission yeast mutants deficient in the H3 K14-specific histone deacetylase (HDAC) Clr3 also showed reduced H3 K9 methylation (Nakayama et al., 2001), leaving open the possibility of a functional interaction between these two modifications (Figure 2). It is interesting to note that there is evidence of physical interaction between the *Drosophila* HMT SU(VAR)3-9 and a histone deacetylase (Czermin et al., 2001) and also between the HAT CBP and a methyl transferase activity (Vandel and Trouche, 2000).

Further evidence for a possible interaction between methylation and acetylation comes from three papers identifying an enzyme (SET8, Table 1) that methylates H4 specifically at lysine 20 (Fang et al., 2002; Nishioka et al., 2002b; Rice et al., 2002). The native enzyme is highly specific for *nucleosomal* H4 K20. Some results suggest that the functional effects of H4meK20 may be modulated by acetylation of H4 K16 or vice versa. For example, Rice and colleagues present immunofluorescence and Western blotting data to show that H4meK20 levels vary through the cell cycle, being highest in mitosis (where H4acK16 levels are lowest) and lowest in S phase (where H4acK16 levels are highest). Also, H4meK20 levels are low on the male polytene X chromosome in *Drosophila*, a chromosome marked by relatively high levels of H4acK16 and increased transcription. It may be that H4meK20 is a mark associated with chromatin condensation and reduced transcription, whereas H4acK16 specifies just the opposite chromatin state (Rice et al., 2002). However, this attractive idea is not consistent with other results, using similar approaches, but a different antibody to H4meK20. These show that levels of H4meK20 are highest during S phase and low-

Table 1. Histone Methyltransferases (HMT) Referred to in the Text and Their Specificities

Primary Specificity	Enzyme *S. cerevisiae*	*S. pombe*	*Drosophila*	Mammals
H3K4	Set1	Set1		Set9[a]
H3K9	-[b]	Clr4	SU(VAR)3-9[c]	SUV39H[d]
H3K27			E(Z)[e]	G9a[e]
H3K36	Set2			
H3K79	Dot1			DOT1L
H4K20	-[f]		dSET8	SET8/PR-Set7[g]
H3K9,K27, H4K20			ASH1	

[a] Nishioka et al. (2002a); originally designated Set7 by Wang et al. (2001).
[b] There is no detectable H3 K9 methylation in *S. cerevisiae*.
[c] Suppressor of position effect Variegation
[d] Human nomenclature (Suv39h in mice); there are two homologs and the enzyme is sometimes referred to as SUV39H1/2.
[e] E(Z) and G9a also methylate H3 K9 (Tachibana et al., 2001; Czermin et al., 2002). E(Z) = Enhancer of Zeste.
[f] No H4meK20 detected by Western blotting in *S. cerevisiae* (Nishioka et al., 2002b).
[g] Alternative nomenclature from different groups (Nishioka et al., 2002b; Müller et al., 2002; Feng et al., 2002).

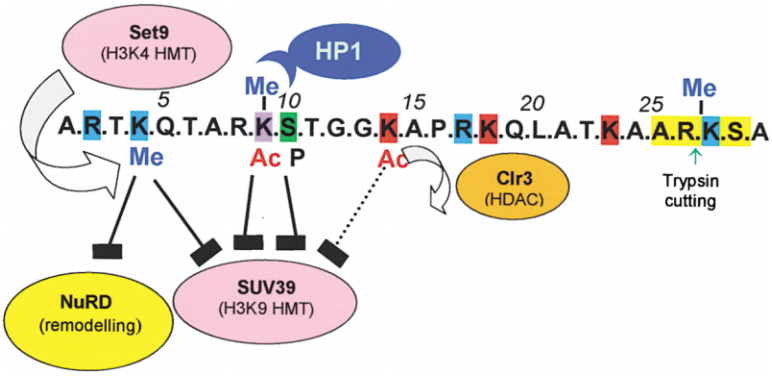

Figure 2. Modifications of the Histone H3 N-Terminal Tail Interact through Their Effects on Chromatin Modifying Enzymes

