Histone acetylation in chromatin structure and transcription

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The amino termini of histones extend from the nucleosomal core and are modified by acetyltransferases and deacetylases during the cell cycle. These acetylation patterns may direct histone assembly and help regulate the unfolding and activity of genes.

Nucleosomes, which fold chromosomal DNA, contain two molecules each of the core histones H2A, H2B, H3 and H4. Almost two turns of DNA are wrapped around this octameric core, which represses transcription *in vivo* and *in vitro*. But despite its similarity to a bead on a string, this building block of the chromosome is unexpectedly dynamic. The histone amino termini extend from the core, where they can be modified post-translationally by acetylation, phosphorylation and methylation, affecting their charge and function. Of such modifications, acetylation and deacetylation have generated most interest since gene activity was first correlated with histone acetylation[1]. Further indications of this correlation emerged when an antibody recognizing ϵ-*N*-acetyl lysine was shown to bind chromatin enriched in a globin gene from chicken erythrocytes. Binding continued even when the gene was inactive[2], indicating that it is the potential for gene activity, rather than the transcriptional process itself, that is associated with hyperacetylated histones. As acetylation neutralizes the positively charged lysine residues of the histone N termini, decreasing their affinity for DNA, this might allow the termini to be displaced from the nucleosome, causing the nucleosomes to unfold and increasing access to transcription factors[3–6].

How acetylation and deacetylation target specific genes or chromosomal domains has long been a mystery. Euchromatin and heterochromatin exhibit different acetylation patterns (euchromatin is the chromatin that decondenses during interphase of the cell cycle and which contains most of the genes coding for cellular proteins; heterochromatin is condensed even in interphase, often contains repetitive DNA, and is generally silent). Antibodies against specific acetylation sites in histone H4 have been used to show that potentially active euchromatin can be modified at all the H4 acetylatable lysines (K5, K8, K12 and K16), whereas H4 in heterochromatin is hypoacetylated[7,8]. None of the four sites seems to be acetylated on the inactive X chromosome in humans[9], and *Drosophila* and yeast heterochromatin seems to be acetylated only on residue K12 (refs 10,11). But the resolution of these studies does not exclude the possibility that other patterns of acetylation are associated with subdomains of hetero- or euchromatin.

One example of an association between acetylation and a function other than gene activity is that of H4 diacetylation and assembly. Unlike histones in chromatin, newly synthesized H4 is preferentially acetylated at K5 and K12 (ref. 12), suggesting that this pattern is associated with assembly. But just as it has been unclear whether certain acetylation patterns are required to activate genes in euchromatin or to silence them in heterochromatin, we have not known whether histone H4 diacetylation is required for nucleosome assembly. This situation is rapidly changing. Not only have the consequences of altering acetylation sites in H3 and H4 been investigated, but many of the genes and enzymes responsible for histone acetylation and deacetylation have been identified. New tools have thus become available to investigate nucleosome assembly, the differences between heterochromatin and euchromatin, and the mechanisms of gene activation and repression.

Nucleosome assembly

In nucleosome assembly, binding of the H3/H4 tetramer to DNA is followed by assembly of H2A/H2B dimers. Newly synthesized histone H4 is diacetylated at K5/K12 in organisms as diverse as *Tetrahymena*, flies and humans. Newly synthesized H3 is also acetylated, albeit in a less conserved manner[12,13]. How important is this acetylation in histone assembly? Chromatin assembly factor 1 (CAF1) is a complex of proteins p150, p60 and p48 from human cells which assembles newly synthesized H3 and H4 onto replicating DNA *in vitro*[14]. The complex is less capable of assembling histones isolated from nuclei, suggesting that the different acetylation pattern of chromosomal histones precludes assembly *in vitro*. CAF1 can bind H4 that is mono-, di- or triacetylated at K5, K8 and K12, or even unacetylated. Some H3 in the complex is monoacetylated, although about 60% is unacetylated[15]. Is K5/K12 diacetylation of H4 not essential for assembly? If K5/K12 diacetylation is important, why can these sites be deleted or mutated in yeast without strongly affecting cellular viability, given that defective nucleosome assembly can be lethal[16–18]?

