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The Ada2/Ada3/Gcn5/Sgf29 histone acetyltransferase module

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Abstract

Histone post-translational modifications are essential for the regulation of gene expression in eukaryotes. Gcn5 (KAT2A) is a histone acetyltransferase that catalyzes the post-translational modification at multiple positions of histone H3 through the transfer of acetyl groups to the free amino group of lysine residues. Gcn5 catalyzes histone acetylation in the context of a HAT module containing the Ada2, Ada3 and Sgf29 subunits of the parent megadalton SAGA transcriptional coactivator complex. Biochemical and structural studies have elucidated mechanisms for Gcn5's acetyl- and other acyltransferase activities on histone substrates, for histone H3 phosphorylation and histone H3 methylation crosstalks with histone H3 acetylation, and for how Ada2 increases Gcn5's histone acetyltransferase activity. Other studies have identified Ada2 isoforms in SAGA-related complexes and characterized variant Gcn5 HAT modules containing these Ada2 isoforms. In this review, we highlight biochemical and structural studies of Gcn5 and its functional interactions with Ada2, Ada3 and Sgf29.

Keywords

Gene regulation; Histone modification; Transcriptional regulation; Epigenetic modification; Histone crosstalks; Protein isoforms

1. Introduction

The discovery that the yeast Gcn5 transcriptional adaptor [1] is a histone acetyltransferase was a watershed moment in eukaryotic gene regulation because it provided a direct link between histone modifications and transcriptional regulation [2]. Previous studies had already established that Gcn5 did not act alone, but was associated with the Ada2 and Ada3 transcriptional adaptor proteins [3-6]. The subsequent fractionation of native Gcn5-containing complexes from yeast revealed that Gcn5, together with Ada2 and Ada3, were associated in the SAGA (Spt-Ada-Gcn5-Acetyltransferase) megadalton complex and a smaller ADA complex [7]. Whereas the isolated Gcn5 protein could only acetylate naked histones *in vitro*, the SAGA and ADA complexes possessed the ability to acetylate the more physiological substrate of nucleosomes in which histones package DNA into disc-like units.

Ada2, Ada3 and Gcn5 form a stable protein complex, first shown by *in vitro* cotranslation and then by *in vivo* reconstitution in *E. coli* [5,6,8]. The Ada2/Ada3/Gcn5 complex is

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necessary and sufficient for nucleosomal HAT activity and thus constitutes the minimal Gcn5 complex for nucleosomal histone acetylation. The SAGA subunit protein Sgf29 binds to the Ada2/Ada3/Gcn5 complex and completes the SAGA HAT module.

This review focuses on Gcn5's histone acetyltransferase activity in the context of the Ada2/Ada3/Gcn5/Sgf29 module, with a particular emphasis on mechanistic and structural findings.

2. Gcn5

Gcn5 (KAT2A) is the catalytic subunit of the HAT module in SAGA (Fig. 1). Gcn5's HAT domain (Fig. 2) has an α/β architecture with a central sheet of six β-strands surrounded by five α-helices [9] (Fig. 3a). The globular fold is conserved across species from yeast Gcn5 to Tetrahymena Gcn5 [10] and humans Gcn5 and PCAF (Fig. 3b, c) [11,12]. The HAT core domain is formed by four antiparallel β-strands (β2-β5) which lie over an amphipathic α-helix (α3). This HAT core domain is shared with other CoA-dependent HATs such as yeast Esa1 (Fig. 3d).

Acetylation by Gcn5 requires two substrates, a histone substrate and an acetyl-CoA cosubstrate. Detailed kinetic studies establish that Gcn5 follows ordered bi-bi kinetics, with acetyl-CoA binding first to the HAT domain before the histone peptide substrate [13]. The crystal structure of the ternary complex of the *Tetrahymena* Gcn5 HAT domain bound to CoA and histone H3 peptide together with additional structures of the *Tetrahymena* Gcn5 HAT domain on its own (apo form) and in a binary complex bound to Coenzyme A (CoA) provided the structural explanation why [10]. The structures show the HAT domain contains two pronounced clefts in an L shape. Acetyl-CoA resides in the smaller cleft while the histone H3 peptide occupies the larger cleft (Fig. 3b). In the apo form, the CoA end of the peptide binding cleft of Gcn5 is obstructed, blocking the H3 peptide from binding (Fig. 4a, b). Binding of the coenzyme A moiety causes a modest opening of the peptide binding cleft, a conformational change which is then apparently used by the histone peptide to access the peptide binding cleft (Fig. 4c). These structures also provided important details for how the catalytic *Tetrahymena* Glu122 (equivalent to yeast Glu173) acts as a general base to increase the nucleophilicity of the substrate Lys14 α-amino group for attack on acetyl-CoA [10,14]. Gcn5's catalytic mechanism is discussed further in the Albaugh and Denu contribution in this special issue.

