Protein acetylation: more than chromatin modification to regulate transcription

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Histone acetyltransferases and deacetylases are involved in the regulation of gene transcription. Recently, tumor suppressor protein p53 has been shown to be a target for transcriptional coactivators that have histone acetyltransferase activity, suggesting acetylation is also involved in the regulation of cell proliferation and tumorigenesis.

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Perhaps the most nebulous role in the control of gene expression has been that of chromatin. Although the rate of transcription of a gene is clearly controlled by a complicated interplay of signaling pathways at the promoter, histones and their modification by acetylation have, until the last few years, been the object of much speculation but little progress. Recently, a combination of biological and chemical approaches have led to the definition of some of the enzymes that add or remove acetyl groups on histones. The possibility that these enzymes and resulting modifications extend their control to processes such as cell proliferation and protection of the genome by modifying the function of p53 has been raised by recent studies and will be the subject of this minireview.

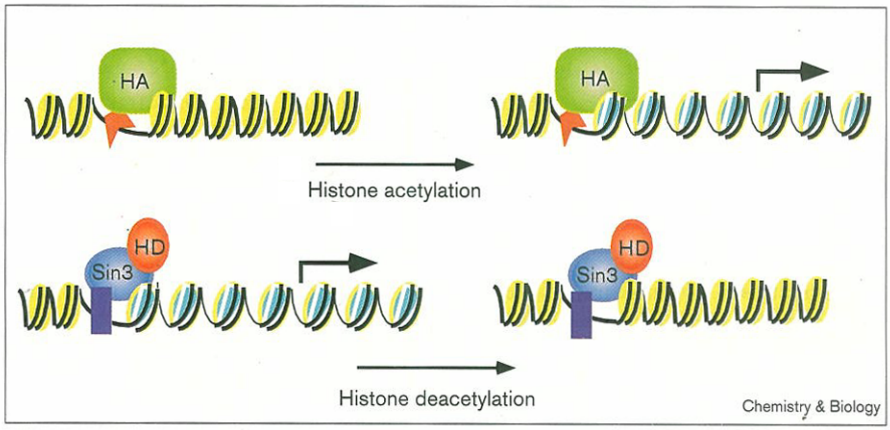
The association of histone acetylation with activated transcription

Chromatin structure has been generally correlated with transcriptional activity: regions of cytologically dense heterochromatin are transcriptionally silent relative to less condensed euchromatin. Biochemical analysis using antibodies that recognize histones acetylated at specific amino-terminal residues established that transcriptionally active chromatin is enriched for acetylated nucleosomes [1], whereas silencing of the mating type cassettes of the yeast *Saccharomyces cerevisiae* correlates with hypoacetylation of nucleosomes [2]. The target sites for acetylation on histones H3 and H4 are specific lysine residues on the amino-terminal tails that lie outside the nucleosome core. The purpose of this acetylation may be to neutralize the cationic charge of the amino-terminal tail with the consequence of destabilizing the interaction of this region with DNA and opening chromatin. Acetylated nucleosomes have an altered conformation that is more accessible to sequence-specific DNA-binding proteins [3].

Recently, a series of studies from widely differing experimental systems have culminated nicely in a new model to show how transcription can be regulated through the specific recruitment of histone acetylases and deacetylases to a promoter (Figure 1). The first evidence implicating the recruitment of histone deacetylases to the promoter as a mechanism for transcriptional repression came with the identification of the biological target of the chemical drug trapoxin. Trapoxin is a cell cycle inhibitor known to irreversibly inhibit histone deacetylase activity. Taunton *et al.* [4] reasoned that trapoxin may mimic the structure of an

Figure 1

Recruitment of histone acetyltransferase (HA) and histone deacetylase (HD) activity to regulate transcription. Sequence-specific DNA binding by activator proteins with associated coactivators that acetylate histones alters the structure of the surrounding chromatin and the activates transcription. Association of complexes containing histone deacetylases with DNA binding repressor proteins produces a closed chromatin structure that is refractory to transcription.



