Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 8807–8808, August 1999

Commentary

Structure and mechanism of action of the histone acetyltransferase Gcn5 and similarity to other *N*-acetyltransferases

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An important posttranslational modification of histones is acetylation of ε-amino groups on conserved lysine residues present in the amino-terminal tails of these proteins. Acetylation neutralizes the positively charged lysines and therefore affects interactions of the histones with other proteins and/or with DNA. Histone acetylation has long been associated with transcriptionally active chromatin and also implicated in histone deposition during DNA replication (1, 2). The first cloning of a histone acetyltransferase (HAT) gene, the yeast *HAT1* gene, was reported in 1995 (3). Subsequently, it was suggested that HAT1 protein is cytoplasmic and involved in histone deposition (4), although the lack of phenotypes of yeast *hat1* mutants, as well as recent evidence that both the human and yeast enzymes are nuclear (ref. 5; S. Tafrov and R.S., unpublished work), makes the *in vivo* function of HAT1 unclear.

A major breakthrough in this field was the purification and cloning of HAT A, a HAT from the macronucleus of the ciliate *Tetrahymena* (6). The sequence of HAT A showed that it was similar to a known yeast transcriptional coactivator, GCN5. Since then, numerous studies have demonstrated that GCN5 (and the related P/CAF) are conserved HATs whose activity on nucleosomes facilitates initiation of transcription (reviewed in ref. 7). Interestingly, GCN5 by itself can acetylate free histones (particularly Lys-14 of H3) but not nucleosomes. Acetylation of nucleosomes by GCN5 requires that it be in one of two large protein complexes called Ada and SAGA in yeast (8). Within the last few years several mammalian proteins, unrelated to GCN5 but also implicated in transcriptional activation, have been shown to have HAT activity. These include CBP/p300, TAF250, ACTR, and SRC-1 (9–12). Thus, it is becoming clear that histone acetylation of nucleosomes is a significant component of the multistep gene activation process.

In this issue of the *Proceedings*, Trievel *et al.* (13) report the crystal structure of the catalytic HAT domain of yeast GCN5. This domain encompasses residues 99–262 of the 439-aa protein. A portion of their structure, consisting of four antiparallel β-strands (β1–4), followed by an α-helix (α3) and another β-strand (β5), is shown in Fig. 1. This part of GCN5 is very similar in structure to that of yeast HAT1 (14), as well as to two other *N*-acetyltransferases whose substrates are not histones, namely aminoglycoside 3-*N*-acetyltransferase (AAT; ref. 15) and serotonin *N*-acetyltransferase (16). All of these enzymes are members of a family of proteins identified by sequence analysis as ones with similarity to known *N*-acetyltransferases such as GCN5, and hence called the GNAT superfamily (17). Members of this superfamily have the common feature that they transfer an acetyl group from acetyl CoA to a primary amino group, albeit very different amino groups for the different enzymes; i.e., onto ε-amino groups on lysines in the case of HATs, onto α-amino groups on the N termini of proteins in the case of enzymes such as ARD1 or MAK3, onto sugars in the case of AAT (15) and GNA1 (18, 19), or onto serotonin for serotonin *N*- acetyltransferase (16). All members of the GNAT superfamily have a conserved motif called motif A (shown in red in Fig. 1), and most of them have two other conserved motifs called B and D.

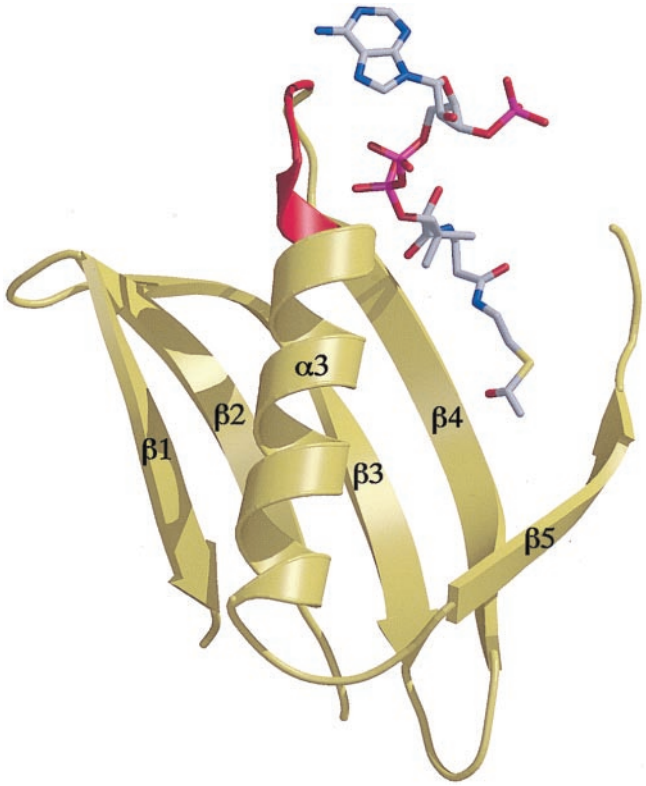

FIG. 1. Ribbon diagram of GCN5 showing the conserved core found in four *N*-acetyltransferases and in an *N*-myristoyl transferase. Motif A of the GNAT superfamily is shown in red. Acetyl CoA as present in HAT1 is modeled into the GCN5 structure. The sulfur in acetyl CoA is shown in yellow.