Much of the investigations into Gcn5's enzymatic activity has focused on its acetyltransferase activity because of the strong association between gene activity and histone acetylation. However, Gcn5 is not limited to modifying lysine side chains with an acetyl group. The Gcn5 HAT domain has been shown to acylate histone lysine side chains with a surprisingly large range of acyl groups (Fig. 5). Human Gcn5 can propionylate and butyrylate histone H3 on Lys14 *in vitro* and *in vivo* and both the H3K14pr and H4K14bu marks are associated with transcriptionally active chromatin [15]. Structural studies had shown that acetyl-CoA, propionyl-CoA and butyryl-CoA all can bind to Gcn5 but the bound butyryl-CoA was not positioned for catalysis and the butyryl group would occupy the same space as the substrate target lysine (Fig. 6) [16]. Significant conformational changes

occur upon the subsequent binding of the substrate peptide, as observed in the ternary *Tetrahymena* Gcn5/CoA/histone peptide structures, where the Gcn5 HAT domain loop 3 is pushed away to accommodate histone peptide binding (Fig. 6d).

In a separate study, human Gcn5 was reported to act as a histone H3 succinyltransferase. However, it has also been reported that the protein bovine serum albumin can be succinylated non-enzymatically by succinyl-CoA and that succcinyl-CoA is signficantly more reactive in non-enzymatic acylation than acetyl-CoA, propionyl-CoA or butyryl-CoA [17-19]. As such, it is not entirely clear whether the observed histone H3 succinylation was actually catalyzed by Gcn5 or if it occurred non-enzymatically. Human Gcn5 has also been reported to glutarylate H4K91 but its glutaryltransferase activity was not confirmed *in vitro* using purified components [20] and like succinyl-CoA, glutaryl-CoA can non-enzymatically acylate proteins. It is worth noting that Gcn5-catalyzed acylation is not limited to saturated fatty acids: the yeast Ada2/Ada3/Gcn5 complex crotonylates nucleosome substrates *in vitro* and Gcn5-dependent crotonylation regulates transcription of selected genes in yeast [21].

Studies of the Gcn5 HAT domain have also been instrumental in elucidating the first molecular basis for histone modification crosstalks [23]. Phosphorylation of H3 Ser10 increases the efficiency of Gcn5 acetylation of H3 peptides by increasing binding affinity to the H3 peptide substrate. In this case, the crosstalk between histone modification (Ser10 phosphorylation and Lys14 acetylation) occurs *in cis* in the same peptide region. Crystallographic structures of H3 peptides with or without Ser10 phosphorylation show a large rearrangement for part of the H3 peptide upon Ser10 phosphorylation (Fig. 7). These changes reposition Thr11 for more extensive interactions with the Gcn5 HAT domain, consistent with the increased binding affinity of the Ser10 phosphorylated H3 peptide. This conformational change is limited to the H3 peptide, with no significant changes to the Gcn5 HAT domain or to CoA binding.

Gcn5 contains a bromodomain at its C-terminus. The bromodomain is a chromatin modification reader of acetylated lysine residues and is found in many transcriptional regulators. The structure of the Gcn5 bromodomain was determined on its own by NMR and in complex with an H4 tail peptide containing acetylated Lys16 by crystallography [24,25]. The structures show a four-helix bundle with a deep hydrophobic pocket which accommodates the acetylated lysine residue (Fig. 8a).

3. Ada2

Recognizable domains in Ada2 include a ZZ zinc finger domain and a SANT domain at the N-terminus and a SWIRM domain at the C-terminus (Fig. 2). Deletion and mutational analysis of the Ada2 ZZ and SANT domains showed that the SANT domain, but not the ZZ domain, is required for interactions with Gcn5 and for normal growth in yeast [6,26]. The SANT (Swi3, Ada2, N-Cor, and TFIIB) domain is an approximately 55 residue α-helical domain with structural homology to the myb DNA-binding domain. Consistent with this, several chromatin remodeling enzymes employ the SANT domain as part of their DNA-binding modules to interact with DNA [27-31]. The SANT domain has also been proposed to act as a histone tail binding motif in chromatin-regulatory enzymes [32]. The

interactions between Ada2 and Gcn5 increase Gcn5's HAT activity on histone substrates *in vitro* and are necessary for transcriptional activation *in vivo* [8,26,33]. The Gcn5 HAT domain is not sufficient to interact with Ada2 and at least 20 residues C-terminal to the Gcn5 HAT domain are required for Ada2 interaction.