The amino acid sequence of the H3 N-terminal tail domain is shown in single letter code. Residues on the N-terminal side of the trypsin cutting site (arrowed) are accessible on the nucleosome surface. Only those residues highlighted in yellow have been located in nucleosome core particle crystals (Luger et al., 1997). Lysines (K) and arginines (R) that can be methylated are blocked in blue, lysines that can be acetylated are in red, and the serine (S) that can be phosphorylated is in green. Lysine 9 can be either acetylated or methylated and is blocked in violet. The enzyme Set9 methylates H3 lysine 4 specifically. Methylation of this residue prevents association of the H3 tail with the NuRD chromatin remodeling and deacetylase complex and the SUV39H histone methyl transferase. Methylation of H3 K9 by SUV39H is also prevented or inhibited by acetylation of K9, phosphorylation of S10, and possibly, acetylation of K14. Methylation of K9 is enhanced by removal of K9 and K14 acetates by deacetylases (HDAC) such as the yeast enzyme Clr3. Methylation of H3 K9 facilitates binding of the heterochromatin protein HP1. For references, see text.

est during mitosis (Fang et al., 2002). The explanation for this discrepancy may lie in the detailed specificities of the antisera involved, and it raises an interesting and important issue. Suppose that two antisera, both specific for H4meK20, differ in that one is inhibited by acetylation at H4K16, whereas the other is unaffected. The antiserum that fails to recognize H4 tails that are also acetylated at K16, but not its acetylation-insensitive counterpart, will give weak staining where H4 acetylation is high, irrespective of the actual level of K20 methylation. It is perfectly possible for two antisera to differ in this way, even ones raised in parallel using the same peptide immunogens. This experimental issue is easily resolved by testing the antisera involved with the appropriate doubly modified peptides, but the general point it raises is an important one, namely that it is crucial to know exactly how the binding properties of a specific antiserum are influenced by modification of residues adjacent to its primary target. Such information allows results to be interpreted in terms of *combinations* of modification on the same histone tail (i.e., in *cis*) and will not only minimize the possible misinterpretation of experimental findings, but will raise antibody-based analysis of the histone code to a new level of detail. In this regard, it should be noted that the lysine ϵ-amino group can carry one, two, or three methyl moieties. All three levels of methylation occur in vivo and seem to exert different functional effects (Santos-Rosa et al., 2002). Antibodies may recognize one level of methylation but not another.

Interplay of Modifications in *trans*

The results outlined so far are not altogether unexpected. Posttranslational modifications within or adjacent to sites of protein-protein interaction are likely to have some sort of effect on that interaction. However, recent experiments in yeast to investigate how H3 K4 methylation itself is regulated have revealed a much more surprising relationship. In budding yeast, H3 K4 methylation requires the *SET1* gene and strains carrying mutations of *SET1* not only lack H3 K4 methylation but show a slow-growth phenotype with loss of silencing of rDNA genes (Bryk et al., 2002). A similar phenotype is seen in cells in which H3 K4 (but not K9) is substituted with arginine or alanine. Several genes in yeast, in addition to *SET1*, are known to be required for efficient telomeric and rDNA silencing, and in a recent paper, Sun and Allis (2002) ask whether mutation of any of these might also alter H3 K4 methylation. They find that mutations in just one, *RAD6*, completely abolish H3 K4 methylation detected by Western blotting. The same result has been reported by Dover et al. (2002), who screened a bank of yeast mutant strains for levels of H3 K4 methylation, also by Western blotting.

The Rad6 protein is a ubiquitin conjugating enzyme involved in many cellular processes, including gene silencing, DNA repair, and sporulation. Rad6 attaches the 76 amino acid peptide ubiquitin to selected protein lysines via an isopeptide bond, thereby targeting these proteins for degradation by the proteasome system (see Sun and Allis, 2002, for references). Certain histone lysines are also targets for ubiquitination, and Rad6 in yeast is the major activity responsible for attachment of ubiquitin to lysine 123 in the C-terminal tail of H2B (Figure 1). This activity seems to provide the link to H3 methylation because substitution of H2B lysine 123 with an alanine, thereby preventing ubiquitination, also abolished methylation of H3 lysine 4 (Sun and Allis, 2002). This substitution mutant also showed defective silencing at telomeres.

The effect on telomeric silencing may be related to a second remarkable property of H2B K123 substitution and *rad6* deletion mutants, namely the complete absence of methylation at H3 lysine 79 (Briggs et al., 2002). H3 K79 is located in the globular domain of the histone, far away from the N-terminal tail, but is exposed (i.e., potentially accessible in vivo) on the upper and lower faces of the core particle (Figure 1). Around 90% of H3 molecules are methylated at this site in wild-type cells (van Leeuwen et al., 2002). H3 K79 methylation is carried out exclusively by the enzyme Dot1, an unusual HMT that lacks a SET domain (Feng et al., 2002; Ng et al., 2002; van Leeuwen et al., 2002). *dot1* mutants are defective in telomeric silencing. Thus, Rad6-catalyzed ubiquitination of H2B K123 seems to be essential for methylation of both H3 K4 and H3 K79 and, consequently, for

appropriate patterns of gene silencing. A third H3 methylation site in *S. cerevisiae*, lysine 36, shows no change in methylation level in either *rad6* or H2B K123 substitution mutants, so there is no generic effect of H2B ubiquitination on histone methylation, nor does it alter levels of the methylating enzymes Set1 and Dot1 (Sun and Allis, 2002).