It was predicted that the conserved motifs in the GNAT superfamily would be involved in binding acetyl CoA because it is the only substrate these diverse *N*-acetyltransferases have in common (17). Indeed, the structure of HAT1 with bound acetyl CoA confirmed that motif A is involved in CoA binding (14), as has the structural work with aminoglycoside 3-*N*-acetyltransferase (15) and serotonin *N*-acetyltransferase (20). In Fig. 1 acetyl CoA has been modeled into the GCN5 structure, based on its position in HAT1 (13, 14). The acetyl CoA is bound in a V-shaped cleft, formed between β-strands 4 and 5. The highly conserved motif A of the GNAT family binds to the pyrophosphate moiety of acetyl CoA. The pantothenate and β-mercaptoethanolamine units of CoA are

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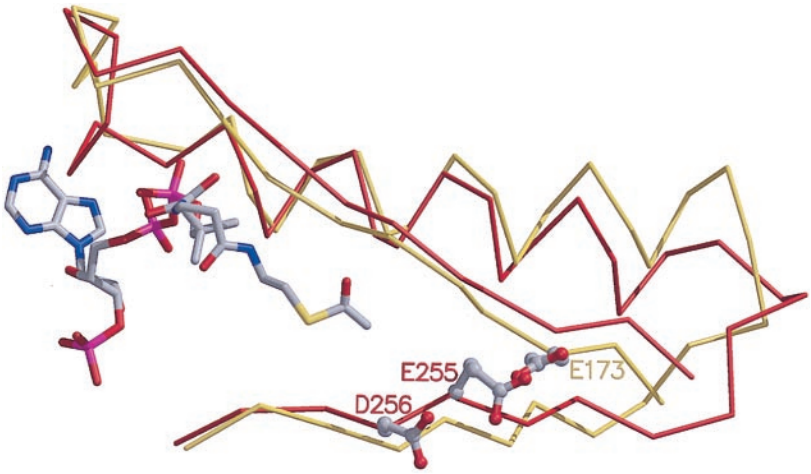

FIG. 2. Superposition of β4-α3-β5 of GCN5 (yellow) and the corresponding region of HAT1 (red). The proteins are represented as Cα traces with bound acetyl CoA. Glu-173 of GCN5, believed to be involved in catalysis, is shown in ball and stick representation, as are residues Glu-255 and Asp-256 of HAT1.

oriented along β4 and hydrogen-bonded to it, thus mimicking a β-strand.

Trievel *et al.* (13) propose a mechanism for catalysis by GCN5. They suggest that a glutamate residue (Glu-173) is positioned to abstract a proton from an NH_3^+ group on the lysine to be acetylated, such that the uncharged amino group then can perform a nucleophilic attack on the carbonyl carbon of the reactive thioester group of acetyl CoA. Fig. 2 shows a superposition of the putative active-site regions of GCN5 (in yellow) and HAT1 (in red) with bound acetyl CoA. Again, notice how structurally similar the two proteins are in this region. According to Trievel *et al.*'s proposal, Glu-173 of GCN5, especially after reorientation of its side chain, would be close enough to the incoming lysine to perform base catalysis. Indeed, mutation of Glu-173 to Gln abolishes activity *in vivo* and *in vitro* (13). There is no Glu or Asp residue at the corresponding position in HAT1. We note, however, that Glu-255 or Asp-256 of HAT1 on the adjacent β-strand of the cleft are positioned so that they could perform the same catalytic function (Fig. 2). Those residues have not been mutated yet.

It is clear from the structures of GCN5, HAT1, and two other members of the GNAT superfamily that they share a conserved core, including the binding site for acetyl CoA (Fig. 1). Interestingly, *N*-myristoyl transferase has a similar structure to the *N*-acetyltransferases discussed above (21, 22), even though this enzyme transfers a much larger acyl group from myristoyl CoA to α-amino groups of glycines at the N termini of substrate proteins. On the other hand, chloramphenicol acetyltransferase, which acetylates a hydroxyl group, does not have a similar structure. It appears that the GNAT enzymes that acetylate amino groups on a diverse set of substrates all bind acetyl CoA in a very similar way, and perhaps share a similar catalytic mechanism. Of course, these enzymes will differ in the regions that bind the substrate to be acetylated. As far as HATs go, the next goal will be to determine a structure with a histone or peptide substrate bound to the enzyme.

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