These apparent inconsistencies partly reflect the redundancy of the H3 and H4 N termini in assembly of the H3/H4 tetramer. Either can be deleted without preventing nucleosome assembly in yeast cell extracts[18] or even in *Xenopus* cell extracts[19], but deletion of both in the same extract blocks assembly[18]. The H3 and H4 N termini can even be exchanged between the two proteins without strongly affecting assembly or viability. The effects of H4 N-terminal mutations on assembly must thus be examined in the absence of the H3 N-terminal tail. Even then, H4 K5, K8 and K12 must all be mutated simultaneously to prevent nucleosome assembly[20]. The redundancy between these sites helps to explain CAF1's interactions with H3 and H4. CAF1 may recognize the H3/H4 histone complex as long as at least one H4 site is acetylated (Fig. 1), serving as a chemical tag or changing protein conformation to allow CAF1 to assemble the H3/H4 complex. Thus, H4 K5/K12 diacetylation may precede the pattern used for assembly by CAF1. Disruption of the CAF1 p160 counterpart in yeast does not block cell viability or nucleosome assembly[24,25], so other pathways of nucleosome assembly are likely to exist.

How H2A and H2B are assembled is less clear. A factor (NAP1) identified in humans, *Drosophila* and yeast[21,22] facilitates the assembly of nucleosome arrays in an ATP-dependent manner[22]. This factor is bound to H2A and H2B in whole-cell extracts and moves from the nucleus in S phase (when much nucleosome assembly is taking place) to the cytoplasm in G2. Its action has not yet been shown to depend on DNA replication or histone acetylation, although some nucleosome assembly probably occurs *in vivo* even without DNA replication[23].

Genes that may be involved in the acetylation of histones for assembly have been identified. Cytoplasmic acetylation for histone assembly involves B-type histone acetyltransferases (HATs), as opposed to A-type HATs, which are nuclear and transcription related. The major B-type *HAT1* gene of yeast has been cloned and can acetylate residues 1–28 of H4 at K5 and K12 *in vitro*[26,27],

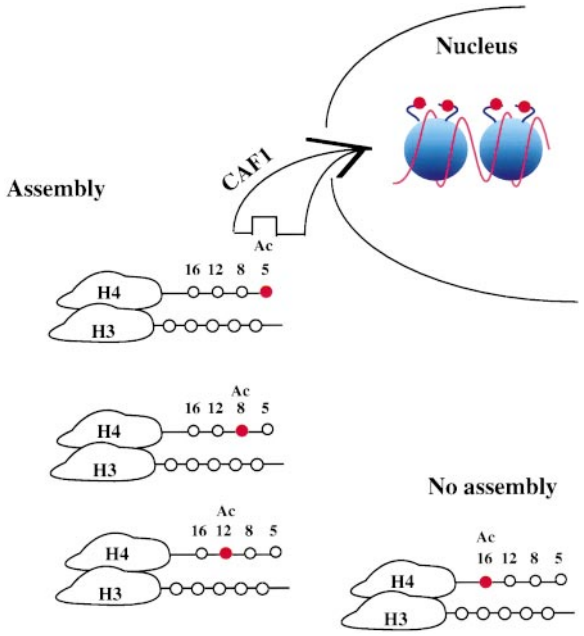

Figure 1 Recognition of the acetylated H3/H4 complex by human CAF1. A single acetyl group (Ac) on H4 K5, K8 or K12, but not K16, may allow CAF1 to recognize the complex. Multiply acetylated H4 K5/K8/K12 would also be recognized. The acetyl group may serve as a chemical tag or alter protein conformation to induce CAF1 binding. Unacetylated H3 would thus be incorporated into nucleosomes by way of H4 acetylation.

indicating that it is a catalytic subunit. HAT1 (M_r 42K) is associated with a second protein HAT2 (M_r 48K), which is required for full HAT1 activity and is similar in sequence to the p48 subunit of CAF1. The p48 subunit is also associated with the human deacetylase HD1 (ref. 28) and a similar histone deacetylase in flies[29]. p48-type proteins may target catalytic subunits to histones, and HAT2 is indeed required for HAT1 to bind to an affinity resin containing the unacetylated H4 N terminus. As yeast extracts containing HAT1 acetylate only K12 in H4, negative regulators may restrict its specificity. Here too, disruption of HAT1 is neither lethal, nor appears to affect histone H4 acetylation *in vivo*. The redundancy of sites K5, K8 and K12 of histone H4 in nucleosome assembly may also extend to other B-type HATs which recognize these sites.