acetylated lysine residue and bind directly to a histone deacetylase. Using the activity of trapoxin as part of a protein purification strategy, they were able to clone the first gene encoding a histone deacetylase termed HD1 or HDAC1 [4]. Surprisingly, the primary structure of HD1 was remarkably homologous with the yeast protein Rpd3. Rpd3 had been identified as part of a transcriptional corepressor complex also containing the Sin3 protein [5], but a biochemical activity had not yet been characterized for the Rpd3 protein. Mammalian homologs of Sin3 can complex with HD1. Corepressor complexes containing Sin3–HD1 appear to lack direct affinity for DNA, but can be recruited to target genes by a variety of DNA-binding transcriptional repressors, the activity of which can be inhibited by trapoxin (reviewed in [6,7]).

The chemical approach to defining histone deacetylases led by Schreiber and colleagues unexpectedly combined with the studies of Brownell *et al.* [8] who succeeded in purifying the principal histone acetyltransferase associated with transcription from *Tetrahymena thermophila*. The cloning of the gene encoding this enzyme revealed that the histone acetyltransferase had extensive sequence similarity to the yeast Gcn5 protein, a component of a coactivator complex necessary for the function of a number of sequence-specific DNA-binding proteins as transcriptional activators [9]. In mammalian cells, a mammalian Gcn5 homolog, termed pCAF, forms a coactivator complex with either of the related proteins CBP or p300 which also have histone acetyltransferase activity (reviewed in [6]). These large proteins can interact with a growing number of sequence-specific DNA-binding transcriptional activator proteins that probably generate the gene specificity for their function.

Acetylation of the p53 tumor suppressor protein

Although p300/CBP histone acetyltransferase activity may be recruited to promoters to open chromatin structure and facilitate transcription, the substrate specificity for the enzymatic activity of these proteins is not limited to histones. The laboratory of Dr. Robert Roeder at Rockefeller University [10] has demonstrated recently that the p53 tumor suppressor protein is also a target for acetylation by p300. p53 is an important regulator of cell growth that is capable of directing the arrest of the cell cycle or the induction of apoptosis in response to stress, most notably DNA damage [11,12]. The biochemical activity best correlated with the antiproliferative effect of p53 is its ability to bind to DNA with sequence specificity and activate transcription. This function is frequently inactivated during carcinogenesis. p53 is found to be mutated in a large proportion of human cancers and most mutations map within the sequence-specific DNA-binding domain [11,12].

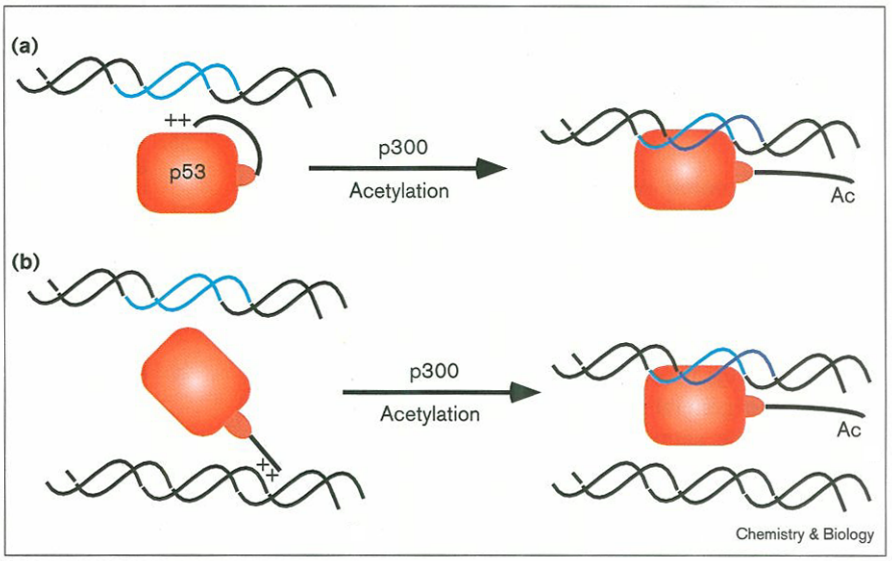
Earlier this year, several groups demonstrated that CBP/p300 can bind to and coactivate transcription with p53 [13–15]. The site of interaction is a well-characterized amino-terminal transactivation domain that can also interact with components of the TAF coactivator complex [16,17]. Gu and Roeder [10] found that the site for acetylation of p53 is at the carboxyl terminus of the protein, apparently distinct from the amino-terminal site of interaction. The carboxy-teminal 26 amino acids of p53 include two clusters in which three of four residues are basic; lysine at the fourth position of each cluster is preferentially acetylated by p300 *in vitro*. The authors also provided important evidence to show that a transiently expressed p53 can be acetylated *in vivo* in this carboxy-terminal region. The proportion of transfected p53 that was acetylated was reported to be low, and it is currently unknown what proportion of endogenous p53 is acetylated in cells.

