Targeting Chromatin Disruption: Transcription Regulators that Acetylate Histones

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An intimate relationship exists between the transcriptional machinery and the chromosomal environment within which it functions. The histones package all chromosomal DNA into nucleosomes, yet mutations in individual histones have specific consequences for the expression of particular genes (Grunstein et al., 1992; Kruger et al., 1995). How can general DNA packaging proteins contribute to the specific regulation of transcription? One mechanism is to target regulatory proteins that modify chromatin structure to particular promoters.

Histone Acetylation and the Functional Specialization of Chromatin

Nucleosomes vary considerably in composition from one chromosomal domain to another. A major variable in nucleosomal composition is the extent to which individual core histones are acetylated (Turner et al., 1992). There is a general correlation between the level of his-tone acetylation and the transcriptional activity of a chromosomal domain (Jeppesen and Turner, 1993). Biochemical analysis reveals that hyperacetylated histones accumulate precisely within particular active chromatin domains (Hebbes et al., 1994) and hypoacetylated histones accumulate within transcriptionally silenced domains (Braunstein et al., 1993). These observations are consistent with the structural consequences of incorporating hyperacetylated histones into the nucleosome. Acetylation occurs on lysine residues within the basic amino N-terminal tail domains of the core histones. These lie towards the outside of the nucleosome (Figure 1). Histone hyperacetylation directs an allosteric change in nucleosome conformation, destabilizes higher-order structure and renders nucleosomal DNA more accessible to transcription factors (Lee et al., 1993; Garcia-Ramirez et al., 1995). These structural transitions are a consequence of the reduction in the capacity of the acetylated N-terminal tails to stabilize the path of DNA in the nucleosome through charge neutralization. Thus acetylation of the histones destabilizes chromatin structure, perhaps alleviating repressive histone-DNA interactions and facilitating the transcription process. As appealing as this hypothesis may be, several major unanswered questions remain before the directed regulation of histone acetylation should be considered to be a causal agent in transcriptional activation.

A major question is whether histone hyperacetylation is a necessary prerequisite for chromatin disruption before transcription initiates, or whether histone hyperacetylation is a consequence of chromatin disruption during the transcription process. A second related question concerns how targeting of particular chromosomal domains leading to the accumulation of hyper- or hypoacetylated histones is accomplished. Changes in histone acetylation are a result of the differential activity of nuclear histone acetyltransferases or deacetylases within particular chromosomal domains. Characterization of these enzymes at the molecular level (Brownell et al., 1996, this issue) provides answers to both of these questions and offers significant insights into the potential role of histone acetylation in the transcription process.

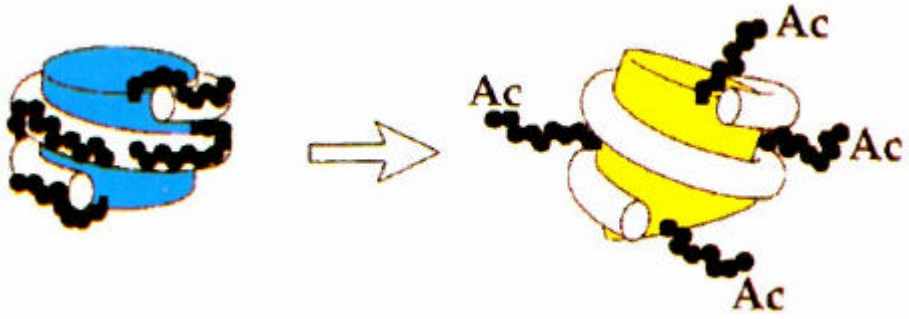

Figure 1. Histone Hyperacetylation Alters Nucleosomal Conformation
A nucleosome with hypoacetylated histones (blue) is more stable than a nucleosome with acetylated histones (yellow). Acetylation renders the histone tails (black) more mobile.

A Transcriptional Regulator That Acetylates Histones

Purification and molecular characterization of a subunit of a Tetrahymena nuclear histone acetyltransferase (p55) reveals a surprising identity to a yeast transcriptional regulator GCN5p (Brownell et al., 1996). Subsequent experiments show that GCN5p itself is a histone acetyltransferase (Brownell et al., 1996). This observation does not establish that GCN5p directs the specific acetylation of core histones in vivo, but it does clearly indicate that the potential for targeted histone modification exists.

There are two extensive regions of greater than 60% identity between the Tetrahymena acetyltransferase and yeast GCN5p. A central domain of 130 amino acids shows homology to other cloned acetyltransferases including a known yeast histone acetyltransferase (Kleff et al., 1995). This domain is predicted to possess the acetyltransferase activity. The second conserved domain is a 60 amino acid segment at the carboxyl terminus that is required for GCN5p function in yeast and that comprises a bromodomain (Marcus et al., 1994). This motif is found in many transcriptional regulators including the yeast SNF2/SWI2 proteins (Tamkun et al., 1992). Two related functions have been proposed for the bromodomain: nuclear compartmentalization and protein-protein interactions (Marcus et al., 1994; Brownell et al., 1996).