The structural basis for how the Ada2 SANT domain interacts with Gcn5 and thus brings Gcn5 into the SAGA complex was provided by the crystal structure of the yeast Ada2/Gcn5 complex [34]. Earlier attempts to crystallize this complex produced crystals with poor diffraction characteristics. This technical limitation was overcome through the use of a synthetic antibody fragment screened *in vitro* for binding to the Ada2/Gcn5 complex as a crystallization chaperone. The structure includes the Gcn5 HAT domain with N-terminal and C-terminal extensions bound to the Ada2 ZZ and SANT domains (Fig. 9). The Gcn5 HAT domain is essentially unchanged on its own or complexed with Ada2. The 20 residues immediately C-terminal to the Gcn5 HAT domain (residues 260-280) shown to be critical for interacting with Ada2 form an α-helix that points away from the HAT domain. This Gcn5 α-helix makes extensive interactions with the Ada2 SANT domain on one side and with the Gcn5 β-hairpin brace which precedes the Gcn5 HAT domain.

At first glance, the Ada2/Gcn5 complex structure creates a predicament: the Ada2 SANT domain is known to increase Gcn5's HAT activity on histone peptides, but modeling an H3 peptide and coenzyme A based on the orthologous *Tetrahymena* Gcn5/H3 peptide/CoA structure positions the Ada2 SANT domain on the opposite side of the Gcn5 HAT domain from the H3 peptide. The Ada2 SANT domain is too far away to make direct contact with the H3 peptide, arguing against the SANT domain acting as a histone tail recruiting module. Instead, it appears that the Ada2 SANT domain activates Gcn5's HAT activity by recruiting the acetyl-CoA cofactor. This mechanism is consistent with Gcn5's ordered bi-bi reaction mechanism where acetyl-CoA binds first to Gcn5 before the histone peptide substrate can bind. However, the distance between the SANT domain and the cofactor precludes direct recruitment of the cofactor. Rather, the Ada2/Gcn5 crystal structure with the modeled H3 peptide and CoA cofactor indicates that the Ada2 SANT domain corrals the Gcn5 HAT domain residue Lys223's side chain to interact with the coenzyme A cofactor. This structural hypothesis was substantiated with isothermal calorimetry binding studies and HAT activity assays which show that Gcn5 Lys223, a residue conserved across species, plays a critical role in acetyl-CoA binding and acetyltransferase activity.

The Ada2/Gcn5 structure shows that the Ada2 ZZ zinc-binding domain interacts with an extended chain of Gcn5(282-312) beyond the Gcn5 α-helix that interacts with the Ada2 SANT domain. This "head module" of the Ada2/Gcn5 complex was observed to rotate with respect to the rest of the structure in two different crystal forms. The physiological significance of this conformational flexibility and in fact, the biological roles of the Ada2 ZZ domain are currently not known. Unlike the Ada2 SANT domain, the ZZ domain is apparently not essential for growth or for activity in an *in vivo* transcription assay [26]. The ZZ domain in some other chromatin proteins binds to unmodified or acetylated histone H3 tails [35], but it is unclear if the Ada2 ZZ domain possesses a similar activity.

The C-terminus of yeast Ada2 and its Ada2a orthologs contain a SWIRM (Swi3, Rsc8 and Moira) domain, comprised of an α-helical core containing a helix-turn-helix motif [36,37]. The structural similarities to the linker histone proteins prompted experiments which show that the yAda2 SWIRM domain binds to DNA and to dinucleosomes, but not to mononucleosomes [36]. This suggests the possibility that the yAda2 SWIRM domain might bind to linker or extranucleosomal DNA.

4. Ada3

In contrast to the substantial structural characterization of Gcn5 and Ada2, much of Ada3's function or structure remains unclear. Ada3 forms a ternary complex with Gcn5 and Ada2, apparently through interactions with Ada2 [5,6,8]. Ada3 is necessary for the Ada2/Ada3/Gcn5 HAT module to acetylate nucleosome versus histone or peptide substrate [8] but the mechanism for this is not known. We also lack clear structural information for Ada3, which does not appear to contain recognizable domains or motifs aside from some predicted coiled-coil regions (Fig. 2). Hybrid methods combining chemical cross-linking, mass spectrometry and molecular modeling have been used to produce a structural model for a mammalian Ada2/Ada3/Gcn5/Sgf29 complex but this model needs validation [38]. The recent cryoelectron microscopy structures for the yeast SAGA complex are important and exciting developments, but unfortunately the Ada2/Ada3/Gcn5/Sgf29 HAT module is poorly defined presumably due to conformational diversity [39,40]. Two α-helical regions in this density interact with the SAGA TAF6 subunit and were attributed to Ada3 in the structure from the Schultz and Ben-Shem groups [39]. More details from structures of the SAGA and related complexes are described in the Hemlinger, Papai, Devys and Tora contribution in this issue.

