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Minireview

Histone Acetylation and Chromatin Assembly: A Single Escort, Multiple Dances?

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The compaction of eukaryotic DNA into chromatin directly affects transmission of genetic material to daughter cells as well as the establishment of specific patterns of gene expression. How chromatin assembly is achieved and regulated in vivo is still largely a mystery. The fundamental building block of chromatin is the nucleosome, which is comprised of an octamer of histone proteins (two molecules each of histones H2A, H2B, H3, and H4) and 146 base pairs of DNA wound around the octamer. Histone synthesis and nucleosome assembly are closely tied to DNA replication in most cells, but how these processes are coupled is not clear. In this issue of Cell, Stillman and colleagues (Verreault et al., 1996, this issue) describe the isolation of a Chromatin Assembly Complex (CAC) from nuclei of human cells which assembles nucleosomes in a replication-dependent manner. Interestingly, CAC consists of the previously identified, three-subunit Chromatin Assembly Factor 1 (CAF-1), H3, and specific acetylated isoforms of H4. Also in this issue, Gottschling and coworkers (Parthun et al., 1996, this issue) describe the identification of a yeast histone acetyltransferase complex of the B type (HAT B, see below) likely to establish the acetylation pattern associated with deposition of nascent H4 into chromatin. Surprisingly, the yeast HAT B complex and the human CAF-1 complex contain structurally related 48 kDa subunits, providing a direct molecular link between histone acetylation and chromatin assembly. Moreover, human p48 has been isolated previously in association with a histone deacetylase (HD1) (Taunton et al., 1996) and the Retinoblastoma (Rb) tumor suppressor protein (Qian et al., 1995) and has therefore acquired the name RbAp48. Together these findings indicate that histone acetylation, deacetylation, and chromatin assembly may be coordinated through common p48 subunits, and that p48 or other factors involved in these processes may provide unique targets for the regulation of cell proliferation.

Histone Acetylation: Who, Where, and Why?

The vast majority of histone synthesis is tightly coupled to DNA replication in somatic cells, and newly synthesized histones are quickly recruited to replication forks in the nucleus for assembly into chromatin. More than 20 years ago, it was reported that nascent H3 and H4 were post-translationally modified in the cytoplasm prior to their transport into the nucleus and assembly into chromatin, and that these modifications were removed after assembly (Ruiz-Carillo et al., 1975; Jackson et al., 1976). Indeed, diacetylation of two specific lysines in newly synthesized histone H4 (K5, K12) is observed in a wide range of organisms (reviewed by Brownell and Allis, 1996) suggesting that this highly conserved acetylation pattern has an important, yet presently unknown, function in H4 assembly into chromatin.

Two major classes of histone acetyltransferases have been operationally defined with respect to their intracellular location and substrate specificity (Brownell and Allis, 1996). Acetylation of newly synthesized H4 is catalyzed by cytoplasmic HAT Bs, while nuclear histone acetylation is carried out by HAT A activities. Until recently, little was known about the regulation of these HATs because none had been isolated and cloned. However, the landmark cloning of the catalytic subunit of a yeast HAT B (Hat1p; Kleff et al., 1995; Parthun et al., 1996) and a ciliate HAT A (highly similar to yeast Gcn5p; Brownell et al., 1996) has changed this situation remarkably (see below). In addition, Parthun et al. provide several important new clues into the regulation of Hat1p by showing that the yeast HAT B complex contains a second protein, Hat2p, that is highly similar to the human Rb-associated protein, p48 (RbAp48, a member of an evolutionarily conserved subfamily of WD-repeat proteins). Association of the Hat2p subunit with Hat1p increases binding to the H4 amino terminus and the specific activity of HAT B. This finding indicates that Hat2p is a key regulatory subunit of the HAT B complex.

What is the function of histone acetylation? Histones in general possess a globular core domain, required for histone–histone interactions central to nucleosome formation, and highly charged, unstructured tail domains that protrude from the octamer. These tails are important both for histone–DNA interactions and for interactions with other nonhistone proteins. Neutralization of the positive charge associated with specific, often highly conserved, lysine residues within the histone tails (see below and Figure 1) by acetylation provides a reversible mechanism for regulating these interactions. In the nucleus, all four histones are subject to acetylation, and acetylation has long been proposed to 'loosen' histone–DNA contacts within the nucleosome to facilitate binding of transacting, regulatory proteins to nucleosomal DNA. Acetylation also appears to play a critical role in influencing histone interactions with specific nonhistone regulatory proteins (Brownell and Allis, 1996).