What might be the mechanism by which modification of one histone tail determines that of another? Sun and Allis show that H3 K4 methylation and H2B ubiquitination occur on the same nucleosome, but also point out a large overall difference in frequency between the two modifications, with about 35% of H3 molecules methylated at K4 and less than 5% of H2B molecules ubiquitinated at K123. It is suggested that ubiquitination of H2B on a single nucleosome acts as a "wedge" to open up a stretch of chromatin, making other nucleosomes accessible to the Set1 methylating enzyme. This requires Set1 to be capable of methylating H3 K4 on nucleosomes in which H2B is not ubiquitinated. Alternatively, the different frequencies of methylation and ubiquitination could reflect their different turnover rates. Methylation is usually a stable modification (Zhang and Reinberg, 2001) and could be retained long after the ubiquitin, which allowed its attachment in the first place, has been lost. In this case, ubiquitination of H2B K123 could be an absolute requirement for Set1-catalyzed methylation of H3 K4 and K79.

There are other puzzles. For one, it is by no means clear how H3 K4 methylation relates to silencing. By combining chromatin immunoprecipitation (ChIP) with DNA microarray analysis, Bernstein et al. (2002) have shown that in budding yeast, levels of H3 K4 methylation along coding regions show a strong positive correlation with levels of transcription. Silent regions, including rDNA genes, are relatively undermethylated. This pattern of association is consistent with results in higher eukaryotes but argues against the proposition that Set1-mediated H3 K4 methylation in the chromatin packaging rDNA is itself responsible for silencing.

A second puzzle is highlighted by the thorough mass spectrometric analysis of H3 K79 methylation levels reported by van Leeuwen et al. (2002). About 90% of H3 molecules in wild-type yeast are methylated at K79, with most (about 50%) being trimethylated, a finding that argues against a role for this modification itself as a silencing signal at telomeres or anywhere else. This high frequency of methylation is consistent with earlier work on H3 and H4 methylation levels in higher eukaryotes (see Zhang and Reinberg, 2001; Spotswood and Turner, 2002, for references). So what role can there be for such a common and widely distributed histone modification? One possibility is that silenced regions are marked by *low* levels of H3 K79 methylation. Van Leeuwen et al. (2002) propose that H3 K79 methylation blocks the binding to active chromatin of "sticky" proteins, such as the silencing proteins Sir2 and Sir3. Absence of H3 K79 methylation in *dot1* mutant cells will allow promiscuous binding of such proteins across the genome, with consequent depletion of their levels at silent regions. Overexpression of Dot1, leading to methylation (almost exclusively trimethylation) of 98% of H3 molecules at K79, was found to diminish silencing, presumably by forcing H3 K79 methylation in potentially silent regions that are sheltered from methylation in wild-type cells. The hypothesis was consistent with ChIP data and the properties of *dot1* and H3 K79 substitution mutants, but there is as yet no biochemical data to show how H3 K79 methylation might influence, directly or indirectly, binding of Sir proteins to the nucleosome.

The *trans* interactions discussed so far all relate to the mechanisms by which the histone code is set. In a recent paper, Sauer and colleagues (Beisel et al., 2002) show that they can also determine how the code is read. They show that the *Drosophila* epigenetic activator ASH1 (*Absent, Small or Homeotic discs 1*), is a histone methyltransferase with a rather broad substrate specificity, methylating H3 at lysines 4 and 9 and H4 at lysine 20. They present a powerful mix of genetic and biochemical evidence to show that this combination of methylated lysines both facilitates binding of the activator BRAHMA (an ATPase component of a SWI/SNF-like remodeling complex) and inhibits binding of epigenetic repressors such as polycomb (PC) and HP1. Thus, in the presence of H3meK4 and H4meK20, H3meK9 is not a determinant of silencing. Instead, it is part of a combination of marks that constitutes a binding platform for a BRAHMA-containing remodeling complex and thereby maintains a transcriptionally *active* state.