Heterochromatin

In *Drosophila* and yeast, heterochromatin is acetylated only at H4 K12, suggesting the importance of this modification in silencing[10,11]. Human heterochromatin does not seem to be similarly acetylated[9], however, and whereas mutations at H4 K16 completely disrupt silencing, similar alteration of K12 (or even simultaneous alteration of K5, K8 and K12) does not[16,17,30,31]. Why is K12 acetylation not required for the integrity of heterochromatin? Either K12 and K16 acetylation may be redundant under certain conditions[11], or the explanation may lie in the condensed nature of heterochromatin. Yeast heterochromatin is packaged by specialized proteins that include the repressor/activator protein RAP1, the silencing information regulators (SIRs)2, 3 and 4, and histones H3 and H4. These proteins are thought to interact as shown in Fig. 2, forming a structure that folds back onto subtelomeric regions to generate a condensed and protective chromosomal cap[32]. As HAT1 acetylation of K12 is the main B-type activity in yeast[26,27], all H4 may be preferentially acetylated at this site during chromatin assembly. Unlike euchromatin, assembled heterochromatin excludes many enzymes[33] and if acetyltransferases and deacetylases are among these, site 12 may remain preferentially acetylated (Fig. 2).

Mutations in the yeast histone deacetylases HDA1 and RPD3 increase general H3 and H4 acetylation levels *in vivo*[34] and would be expected to unfold heterochromatin. Curiously, however, mutations in these enzymes increase repression of telomeric URA3 (refs 34–36). Similar hyper-repression of a gene adjacent to heterochromatin occurs when RPD3 in *Drosophila* is mutated[36]. However, deacetylase disruption alters expression of many genes in yeast and mammals[34,37,38], and is likely to do so in *Drosophila*. In both yeast and flies, even small increases in certain components of heterochromatin can improve silencing and increase the extent of heterochromatin[39–41]. Thus histone hypoacetylation in heterochromatin may still be a result of its condensed state.

Transcription

The core TATA promoter is recognized by the TATA-binding protein (TBP), TBP-associated factors (TAFs), and other proteins making up the proposed holoenzyme of RNA polymerase[42]. The activity of this basal transcription complex is enhanced by activator proteins, which recognize upstream activation sequences (UAS). In yeast, activator binding sites are usually nucleosome-free, and may be kept that way by specialized proteins (such GRF2 at the GAL1 UAS[43,44]), but TATA elements are often found in or near nucleosomes[43]. How activators function, especially in the context of chromatin, has been a mystery.

Histone acetylation was known to affect transcription *in vivo*. Simulating acetylation by mutating lysines in H4 to uncharged residues, for example, can alter both nucleosome positioning[45,46] and gene activity, often increasing uninduced transcription and reducing activated transcription[46,47]. But actual acetylation of the histone N termini, in particular that of H4 (ref. 48), may trigger orderly nucleosomal disruption near TATA elements conducive to efficient transcription. How could this occur?

The HATs are likely to participate in gene activation. Histone acetyltransferase A in *Tetrahymena* is homologous to the yeast GCN5 protein which is required for activation of several genes in yeast and has intrinsic activity[49]. GCN5 may function as part of a complex of proteins (including ADA2 and ADA3) connecting activators to the basal transcription machinery[50–52]. In vertebrates, other transcription factors also have HAT activity. In particular, the homologous proteins CBP and p300 (often termed p300/CBP) link activators to the transcription machinery at promoters regulated by nuclear hormone receptors, the adenoviral oncoprotein E1A and other regulators[53]. p300/CBP interacts with a second factor, pCAF, which, like p300/CBP, has intrinsic HAT activity[54–56]. E1A represses many activators[57] and competes with pCAF for binding to p300/CBP, so pCAF and p300/CBP may associate to acetylate chromatin at certain promoters, activating transcription[54].

Recent findings have strengthened the link between histone acetylation and nucleosomal disruption in yeast. Tethering of TBP or a component of the RNA polymerase holoenzyme upstream of the TATA element by a sequence-specific DNA-binding protein was known to bypass the need for an activation domain, indicating that the activator may recruit the RNA polymerase holoenzyme to the downstream promoter[58]. However, a protein associated with TBP (TAF130 in yeast, TAF230 in *Drosophila*, and TAF250 in humans) has intrinsic HAT activity[59]. If TAF130 or GCN5 (or related proteins) dislodge histone N termini from nucleosomes at the promoter, tethering of these proteins by activators can explain how activators can disrupt nucleosomes even without a functional TATA element[60].