5. Sgf29

Sgf29 (SAGA associated factor of 29 kDa) was identified as a SAGA subunit in a proteomics analysis of the SAGA complex [41]. Sgf29 is not required for the integrity of the SAGA complex but global histone H3 acetylation is decreased *in vivo* in the absence of Sgf29 [42] even though Sgf29 is not necessary for the SAGA complex to acetylate histones or nucleosomes *in vitro* [43]. Proteomic and biochemical analyses confirm that Sgf29 is a core subunit of SAGA and ADA family of complexes [44]. The rat ortholog of Sgf29 appears to associate with the Ada2/Ada3/Gcn5 HAT module via coiled-coil interactions with Ada3 [45] and the recombinant *S. pombe* four protein complex of Ada2, Ada3, Gcn5 and Sgf29 has been expressed and characterized [46] showing that these four proteins are sufficient to form a subcomplex.

Sgf29 exerts its effect on H3 acetylation though histone modification crosstalk with H3 methylation. The C-terminal tandem Tudor domain of human Sgf29 was determined to be necessary and sufficient for binding to H3K4me3, with the second Tudor domain playing a critical role in the binding [47]. Crystal structures of the yeast and human Sgf29 tandem Tudor domains bound to unmodified and methylated H3 peptides show that each Tudor domain forms a typical twisted five-stranded anti-parallel β-barrel Tudor fold with a not so typical face-to-face orientation of the Tudor domains [43] (Fig. 8b). The bound H3K4me3

peptide binds in a negatively charged pocket created by the two Tudor domains with the Tudor 1 domain binding to the first H3 residue (Ala1) and the Tudor 2 domain binding to the di- or trimethylated Lys4 residue. The physical association of a H3K4me3 binding module in Sgf29 with the Ada2/Ada3/Gcn5 histone acetyltransferase module thus provides a mechanism to account for why H3 acetylation is correlated with H3K4 methylation [43,48].

This histone H3 acetylation-histone H3 methylation crosstalk appears to explain why the SAGA complex is particularly active at promoters. Promoters are enriched for H3K4me3, which recruits the SAGA complex through Sgf29 binding to this histone mark at promoters where increased histone acetylation is mediated by the SAGA HAT module. Elegant *in vitro* studies employing recombinant S. pombe Ada2/Ada3/Gcn5/Sgf29 complexes and recombinant true H3K4me3 nucleosomes indicate that this four subunit HAT module binds to H3K4me3 and stimulates acetylation on the same H3 tail *in cis* [46]. The presence of H3K4 trimethylation affected the Michaelis constant K_m, but not the catalytic rate constant k_{cat} for histone peptide substrates. This indicates that H3K4 methylation improves HAT activity by increasing binding of the Ada2/Ada3/Gcn5/Sgf29 module to the methylated substrate and not by directly influencing the HAT module. The Ada2/Ada3/Gcn5/Sgf29 module was also found to preferentially acetylate H3K4me3 nucleosomes over unmodified nucleosomes when presented with both substrates simultaneously, thus explaining how the SAGA complex can hyperacetylate gene promoters while leaving gene bodies less acetylated.

6. The Ada2/Ada3/Gcn5 and Ada2/Ada3/Gcn5/Sgf29 HAT modules

Gcn5 preferentially acetylates free histone H3 at position K14, but association with subunits of the SAGA or ADA complexes expands this target preference [49]. The minimal complex for nucleosomal acetylation, Ada2/Ada3/Gcn5 was observed to possess the same lysine specificity as the full SAGA complex [8]. Steady-state kinetic studies using histone H3 polypeptide substrate show that once H3K14 has been acetylated, Gcn5 can acetylate H3K9, H3K23, H3K18, H3K27 and H3K36 in decreasing order of preference [50]. A similar but not identical substrate specificity was detected for the Ada2/Ada3/Gcn5 HAT module using the same histone H3 substrate [51]. The Gcn5 bromodomain plays an important role in both cooperativity and selectivity of histone acetylation. Cooperative acetylation of nucleosomes by the Ada2/Ada3/Gcn5 HAT module and by the SAGA complex was determined to be mediated by the Gcn5 bromodomain acetyl-lysine binding activity [52]. This acetyl-lysine binding activity also regulates lysine-site-specificity of the Ada2/Ada3/Gcn5 HAT module, which acetylates H3K14 and H3K23 independent of the Gcn5 bromodomain and H3K9, H3K18, H3K27 and H3K36 dependent on the Gcn5 bromodomain [51]. It appears that the Ada2/Ada3/Gcn5 HAT module binds to acetylated H3K14 via the Gcn5 bromodomain, stimulating acetylation of H3K18. This tethering mechanism of Gcn5 to H3K14ac is reminiscent of Sgf29's binding to H3K4me3 to achieve hyperacetylation, and both tethering schemes appear to be employed by the SAGA complex [46].