What governs the targeting and specificity of these interactions? The discovery that a yeast transcriptional adaptor protein, Gcn5p, functions as the catalytic subunit of a HAT A activity (Brownell et al., 1996) suggests that HAT As are "recruited" to specific genes through selective protein–protein interactions with a subset of transcription factors. Perhaps HAT B's are similarly targeted to different cytosolic complexes and nuclear replication forks through interactions between the WD40 domain (a known protein–protein interaction domain) in Hat2p and other proteins. Remarkably, the acetylation site specificity of these two classes of enzymes, which both recognize the amino terminus of H4, is nonoverlapping and is apparently generated by residues flanking the lysines to be acetylated. Sites in H3 (K14) and H4

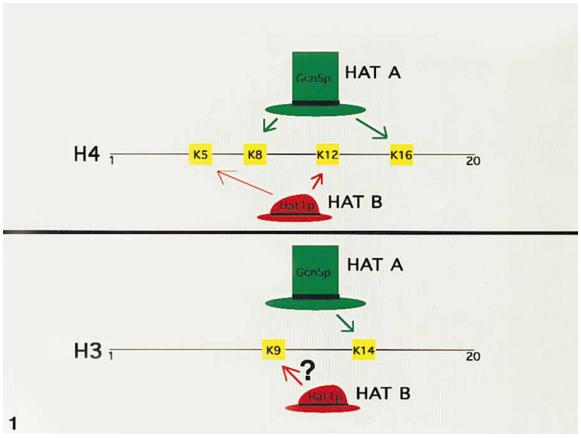

Figure 1. Deposition- and Transcription-Related Acetylation Sites Are Distinct and Nonoverlapping

In vivo and in vitro studies suggest that sites of acetylation in H3 and H4 are remarkably nonrandom. For example, lysines (K) 5 and 12 are acetylated in newly synthesized H4 in a wide range of eukaryotes. Cytosolic HAT B (Hat1p/Hat2p in yeast, shown as a red HAT) preferentially acetylates H4 at K12 and may also modify K5 (rHat1p). In contrast, transcription-related acetylation sites, catalyzed by A-type HATs such as yeast Gcn5p (shown as a green HAT), preferentially acetylate H4 lysines 8 and 16. Potential sequence recognition sites for the A and B-type HATs have been reported; interestingly, the second residue following the acetylation site is typically a G in HAT Bs and a non-G residue in HAT As. Although depositon-related acetylation of H3 has been reported, the extent and sites of this modificaiton are variable among diverse organisms, and it is presently unclear if a distinct HAT B type enzyme labels nascent H3.

(K8, K16) acetylated by yeast Gcn5p (Kuo et al., 1996) are completely different from Hat1p-mediated sites of acetylation in H4 (K12 for native yeast enzyme; K5 and K12 for recombinant Hat1p), and from sites acetylated in newly synthesized yeast H3 (K9) (Figure 1). The transcription related acetylation events could be introduced after removal of deposition-related acetylations to reprogram gene expression. Alternately, HAT A activities may build upon patterns of acetylation introduced by HAT Bs to achieve more highly acetylated histone H4 isoforms.

Nucleosome assembly is a two-step process, wherein H3–H4 tetramers are first deposited onto newly replicated DNA, with the subsequent addition of H2A–H2B dimers. Interestingly, CAF1 performs the first step of the assembly process bringing H3 and H4 to replicating DNA. The cytoplasmic acetylation of H3 and H4 may be important for several aspects of this process. Since the histones are highly charged, it may be necessary to sequester them in some way to prevent promiscuous binding to other proteins or nucleic acids. Acetylation would neutralize part of this charge, as could specific binding of escort proteins such as Hat2p (RbAp48). The unique combination of deposition-related acetylation sites might also allow the newly synthesized histones to be distinguished easily from bulk histones in the nucleus. These unique acetylation patterns may facilitate the targeting of newly synthesized histones to replication forks. Moreover, since CAF1 preferentially utilizes the modified, cytoplasmic isoforms of histones H3 and H4 (Kaufman et al., 1995), chromatin assembly by CAF1 is restricted to a time in the cell cycle when these nascent, modified histones are available. This restriction may form part of the tight link between chromatin assembly and S phase and may provide a window during chromosome replication when histones are uniquely modified for changes in gene expression patterns.