How histone deacetylases function at specific promoters has recently attracted considerable attention. An affinity matrix containing trapoxin, a deacetylase inhibitor, was used to isolate a human protein (HD1 or HDAC1) that co-immunoprecipitates histone deacetylase activity[28]. HD1 is homologous to yeast RPD3, mutations in which alter expression of several genes[34,37]. A second mammalian protein, HDAC2, is 85% identical to RPD3 and is associated with the transcriptional activator/repressor YY1 (ref. 61). A 350K histone deacetylase complex (HDA) from yeast nuclei

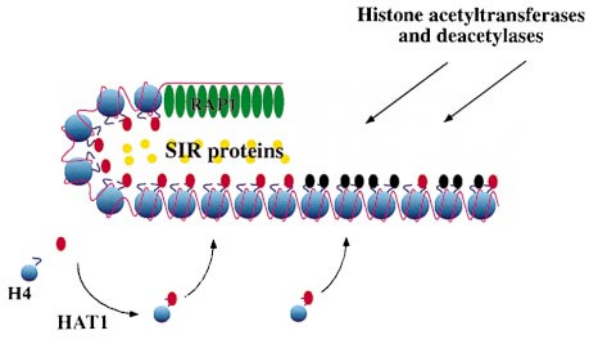

Figure 2 Preferential acetylation of H4 K12 in heterochromatin. Yeast heterochromatin is shown as a looped condensed structure formed by the interaction of RAP1, which binds to telomeric DNA, with SIR proteins, which interact with H3 and H4 (ref. 32). H4 K12 is thought to be acetylated by HAT1 during histone assembly so that the site is modified in both heterochromatin and euchromatin. Subsequent acetylation and deacetylation of H4 in euchromatin would generate H4 acetylated at residues K5, K8, K12 and K16 (red dots refer to acetylated K12; black dots to acetylated K5, K8 or K16), while heterochromatin blocks access to both histone acetyltransferases and deacetylases and so remains enriched in only K12.

containing three polypeptides, HDA1, HDA2 and HDA3, has also been purified to near homogeneity[62]. HDA1 is related to RPD3 and to three other histone deacetylases from yeast, HOS1, HOS2 and HOS3 (ref. 34). These enzymes probably act on histones *in vivo*, as their mutagenesis increases histone H3 and H4 acetylation[34]. They may have overlapping but non-identical functions[34], targeting specific genes, chromosomal domains, or portions of the cell cycle.

How certain deacetylases act in a gene-specific manner is now becoming clear. Histone deacetylation should lead to repression, and a yeast histone H4, in which all four acetylation sites have been mutated to simulate hypoacetylation, represses every gene tested[47]. Similarly, tethering HDAC2 to a GAL4 DNA-binding domain represses an adjacent gene even in the absence of the repressor YY1, suggesting that YY1 represses by tethering the histone deacetylase[61]. The yeast co-repressor SIN3, also known as RPD1, is thought to function in the same pathway as RPD3, because mutations in either have similar yet non-additive effects on the same genes. These effects mimic those of histone acetylation-site mutations, increasing uninduced but decreasing induced activity[37,63]. Even before mammalian RPD3 homologues were discovered, SIN3 homologues (mSIN3A and mSIN3B) were known to form a complex with the mammalian repressor Mad. Mad forms a complex with the protein Max to repress certain genes. Regulatory sequences would otherwise be activated by the Myc/Max heterocomplex[64,65], which recognizes the same E-box-related DNA sequence. Mad/Max and mSIN3 form a ternary complex, and tethering mSIN3 to DNA represses adjacent transcription, suggesting that Mad/Max tethers mSIN3 to the E box. The discovery of HD1 (HDAC1) in mammals indicated that this type of repression might occur by interaction between the histone deacetylase and mSIN3, deacetylating adjacent histones and allowing their tails to displace transcription factors[66]. mSIN3A indeed interacts with the histone deacetylases HDAC1 and HDAC2 *in vivo* and this complex contains deacetylase activity. Similar Mad-mediated repression is prevented by the deacetylase inhibitors trapoxin or trichostatin A[67,68]. Moreover, a fusion protein containing the mSIN3A domain that interacts with HDAC and the GAL4 DNA-binding domain represses a reporter gene. mSIN3A mutants lacking this domain can still repress, however, suggesting that interaction with the deacetylase is not the only pathway responsible for repression[68]. The Mad repression complex is also associated with a large (270K) protein (N-CoR), which, together with a related co-repressor (SMRT) is also involved in repression by the ligand-free receptors for thyroid hormone and retinoic acid. These receptors both heterodimerize with the 9-*cis*-retinoic-acid receptor, RXR, and require mSIN3 and histone deacetylase for repression. N-CoR and SMRT may interact directly with mSIN3[69–71].