The Ada2/Ada3/Gcn5 HAT module is the minimal SAGA complex that will acetylate nucleosomes, but the nucleosome core particle with 145-147 bp of DNA wrapped around the histone octamer core is apparently not the preferred binding or acetylation substrate of

SAGA. Mittal et al were able to perform technically demanding kinetic studies of the yeast SAGA complex to show SAGA bound nucleosomes containing 15 bp of extranucleosomal or linker DNA on one side and 95 bp extranucleosomal DNA on the other side twice as tightly as a 147 bp 601 nucleosome with minimal extranucleosomal DNA [53]. The +95/+15 nucleosome also increased SAGA's acetylation catalytic rate constant (k_{cat}) by a factor of 4. Although the specific SAGA subunit or domain that mediates this increased binding is not known, the Ada2 SWIRM domain which appears to interact with extranucleosomal DNA is a reasonable candidate [36].

The SAGA complex's deubiquitylase module contains the yeast Sca7/Sgf73 or the human ataxin-7 subunit. Expansion of the polyglutamine sequence near the amino terminus of the ataxin-7 protein causes the neurodegenerative disease spinocerebellar ataxia type 7 (SCA7). The pathogenic ataxin-7-60Q protein containing 60 glutamines, but not the non-pathogenic ataxin-7-10Q protein containing 10 glutamines, formed a complex with the yeast Ada2/Ada3/Gcn5 complex apparently through direct interactions with Gcn5 [54]. It is worth noting that SAGA's deubiquitylase and HAT modules appear to be physically close to each other in the cryoelectron microscopy structures of the SAGA complex [39,40]. Ataxin-7-60Q significantly reduced the HAT activity of the Ada2/Ada3/Gcn5 complex on both histone and nucleosome substrates *in vitro.* These *in vitro* results were substantiated by studies in yeast cells which show that the ataxin-7-60Q protein, but not the ataxin-7-10Q protein, inhibited H3K9 acetylation at both GAL1 and GAL7 promoters.

7. Ada2 isoforms in the HAT module

In contrast to yeast which contains only one Ada2 isoform, higher eukaryotes contain Ada2a and Ada2b isoforms with 25-50% sequence identity between isoforms in plants, Drosophila and humans [55-58]. The Ada2b isoform is found in the SAGA complex while the Ada2a isoform is found in the ATAC (Ada Two A-containing) complex. Both SAGA and ATAC complexes contain the catalytic core of Ada2a or Ada2b plus Ada3, Gcn5 and Sgf29, and the corresponding human ternary and quaternary complexes of Ada2a/Ada3/Gcn5, Ada2b/Ada3/Gcn5, Ada2a/Ada3/Gcn5/Sgf29 and Ada2b/Ada3/Gcn5/Sgf29 possess HAT activity on histones [59]. On mononucleosome substrates, a recombinant human Ada2a/Ada3/Gcn5 ATAC subcomplex possessed significantly lower H3 HAT activity compared to the equivalent SAGA Ada2b/Ada3/Gcn5 subcomplex [60]. However in a different study, both the human ATAC and SAGA complexes showed similar, weak H3 HAT activity using mononucleosome or polynucleosome substrates [61]. In addition to the Gcn5 HAT subunit, *Drosophila* and human ATAC complexes contains a second catalytic HAT subunit, Atac2. The Atac2 HAT subunit confers upon ATAC complexes histone H4 HAT activity, at least on histone substrates [61-63]. The C-terminal regions of Ada2a and Ada2b appear to mediate incorporation into *Drosophila* SAGA versus ATAC complexes [64].