Regulation of p53 activity through the carboxyl terminus

The acetylation of the carboxyl terminus of p53 has important implications for the ability of p53 to bind to its DNA target site. Sequence-specific DNA binding is directed by a central domain of p53; in DNA-binding studies, however, p53 is latent for its interaction with the DNA target site through an autoregulatory inhibition mediated by the carboxy-terminal 26 amino acids. Binding of the carboxyl terminus of p53 by a specific monoclonal antibody, Pab 421, can reverse the inhibition and activate p53 for sequence-specific DNA binding [18]. Alteration of the carboxy-terminal 26 amino acids by truncation or alternative mRNA splicing yields a p53 protein that is constitutively active for sequence-specific DNA binding [18–21]. Acetylation of p53 by p300 thereby is an attractive means of regulating p53 function. In fact, acetylation at the carboxyl terminus was shown to dramatically activate the latent DNA-binding activity of p53 [10]. Inhibition of sequence-specific DNA binding is likely to be directed by electrostatic interactions through basic residues present in the carboxyl terminus; acetylation serves to neutralize the charge of the lysine sidechain and activate p53.

The mechanism by which the carboxyl terminus of p53 inhibits the core sequence-specific DNA-binding domain is still debated (Figure 2). One model proposes that residues at the carboxyl terminus interact with the core DNA-binding domain, placing p53 in a conformation that is refractory to DNA binding [22,23]. Such an interaction is proposed to be directed by the basic residues at the carboxyl terminus and can be blocked by modification of the carboxyl terminus, such as acetylation, leading to an allosteric transition within p53 to a sequence-specific DNA-binding conformation. Support for this model has come from the observation that the carboxy-terminal domain or synthetic peptides derived from this region are able to activate latent p53 *in trans* for sequence-specific DNA binding *in vitro* [22,23]. The peptides are proposed to block the intramolecular interaction of the carboxyl terminus with the core DNA-binding domain by competition,

Figure 2



Two models to explain how acetylation of the carboxyl terminus of the p53 protein can activate latent sequence-specific DNA binding. **(a)** The carboxyl terminus (depicted as a tail) interacts with the core sequence-specific DNA-binding domain of p53. Acetylation of lysine residues by p300/CBP disrupts this binding and changes the conformation of p53 to allow binding to its target sequence (depicted in blue). **(b)** binding to excess nonspecific competitor DNA by the basic carboxyl terminus of p53 sequesters the sequence-specific DNA-binding domain from interacting with its target sequence. Acetylation of the carboxyl terminus inhibits its nonspecific DNA binding and allows sequence-specific DNA binding to occur. Details are presented in the text.

while lacking inhibitory activity themselves. Very high concentrations of peptide or the carboxy-terminal domain are required to direct the activation of p53 *in trans* which may indicate that the interaction of these peptides with the core domain is very weak relative to the same sequences on the intact protein.

