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Minireview

Structure and functions of the GNAT superfamily of acetyltransferases☆

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Abstract

The Gcn5-related *N*-acetyltransferases are an enormous superfamily of enzymes that are universally distributed in nature and that use acyl-CoAs to acylate their cognate substrates. In this review, we will examine those members of this superfamily that have been both structurally and mechanistically characterized. These include aminoglycoside *N*-acetyltransferases, serotonin *N*-acetyltransferase, glucosamine-6-phosphate *N*-acetyltransferase, the histone acetyltransferases, mycothiol synthase, protein *N*-myristoyltransferase, and the Fem family of amino acyl transferases.

Keywords: Acetyltransferase; Three-dimensional structure; Steady-state kinetics; GNAT; Histone acetyltransferase; Chemical mechanism; Structure and function of enzymes; Protein modification; Antibiotic resistance

The GNAT superfamily

The first members of what is now termed the GCN5-related *N*-acetyltransferase (GNAT)[1] superfamily were identified as aminoglycoside acetyltransferases in bacteria that became resistant to the action of the antibiotics gentamicin and kanamycin [1]. Numerous genes encoding aminoglycoside *N*-acetyltransferases that regioselectively acetylated one of the 4–5 amino groups in aminoglycosides were cloned and sequenced in the 1980s and shown to contain four amino acid "motifs" spanning approximately 100–120 residues, in spite of their low overall sequence homology [2]. Subsequently, they were shown to exhibit sequence homology to a class of eukaryotic transcription factors, the first being the yeast GCN5 in 1992 [3]. The activity of a *Tetrahymena* homologue of the yeast GCN5 as an *N*-acetyltransferase that acetylated histones was reported in 1995 [4], providing a direct link between histone acetylation and transcriptional regulation. The subsequent revelation that yGCN5 was, in fact, a histone acetyltransferase (HAT) provided a model for HAT recruitment to specific promoters by DNA-bound activating proteins [5]. The numerous genome sequencing efforts of the last decade, coupled with powerful bioinformatics approaches to protein superfamily identification, have revealed some 10,000 members of the GNAT family in all kingdoms of life [see http://supfam.mrc-lmb.cam.ac.uk/SUPERFAMILY].

To date, over two dozen members of the family have been structurally characterized, revealing a structurally conserved fold comprised of an N-terminal strand

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[1] *Abbreviations used:* GNAT, GCN5-related *N*-acetyltransferase; HAT, histone acetyltransferase; AgNATs, aminoglycoside *N*-acetyltransferases; SNAT, serotonin *N*-acetyltransferase; AANAT, arylalkylamine *N*-acetyltransferase; YGCN5, yeast GCN5; UDP–MPP, UDP–UDP–*N*-acetylmuramyl pentapeptide.

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followed by two helices, three antiparallel β strands, followed by a "signature" central helix, a fifth β strand, a fourth α helix and a final β strand (Fig. 1A). The superposition of 15 GNAT structures (Fig. 1B) reveals that these elements are nearly universally conserved in spite of structure-based sequence alignments that reveal 3–23% pairwise sequence identity. Beta strands four and five splay apart, presumably as the result of a "β bulge" in strand four, and the disruption of the antiparallel interaction between these two strands provide backbone hydrogen bonding partners for the β-alanine portion of the pantetheine arm of AcCoA. Differences between GNAT structures are generally confined to the immediate N-terminus, with much greater variation at the C-terminus, which can be extended significantly. An earlier structural overview of this superfamily appeared in 2000 [6]. This review will focus on recent advances in our understanding of the structures and chemical mechanisms of members of this large superfamily for which both have been interrogated.