Further complicating matters, *Drosophila* contains two splice isoforms of Ada2b: Ada2b-PA and Ada2b-PB [65,66]. The shorter Ada2b-PA isoform forms the Chiffon histone acetyltransferase (CHAT) complex with Ada3, Gcn5, Sgf29 and Chiffon [67]. Chiffon is the *Drosophila* ortholog of the Dbf4 regulatory subunit of the Cdc7 kinase required for chromosomal DNA replication. The longer Ada2b-PB isoform is incorporated into a

complex together with Ada3, Gcn5 and Sgf29. This *Drosophila* ADA complex possesses histone and nucleosomal H3K9 and/or H3K14 acetyltransferase activity [68], and appears to be a metazoan counterpart to the yeast ADA complex which contains Ada2, Ada3, Gcn5, Sgf29 and two yeast ADA complex-specific subunits, Ahc1 and Ahc2 [7,44,69]. The functional role of these smaller Gcn5-containing HAT complexes is not clear, but the absence of the Tra1 subunit which binds to acidic transcriptional activator suggests functions besides activator-dependent transcriptional regulation.

8. Outlook

Much has been learned about the structure and function of Gcn5 on its own and in the context of the Ada2/Ada3/Gcn5/Sgf29 HAT module. We understand the molecular mechanisms for how the Gcn5 HAT domain binds to and acetylates its H3 histone tail substrate, how phosphorylation can affect acetylation *in cis* and how Ada2 binding increases Gcn5's HAT activity. Nevertheless, much remains to be discovered. We need to better understand conformational changes in Gcn5 that occur upon acetyl-coenzyme A cofactor and histone peptide substrate binding and that enable non-acetyl acylation of histone substrates. We still need to determine the mechanism of nucleosome acetylation by the Ada2/Ada3/Gcn5/Sgf29 HAT module. This will require elucidating what regions or domains of the HAT module interact with the nucleosome and what surfaces of the nucleosome are engaged by the HAT module. Structural descriptions of the intact HAT module on its own and in complex with the nucleosome would be highly desirable. We will also need to investigate how histone crosstalks mediated by the Gcn5 bromodomain and the Sgf29 Tudor domain occur when the Ada2/Ada3/Gcn5/Sgf29 HAT module interacts with its nucleosome substrate. Given the apparent modularity of the SAGA complex with respect to its acetylation and deubiquitylation enzymatic activities, it is likely that studies of the Ada2/Ada3/Gcn5/Sgf29 HAT module will be highly relevant to understanding the acetylation activity of SAGA and SAGA-like complexes.

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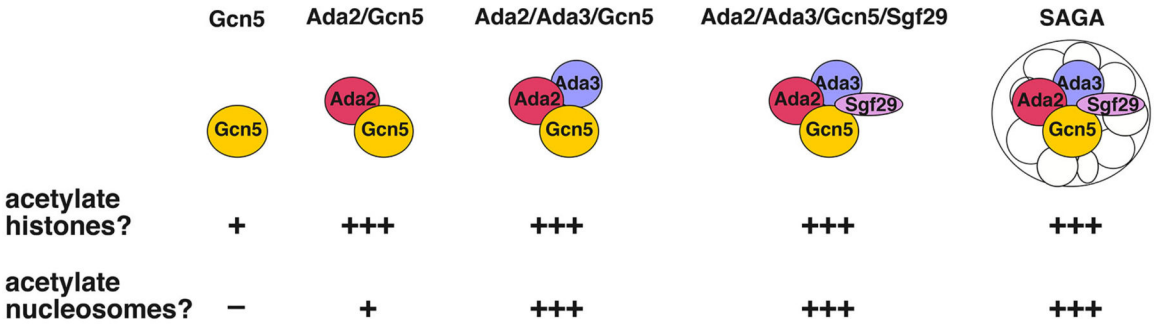

Figure 1: Acetylation efficiency of different Gcn5-containing complexes

The Gcn5 catalytic subunit possesses weak histone acetyltransferase activity on its own. Ada2 increases HAT activity on histones, and Ada3 is needed for robust nucleosome acetyltransferase activity. Sgf29 mediates processive acetylation and completes the SAGA HAT module. Comparison of HAT activity based on Balasubramanian et al, 2002 [8] and Ringel et al, 2015 [46].