GCN5p is a regulatory molecule that facilitates the action of acidic activators in yeast such as GCN4 and GAL4-VP16 (Georgakopoulos and Thireos, 1992; Marcus et al., 1994). GCN5p functions as a complex with two other proteins, the coactivator ADA2p and ADA3p. ADA2p interacts directly with the acidic activation domain of VP16 and with TATA-binding protein (TBP) (Barlev et al., 1995). Thus the potential exists for GCN5p to be recruited to a specific gene through selective interactions with a subset of transcription factors. Once in the vicinity of the promoter, GCN5p may interact with the basal transcriptional machinery and bias the existing equilibrium between histone acetylation and deacetylation towards the progressive accumulation of nucleosomes containing acetylated histones. Targeted histone acetylation could thus directly contribute to the transcriptional activation process by disrupting repressive chromatin structure and facilitating the sequestration of the basal transcriptional machinery (Figure 2). Other, more elaborate possibilities should, however, also be considered.

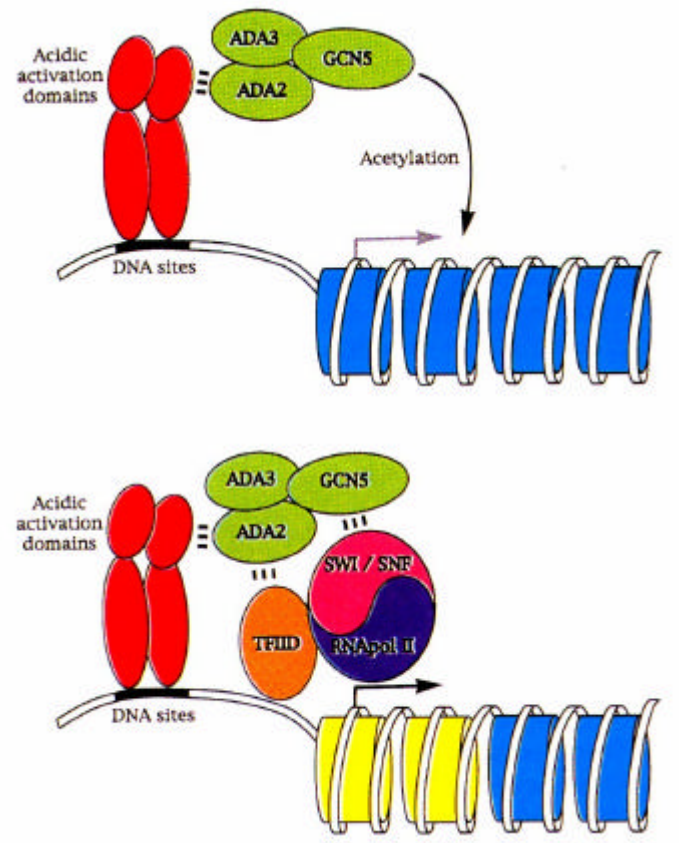

Figure 2. A Model for Interactions and Potential Functions of the ADA2p-ADA3p-GCN5p Complex (see text for details)

Compartmentalizing Chromatin Disruption

Histone hyperacetylation directed by GCN5p could potentially occur throughout the entire nucleus, within a chromosomal domain, within an array of nucleosomes, or within a single nucleosome. Each of these possibilities require targeting, however, there is significant variation in precision and structural consequences. Any mechanism that increases the level of histone acetylation in chromatin might be expected to facilitate transcription. If GCN5p acetylates all of the histones within the nucleus, then general effects on the transcription of many genes would be anticipated from alterations in GCN5 function. In fact GCN5p appears selective in the genes that it regulates (Georgakopoulos and Thireos, 1992; Marcus et al., 1994). The recruitment of the regulatory complex ADA2p-ADA3p-GCN5p to an individual promoter through contacts with a particular set of specific DNA binding transcription factors clearly implies that histones and potentially other proteins in the immediate vicinity of the regulatory complex will become hyperacetylated. This hyperacetylation process might be targeted not only by the tethering of the acetyltransferase but also by the architecture of the transcription-factor-coactivator complex and specific features of local chromatin structure such as positioned nucleosomes (Figure 3, top). Disruption of repressive histone-DNA contacts in one or two nucleosomes could easily facilitate the stable association of components of the basal transcriptional machinery (Lee et al., 1993). It is also possible that several adjacent nucleosomes might be acetylated further disrupting higher-order chromatin structure (Garcia-Ramirez et al., 1995). Such disruption would enhance not only recruitment of the basal machinery but also the generation of a productive transcription elongation complex including RNA polymerase II.