Another plausible mechanism for the inhibition of sequence-specific DNA binding by the carboxy-terminal domain of p53 concerns a biochemical activity intrinsic to the domain: binding to nucleic acids without sequence specificity. The carboxy-terminal domain of p53 is sufficient to direct binding to DNA and can actually catalyze the annealing of single strands of complementary DNA or RNA sequences [19,24,25]. This activity can be disrupted by antibody binding or by alteration of the structure of the carboxyl terminus [21,24]. Because the same modifications to the carboxyl terminus can also activate sequence-specific DNA binding, it was proposed that latency of sequence-specific DNA binding is mediated by nonspecific DNA binding by the carboxyl terminus, precluding the binding of p53 to its specific DNA target. In fact, it has been observed that increasing amounts of nonspecific double-stranded DNA can strongly inhibit sequence-specific DNA binding by p53, but not when the carboxyl terminus is altered by deletion or by antibody binding [21]. Interestingly, however, short (< 25 nucleotide) oligonucleotides were observed to activate latent p53 [22].

Sequence-nonspecific DNA binding by the carboxyl terminus of p53 may be mediated through the interaction of the basic residues in the carboxy-terminal region with the phosphodiester backbone. Acetylation of lysine in this region is likely to disrupt the interaction with nonspecific DNA and thereby free p53 to bind to its target site and activate the transcription of downstream targets, such as p21 and bax, that inhibit cell proliferation. Such a mechanism may explain why very high concentrations of carboxy-terminal peptide are required to activate p53 *in trans*, as these concentrations approach that of the nonspecific competitor DNA typically added to *in vitro* DNA-binding reactions and may saturate the DNA preventing the intact p53 from interacting with this inhibitor.

Prospects and predictions

Whatever the mechanism of the latency of sequence-specific DNA binding might be, it is clear that carboxy-terminal acetylation may be an important activation mechanism for p53 function. The relevance of acetylation at the carboxyl terminus of p53 to p53 function *in vivo* promises to be an active avenue of investigation. It will be essential to determine whether acetylation of p53 is altered during the cellular response to DNA damage. p53 levels and transcriptional activity are dramatically increased in response to DNA damage caused by a stabilization of the normally labile p53 protein. The proportion of acetylated p53 that is acetylated *in vivo* is reported to be low [10]. Is the proportion of transfected p53 increased in response to DNA damage? Does acetylation of p53 influence the half-life of the protein as well as the DNA-binding activity?

The reversibility of p53 acetylation will also be an interesting issue to address. The tonic level of p53 activity may be controlled then by the balance of acetylase activity through p300/CBP and by as yet uncharacterized deacetylases. In this regard, it is curious that trapoxin, an irreversible inhibitor of HDAC1, induces cell cycle arrest or apoptosis in some cell types, each responses that can also

be directed by induction of wild-type p53. Perhaps the effects of trapoxin are caused by inhibiting the deacetylation of p53, shifting the balance of p53 towards an acetylated and activated form that can arrest cell proliferation. It is probably worth determining whether trapoxin is able to induce growth arrest or apoptosis in cells lacking p53.

The demonstration of p53 acetylation by transcriptional cofactors previously known as histone specific acetyltransferases suggests that protein acetylation and deacetylation may possibly be important regulatory modifications in other systems. Although the CREB transcription factor, for which CBP is a coactivator, is not apparently a substrate for acetylation [10], a recent paper has shown that two components of the general transcription machinery, TFIIE and TFIIF, can be acetylated by several histone acetyltransferases [26]. These coactivators may therefore influence transcriptional activation and elongation by mechanisms distinct from their effect on chromatin structure. The design of inhibitors of acetylases will probably be important for determining the specific roles of these proteins during a process as complex as transcription, and it may help to define roles for these proteins in other cellular processes.

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