Memory Mechanisms

Histone acetylation does not provide an obvious candidate for a component of cellular memory, in that it is generally a dynamic modification, maintained by the ongoing activity of histone acetyltransferases (HATs) and deacetylases (HDACs). However, it has been known for some time that certain bromodomains, sequence elements found in many chromatin-associated proteins and most HATs (Zeng and Zhou, 2002), bind preferentially to acetylated peptides in in vitro binding assays, leading to speculation that acetylated histone tails could form targets for the binding of bromodomain-containing proteins in vivo. Recent experiments provide direct evidence for this.

Hassan et al. (2002) in this issue of *Cell* use nucleosome arrays attached to magnetic beads to study the binding of the SWI/SNF chromatin remodeling complex and the SAGA and NuA4 HAT complexes to chromatin in vitro. The SWI/SNF complex is attracted to chromatin by the activator Gal4-VP16 (the assembled arrays contain a block of four Gal4 binding sites). The authors have shown previously that prior acetylation of the array with SAGA or NuA4 complexes does not enhance SWI/SNF binding but does allow retention of the complex when the activator Gal4-VP16 is removed by treatment with competing oligonucleotides. In the present paper, they show that this retention requires the bromodomain of the Swi2/Snf2 subunit of the SWI/SNF complex. Further, the SAGA complex itself is anchored to acetylated arrays following removal of VP16, but only if the bromodomain of the Gcn5 subunit is intact. The bromodomain of the Spt7 subunit is not required. In contrast, the NuA4 complex, which lacks a bromodomain, is not retained following removal of Gal4-VP16. To study the role of the Swi2/Snf2 bromodomain in vivo, SWI/SNF binding to the promoter of the *SUC2* gene was studied by ChIP in Swi2/Snf2-deficient cells carrying plasmids encoding

epitope-tagged, wild-type Swi2/Snf2 or Swi2/Snf2 lacking a bromodomain. Only the wild-type Swi2/Snf2 showed enhanced association with the *SUC2* promoter under inducing conditions. Thus, the SAGA HAT complex binds to acetylated nucleosomes through the Gcn5 bromodomain, providing a self-perpetuating, epigenetic mark tethered to a small chromatin domain.

Silencing complexes may also have the self-perpetuating potential required of an efficient memory mechanism. Proteins of the Polycomb group (PcG) were first identified in *Drosophila* on the basis of their ability to associate with genes and gene clusters silenced during development and thereby to maintain the silent state through subsequent developmental stages. Two recent papers show that E(Z) (*Enhancer of Zeste*), a component of PcG silencing complexes isolated from *Drosophila* embryos, has histone methyltransferase activity (Czermin et al., 2002; Müller et al., 2002). Mutations in the E(Z) SET domain remove this activity and also disrupt silencing of HOX genes in vivo (Müller et al., 2002). With nucleosome substrates (which it prefers), the E(Z) HMT methylates H3 K27 exclusively (Müller et al., 2002), but can methylate both H3 K27 and H3 K9 when presented with peptide substrates (Czermin et al., 2002). H3 methylated by the E(Z) complex binds specifically to Polycomb protein (Czermin et al., 2002), arguing for a direct relationship between H3 methylation by E(Z) and assembly of the PcG silencing complex. Further, immunofluorescence microscopy shows an almost perfect colocalization of H3 *tri*methylated at K9 and PSC, a component of the PcG silencing complex (Czermin et al., 2002). In contrast, there is no correspondence between PSC and H3 *di*methylated at K9. The authors note the important caveat that their antibody to H3 trimethylated at K9 crossreacts (albeit weakly) with H3 peptides trimethylated at K27 (the two sites share an ARKS tetrapeptide motif). It remains possible that H3 methylated at K27 contributes to the staining pattern. But, whatever lysines may be involved, the results show that E(Z)-containing PcG silencing complexes have the potential to place a self-sustaining epigenetic mark.