One outstanding question is whether both H3 and H4 need to be concomitantly acetylated for assembly in vivo. Clearly deposition of H3 and H4 is linked via their association with CAF1 in the CAC assembly (Kaufman et al., 1995). Interestingly, deletion of either the amino-terminal tail domain of H3 or H4 is viable in yeast, but deletion of both is lethal (Morgan et al., 1991; Ling et al., 1996). These tail domains, then, may serve redundant functions in nucleosome assembly. This concept is further illustrated by the finding that the H3 tail can be replaced by the H4 tail, and vice versa, without loss of nucleosome assembly or viability. These substitutions, however, do affect the regulation of specific genes, supporting the idea that these tail domains have separate functions in nucleosome assembly and transcriptional regulation (Ling et al., 1996).

Acetylation/deacetylation of H4 (and presumably H3) may also be important for chromatin maturation and protection of genome integrity. Substitution of all four lysines for arginine in H4, which should mimic the fully unacetylated form of the histone, leads to either loss of viability or extremely slow growth, even in the presence of wild type H3 (Megee et al., 1995). Substitution of all four H4 lysines for glutamine (Q), which should mimic the fully acetylated H4, causes a delay in G2/M progression and thus, neutralization of the lysine charges in H4 are apparently not enough to allow completion of the cell cycle. Although the quadruple Q substitution may have an effect on the overall structure of the tail domain, it is interesting that the insertion of an additional lysine into this mutant tail suppresses the G2/M block. It is especially intriguing that the peptide used to insert the extra lysine in these experiments fortuitously recreates at least part of the consensus sequence (GKXG) suggested by Parthun et al. as the recognition site for Hat1p. Perhaps acetylation of a lysine in the H4 tail by this enzyme not only serves to direct newly synthesized H4 to CAF1 for chromatin assembly, but also serves as a signal for the further maturation of chromatin and progression of the cell cycle.

The p48 Family: Histone Escorts

This has been a banner year for the cloning of histone-modifying activities, with the identification of the HAT A and HAT B catalytic subunits described above, and the isolation and cloning of mammalian HD1, a histone deacetylase with remarkable similarity to the yeast transcriptional regulator, Rpd3p (Taunton et al., 1996). HD1 was isolated by its ability to bind the irreversible deacetylase inhibitor, trapoxin. Interestingly, incubation of mammalian cell cultures with trapoxin and other deacetylase inhibitors (trichostatin and sodium butyrate) causes G1 and G2 arrests, indicating that histone deacetylation is also linked to cell cycle control. Notably, HD1 is associated with a p48 family member (RbAp48) providing a further molecular link between histone acetylation, chromatin assembly and histone deacetylation (see below).

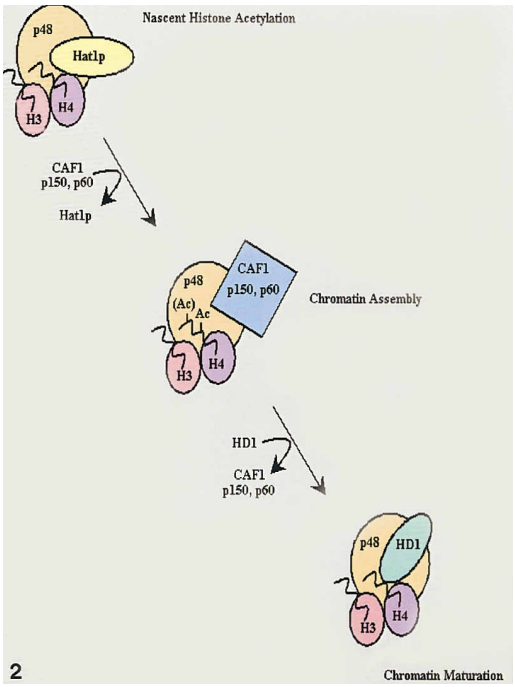

Figure 2. Model for Linkage of Histone Acetylation, Chromatin Assembly, and Chromatin Maturation through a Common Regulatory Subunit

Newly synthesized H3 and H4 associate in the cytoplasm and H4 is rapidly acetylated by HAT B activities, which include a member of the p48 family of histone escorts. Acetylated H4 and H3 subsequently become closely associated with the chromatin assembly factor 1 (CAF1), which also contains a closely related p48 family member. This chromatin assembly complex (CAC) facilitates the formation of new H3–H4 tetramers onto replicating DNA. Removal of deposition-related acetyl groups (in tetramers or completed octamers) by histone deacetylase (HD1 or Rpd3 in yeast), which is also associated with a p48 family member, may be prerequisite for maturation of chromatin into more stable, higher order states and for the establishment of transcription-related patterns of acetylation. The high degree of similarity of the p48-like subunits in HAT B, CAF1, and the deacetylase complex suggest coordinate regulation of these events. However, this model relies upon functional identity between p48 family members which has not yet been established.