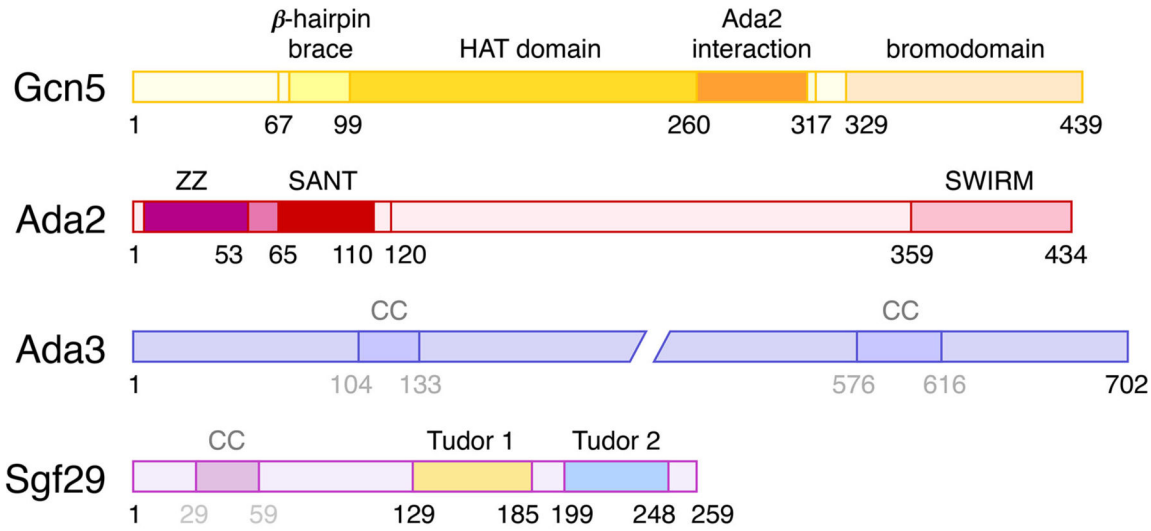

Figure 2: Domain organization of the SAGA HAT module subunits

The known domains and motifs in Gcn5, Ada2, Ada3 and Sgf29 are shown. In addition, predicted coiled-coil regions (CC) are shown for Ada3 and Sgf29. Little is currently known about the structure of Ada3.

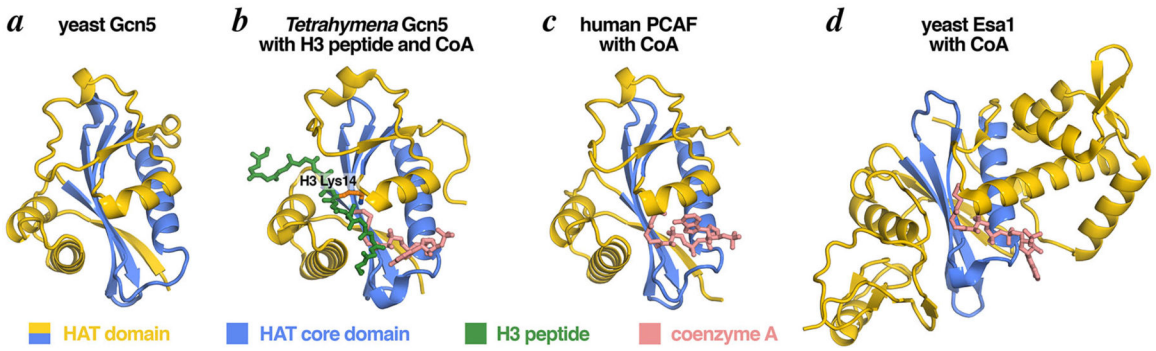

Figure 3: The conserved structure of the Gcn5 HAT domains

The Gcn5 HAT domain contains a central HAT core domain (blue) and structurally more divergent N- and C-terminal segments (yellow). The HAT core domain is structurally conserved among orthologous Gcn5 enzymes (yeast in panel a, *Tetrahymena* in panel b), closely related HAT enzymes like PCAF (panel c) and more distantly related HAT enzymes like Esa1 (panel d). The crystal structure of the *Tetrahymena* ternary complex (panel b) shows Gcn5 bound to its two substrates, CoA (a nonenzymatic surrogate for acetyl-CoA) and an H3 histone peptide. Molecular graphics prepared in PyMOL [66] using PDB coordinates 1YGH, 1PU9, 1CM0, 1MJA.