Recent observations indicate that the RNA polymerase II holoenzyme contains components of the SWI/SNF family of global transcriptional regulators (Wilson et al., 1996). SWI/SNF global regulators also contend with chromatin (Tamkun et al., 1992; Kruger et al., 1995). To consider how more extensive domains of chromatin are targeted for modification, we must consider the properties of the SWI/SNF complex. SWI/SNF facilitates the association of components of the basal transcriptional machinery with nucleosomal DNA under particular conditions: a large molar excess of SWI/SNF proteins over nucleosomal DNA and a very precise positioning of the TATA box relative to the surface of the histone octamer (Imbalzano et al., 1994). Histone hyperacetylation is also reported to allow association of the basal transcriptional machinery in the absence of SWI/SNF (Imbalzano et al., 1994). Thus SWI/SNF activities and histone hyperacetylation might have a comparable impact on chromatin structure. The process of transcriptional elongation with the concomitant movement of the RNA polymerase through chromatin (or vice versa) might enable the SWI/ SNF complex and histone acetyltransferases to destabilize nucleosomes within an entire transcription unit or chromosomal domain (Figure 3, bottom). In fact, histone-DNA crosslinking experiments strongly support a precise demarkation of histone acetylation states within individual chromosomal domains (Hebbes et al., 1994).

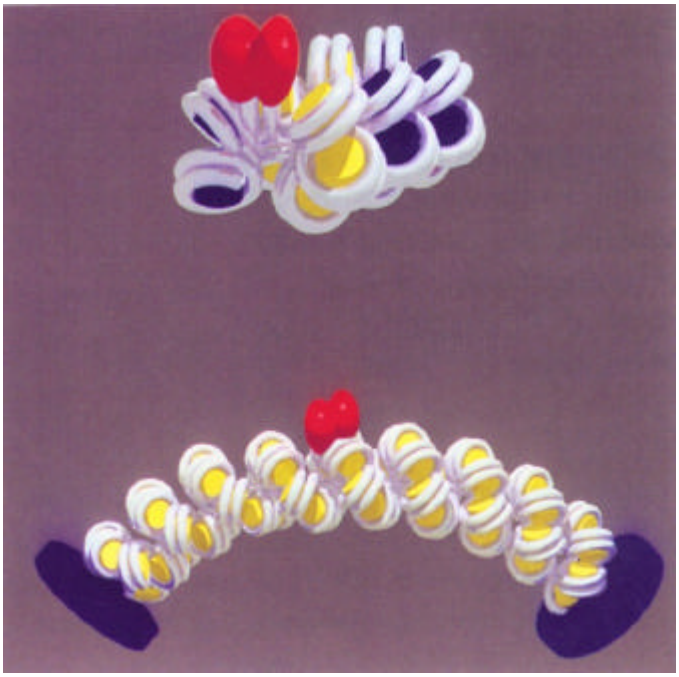

Figure 3. Targeting Histone Hyperacetylation
Nucleosomes with hypoacetylated histones are shown in blue; nucleosomes with hyperacetylated histones are shown in yellow. (Top) A restricted number of nucleosomes (six) in the vicinity of the targeted acetyltransferase (red) are modified. (Bottom) Histone hyperacetylation extends over nine turns of the chromatin fibre comprising an entire chromatin domain. The boundaries of the domain are indicated by the large blue discs at each end of the acetylated chromatin domain. (See text for details).

Perspective

The discovery that a transcriptional regulator GCN5p is a histone acetyltransferase (Brownell et al., 1996) provides a new set of possible mechanisms by which transcription might be regulated. These mechanisms lead to a model for the targeted disruption of chromatin structure that requires the selective recruitment of GCN5p to a particular regulatory element. It is of course possible that GCN5p acetylates transcription components other than histones, or that the acetyltransferase activity has no influence on transcription, however, these possibilities seem unlikely in view of the known properties of the Tetrahymena p55 protein and the strong correlation between histone acetylation and transcription.

Several observations indicate that there may be other targeted transcriptional regulators in addition to GCN5p with the capacity to modify histones. GCN5p is not an essential gene for viability in yeast (Georgakopoulos and Thireos, 1992) and the Tetrahymena histone acetyltransferase appears to selectively modify histone H3 (Brownell et al., 1996). Several distinct patterns of histone acetylation have been defined for individual core histones. Thus, other transcriptional regulators might acetylate different core histones with distinct specificities for different lysine residues in the N-terminal tails. Such capacity for covalent modification through acetylation emerges as a novel function for the transcriptional machinery.

The question why mutation of the N-terminal tail domains of individual histones has specific consequences for the expression of particular genes (Grunstein et al., 1992) is answered by the specificity and targeting of acetylation patterns as a component of the transcription process. The interaction of the histone acetyltransferase with regulators that themselves interact with DNA binding proteins explains the targeting phenomenon. The potential specificity of histone acetylation patterns directed by a particular acetyltransferase, or the specific requirements for acetylation at an individual promoter can account for why mutations in the N-terminal tails of the histones influence transcription of a restricted set of genes. What emerges from these observations is the opportunity for chromatin structure to be precisely modulated through highly regulated reversible mechanisms. Such modifications might be a prerequisite for transcriptional activation.

The recognition that transcription factors might function through enzymatic activities that modulate chromatin structure is important for our understanding of both transcriptional regulation per se and the role of chromatin structure in the nucleus. Gene regulation in eukaryotes involves substantial communication between architectural proteins such as histones and the transcriptional machinery itself.

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