The Histone Code in Transcription Initiation

So far, we have focused on the possible roles of the histone code in the long-term maintenance and heritability of transcriptional states. However, changes in histone modifications, particularly acetylation, have long been associated with more short-term aspects of transcriptional regulation, particularly initiation. Recent data strongly suggest that transcriptional initiation involves the progressive setting of precisely-defined patterns of histone modification. Agalioti et al., in this issue of *Cell*, use ChIP to show that activation of the endogenous *IFN*-β gene in response to viral infection is accompanied by the progressive acetylation of H4 K8 and H3 K9 and K14. In vitro experiments with immobilized mononucleosomes carrying an *IFN*-β promoter fragment showed that H4acK8 was necessary for recruitment of the SWI/SNF complex and that H3acK9 and H3acK14 were necessary for recruitment of TFIID, probably through binding of the double bromodomain of the TAFII250 subunit. Acetylation and transcription complex assembly required GCN5/PCAF but occurred as normal in extracts immuno-depleted of another HAT, CBP. The results lead to a model in which transcription initiation involves sequential modifications at defined sites on the histone tails, each of which facilitates recruitment/binding of the next element in the assembly pathway. A similar model can accommodate the recently described changes that occur on activation of the *pS2* promoter by estrogen. Here, CBP is recruited within 15 min of estrogen stimulation, leading to acetylation of H3 K18. This is followed by acetylation of H3 K23, recruitment of the arginine HMT CARM1, methylation of H3 R17, and transcription complex assembly (Daujat et al., 2002).

What Should We Expect of a Histone Code?

It is in the nature of scientific progress that simple ideas, like people, grow more complex with age. It is now ten years since evidence was first presented that the modification (acetylation) of particular residues on the histone tails was functionally significant (see Turner, 1993, for references). Since then, it has been established beyond all reasonable doubt that specific modifications and combinations thereof mediate protein-protein interactions crucial for the long-term and short-term regulation of transcriptional activity. Some of these interactions involve the modifying enzymes themselves, and much recent data, some of which is discussed here, shows how particular histone tail modifications can interact, both to put in place defined *patterns* of modification and to control their recognition. But do these findings support the existence of a histone code, or even more grandly, an epigenetic code?

In attempting to answer this question, it is useful to distinguish between short-term and long-term transcriptional effects. Studies on the rapid transcriptional upregulation of inducible genes provide evidence for a "cascade" of events, each dependent on the one preceding it and each involving specific histone tail modifications (Agalioti et al., 2002; Daujat et al., 2002, and references therein). The final pattern of modifications will represent the end result of this cascade and thus may have no significance in itself. Also, high levels of induced transcriptional activity are often necessarily transient, with no requirement for heritability from one cell generation to the next. It is also noteworthy that induction of the *pS2* and *IFN*-β genes involves quite different sets of histone modifications. This is perhaps not surprising given that different HATs are involved, and further studies may yet reveal other modifications shared by the two systems, but there is as yet no evidence for a universal histone code involved in induced transcriptional upregulation.

A histone code could be particularly valuable where there is a requirement for long-term maintenance of a transcriptional state, i.e., for heritability. What could be the nature of this long-term code? The idea that a *single* modification of a particular tail residue can, unfailingly, specify a particular functional effect becomes, at least as a general principle, increasingly unlikely. Specific modifications certainly have the *potential* to exert such effects, but whether they do or not is largely dependent on the context in which they are presented. For example, H3 methylated at K9 has the potential to initiate chromatin condensation and silencing, in part through its ability

to bind proteins such as HP1 and possibly PcG proteins (see above). However, in the context of a nucleosome also methylated at H3 K4 and H4 K20, it provides part of a binding platform for the chromatin remodeling component BRAHMA and a mark for the long-term maintenance of a transcriptionally *active* state (Beisel et al., 2002). The silencing potential of H3meK9 may also be overridden by more transient modification of adjacent sites, such as phosphorylation of the adjacent residue (S10) or acetylation of K14. It may even be removed altogether, along with other H3 and H4 tail modifications, through replication-independent chromatin assembly, a process that replaces existing H3/H4 tetramers with new tetramers containing the H3.3 histone variant and that operates within transcriptionally active chromatin (reviewed by Ahmad and Henikoff, 2002 [this issue of *Cell*]). To add further complexity, the setting of chromatin marks must also involve DNA methylation, which, at least in fungi, can be triggered by histone methylation (Selker et al., 2002) and binding of silencing RNAs (Volpe et al., 2002; see also Richards and Elgin, 2002). Viewed in this light, the histone code can be seen as part of a sequence of events, possibly involving structural and catalytic proteins and RNAs, whose end result is a functionally stable chromatin state. The *heritable* component of such a stable transcriptional state could usefully be defined as an epigenetic code. This heritable code would involve both DNA methylation, which is stable and has a defined maintenance mechanism (Bird, 2002), along with histone modifications such as methylation and acetylation, both of which have the potential to self-perpetuate through binding of HATs and HMTs to specifically modified tail domains. Thus, the necessary mechanisms have already been identified. It remains to be seen exactly how they are used.

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