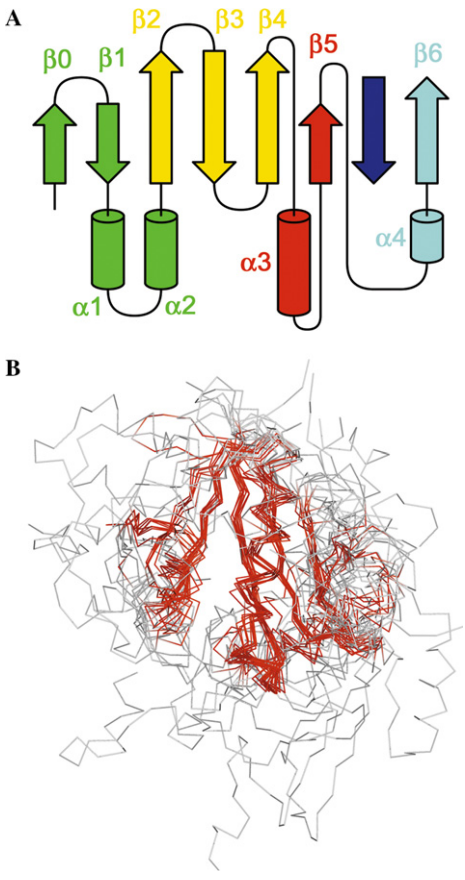

Fig. 1. (A) Topology of the core GNAT fold. From the N-terminus, secondary structural elements are colored green (β1,α1,α2), yellow (β2–4), red (α3,β5) and blue (α4,β6). The dark green (β0) N-terminal strand is not completely conserved and the deep blue C-terminal strand may be from the same monomer, or contributed by another. (B) Superposition of 15 GNAT structures. Residues in which the rmsd is <2.7 Å are highlighted in red.

Aminoglycoside *N*-acetyltransferases

Aminoglycoside *N*-acetyltransferases (AgNATs) catalyze the regioselective acetylation of one of the four amino groups found on a diverse set of aminoglycosides with antibiotic properties. Acetylation reduces the affinity of these compounds for the acceptor tRNA site on the 30S ribosome by four orders of magnitude [7], effectively making bacteria expressing these genes resistant to the antibiotic. They were the first of the GNAT superfamily members to be identified and were the subject of the first detailed sequence comparison studies that allowed for subsequent identification of the GNAT superfamily [2]. They have been the subjects of intensive kinetic, enzymological and structural study due to the clinical importance of aminoglycoside resistance (Fig. 2). Northrop performed some of the earliest kinetic studies [8], but the gentamicin acetyltransferase with which these studies were performed was never crystallized. Similarly, the first of the aminoglycoside *N*-acetyltransferases to be structurally determined, the *Serratia marascens* 3-*N*-acetyltransferase, was never kinetically characterized [9]. This discussion will thus focus on the *Mycobacterium tuberculosis* 2′-*N*-acetyltransferase, the *Enterococcus faecium* and *Salmonella enterica* 6′-*N*-acetyltransferases for which both detailed structural and kinetic data have been recently reported.

The *M. tuberculosis* 2′-*N*-acetyltransferase is a chromosomally encoded enzyme that was identified [10] based on sequence homology to an aminoglycoside 2′-*N*-acetyltransferase identified in *Providencia stuartii* [11]. The gene, encoded by the Rv0262c open reading frame, was PCR amplified from genomic *M. tuberculosis* DNA, expressed in soluble form, purified to homogeneity and shown to have aminoglycoside 2′-*N*-acetyltransferase activity with a broad range of 4,6- and 4,5-substituted aminoglycosides [12]. The specificity for acyl-CoAs was much stricter, with acetyl-CoA being preferred over proprionyl-CoA by a factor of 45, and by factors of greater than 200 for all other acyl-CoAs tested. The initial velocity pattern was intersecting, supporting a sequential kinetic mechanism involving ternary complex formation. The use of alternate substrate kinetic methods, pioneered by Northrop [8], supported a random kinetic mechanism.

The enzyme was crystallized and the three-dimensional structures of the unliganded enzyme and three ternary complexes containing CoA and either tobramycin, kanamycin A or ribostamycin were determined [13]. The enzyme is active as a dimer in solution, and the dimer is observed in the crystal, with all 181 amino acid residues of the monomer being visible. Each monomer adopts a classic GNAT fold, with the major difference being the presence of four additional short β strands (β7–10) at the C-terminus. In comparison to other dimeric GNATs that will be discussed below, the dimer