Some aspects of this mechanism are highly conserved. The yeast protein UME6, which represses some genes involved in meiosis, may tether SIN3 and RPD3 to an upstream repressor DNA sequence. UME6 and SIN3 interact directly[72], but there is little evidence that SIN3 interacts directly with histone deacetylases. Although HDAC1, mSIN3A and RbAp48 can be co-immunoprecipitated when expressed in insect cells, conserved insect proteins may hold the complex together[67]. But other components of deacetylase complexes continue to emerge. Components of the RPD3-like human complex include HDAC1 and HDAC2, p48-like proteins (RbAp46 and RbAp48), which could target the deacetylase to histones, and other proteins (SAP18 and SAP30) of unknown function. SAP18 interacts directly with mSIN3 *in vitro*[73]. Binding to specific target sequences may alter the components of some complexes, however, and only a small fraction of deacetylase complexes may contain RbAp48-like proteins. Figure 3 summarizes one mechanism of deacetylase mediated repression[74].

Future directions

The conservation of histones, HATs and histone deacetylases indicates that modifications of nucleosome structure occur in all eukaryotes, although the mechanisms are still poorly understood. Whether HATs unfold histone N termini from nucleosomal DNA or displace nucleosomes at specific promoters, what histone modifications are required for efficient transcription, or how different acetyltransferases and deacetylases cooperate to produce them, are all still unclear. Moreover, acetylation experiments using free histones, rather than chromatin, *in vitro* can be misleading, and proteins other than histones may also be substrates for these enzymes *in vitro* or *in vivo*. High-mobility-group (HMG) proteins, for example, can be acetylated, and their deacetylation is inhibited by a histone deacetylase inhibitor[74].

Transcription factors that can activate or repress transcription may do so by associating with HATs or deacetylases as appropriate. Nuclear hormone receptors, for instance, with and without ligand, may bind to HATs (p300/CBP and pCAF) and to deacetylases (HDAC1), respectively.

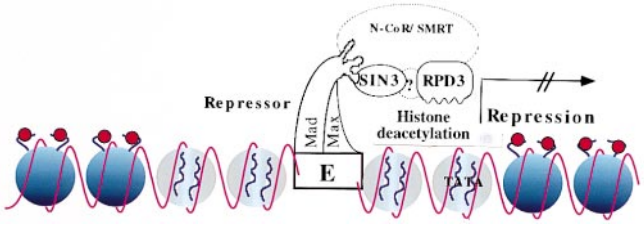

Figure 3 Transcription factors and repression by histone H3/H4 deacetylation. Repressors include Mad (when interacting with Max), UME6 or heterodimerized nuclear hormone receptors. The repressor recognizes its DNA-binding site and interacts directly with SIN3. The interaction of SIN3 with RPD3 (HDAC) may not be direct. Proteins such as SMRT and N-CoR form part of the complex at certain promoters, and other proteins such as SAP18, SAP30, RbAp46, RbAp48 may form part of the complex before or after it interacts with a DNA-bound repressor. Deacetylation would then allow the otherwise extended and acetylated histone N termini (red dots) to bind DNA, preventing access to transcription factors. It is assumed that repression can occur on either side of the repressor even when the repressor is not upstream of the transcription start site. E, E box.

Five histone deacetylases are known in yeast, suggesting that new ones will be found in higher eukaryotes. Are they involved in gene-specific repression, or do they act on larger chromosomal domains, perhaps at specific periods of the cell cycle? Which sites on which histones do they affect? And do they affect all promoters or chromosomal domains equally? Mutations in HATs, deacetylases and components of these complexes are now available in yeast, and a combination of genetics and biochemistry should clarify the relation between chromatin structure and gene activity. The results may have implications for human disease. The interaction of Mad and Max is important in tumour suppression[74], as is the integrity of the thyroid-hormone repressor. Moreover, drugs targeting histone deacetylases have been used against malaria and toxoplasmosis[75]. This much is certain: histones are important regulators of gene activity. □

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