The unexpected finding that the yeast HAT B complex, human CAF1, and HD1 all share related subunits suggests that p48 family members may act as "integrators" to coordinate biochemical steps in the processes of histone acetylation/deacetylation, chromatin assembly, and chromatin maturation (Figure 2). In the cytoplasm, a p48 family member such as Hat2p could "escort" newly synthesized H3 and H4 to Hat1p for acetylation of H4 and then, recruit other CAF1 subunits for movement into the nucleus and assembly of tetramers at replication forks. The absence of HAT activity in isolated CAF1 fractions (Verreault et al., 1996) argues against concomitant interaction between p48, Hat1p, and the other CAF1 subunits. Upon deposition of histones onto replicating DNA, the p48 subunit could attract HD1 to remove the deposition-related acetyl moieties (at K5 and/or K12 in H4). Indeed, mutations in *RPD3*, a yeast HD1 homolog, result in increased acetylation of K12 in H4, suggesting Rpd3p normally deacetylates this residue (Rundlett et al., 1996). Deacetylation of the histones could be prerequisite to release of CAF1 subunits and subsequent maturation of chromatin. The model as depicted in Figure 2 presumes that these highly related family members have overlapping or identical functions. It is also possible that individual p48 family members specifically escort histones for a single process. In this case, regulation might still be coordinated through interactions between p48 family members, which could transfer the histones sequentially from one process to the next. Interestingly, an 'integrator' function has also been suggested for CBP (and is likely for the highly related p300; Yang et al., 1996), which coordinates interactions between various nuclear activators, coactivators and Gcn5p-related HAT A activities required for the regulation of gene expression (Kamei et al., 1996; Yang et al., 1996).

Disruption of p48-histone interactions or occlusion of p48 family members from HAT B, CAF1, or the HD1 complex could halt the above cascade of events, and thereby inhibit progression of the cell cycle (through S-phase and G2/M). Indeed, sequestration of p48 by the Rb tumor suppressor protein could contribute to the inhibition of cellular proliferation by interfering with any or all of these partnerships. At present, however, the physiological significance of Rb interactions with p48 is unclear. Since CAF1 is also needed for reassembly of chromatin after DNA repair (Gaillard et al., 1996), p48 might also act as a sensor to limit cell cycle progression in the face of DNA damage.

If the transfer of histones from one p48-containing complex to another is crucial to chromatin assembly and cell cycle advance, loss of Hat1p, Hat2p, or other members of the cascade should result in severe growth defects. However, disruption of the *HAT1* or the *HAT2* gene, or both, has little consequence on cell growth or viability. This may be due to the presence of redundant HAT or p48-related activities in yeast. Indeed, Parthun et al. have observed multiple HAT activites in yeast cytoplasmic extracts, and at least one other yeast gene homologous to p48, *MSI1*, has been noted by these and other researchers (Qian et al., 1995).

Chromatin Modifying Activities, Cancer, and the Future

The remarkable flurry in identification of chromatin-modifying activities in recent times has been accompanied by unexpected connections between these activities and cellular transformation. The possible link between Rb function as a tumor suppressor and the function of p48 as a "histone escort" is intriguing, but is at present very speculative. However, disruption of interactions between a Gcn5p (HAT A) homolog, P/CAF, and its cofactors, p300 or CBP, by the product of the viral E1A oncogene is required for E1A-mediated cellular transformation (Yang et al., 1996). Translocation of another putative acetylase, MOZ, and in-frame fusion to CBP is associated with specific subtypes of acute myeloid leukemias (Borrow et al., 1996). MOZ is homologous to the yeast gene *SAS2* (Something About Silencing) which is required for silencing in yeast (Reifsnyder et al., 1996), suggesting the human gene may also participate in silencing functions. Understanding the nature, regulation, and specificity of these activities is no longer just the pursuit of those interested in understanding the

structure and function of chromatin, but is now directly relevant to our understanding of both normal cellular regulatory processes and abnormal processes which lead to oncogenesis.

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