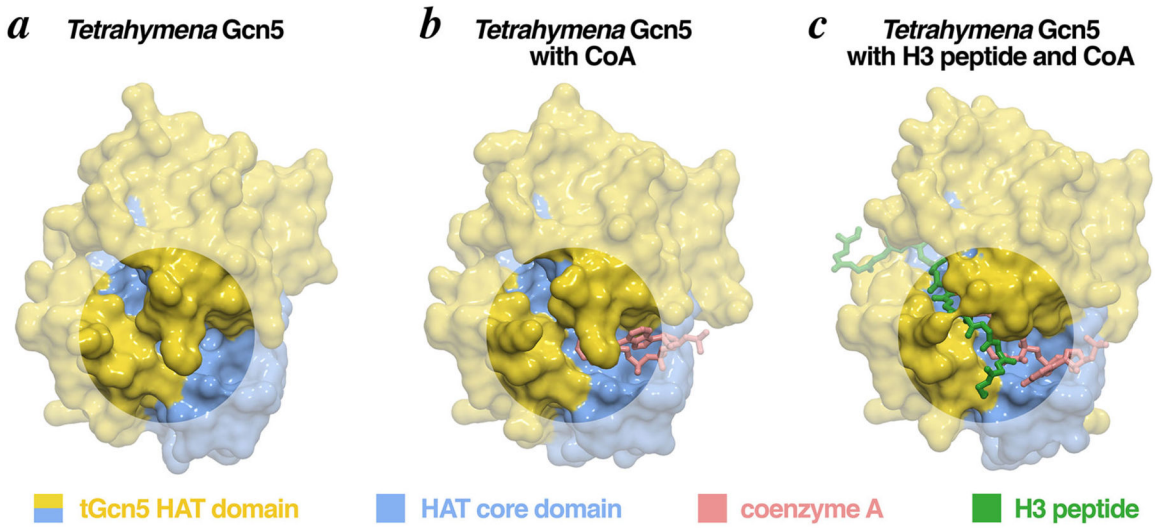

Figure 4: Surface representations of the Gcn5 HAT domain in complex with its substrates

The Gcn5 HAT domain contains two pronounced clefts which accommodate the histone peptide and acetyl-CoA substrates. Binding of acetyl-CoA to the smaller cleft causes a conformational change that opens the larger, peptide binding cleft, allowing the H3 peptide to bind (circles highlight the conformational changes). Molecular graphics prepared in PyMOL using PDB coordinates 1QST, 1QSR and 1PU9.

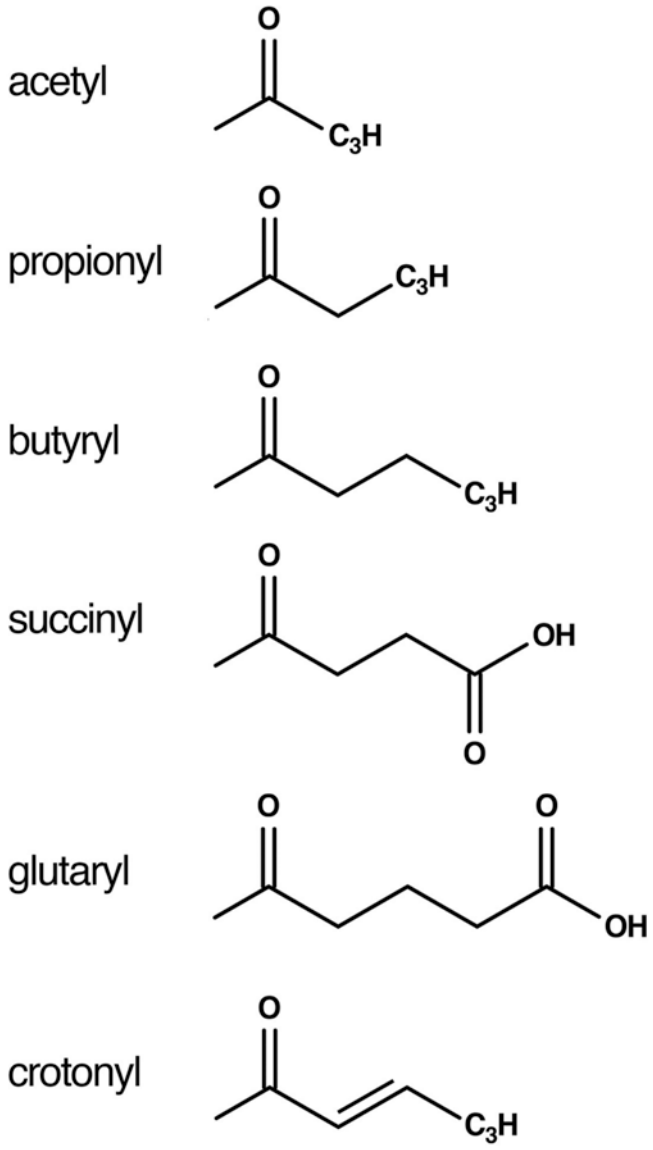

Figure 5: Acyl groups used by Gcn5

Chemical structures of acyl groups in acyl-CoA cosubstrates used by Gcn5 to acylate lysine residues.

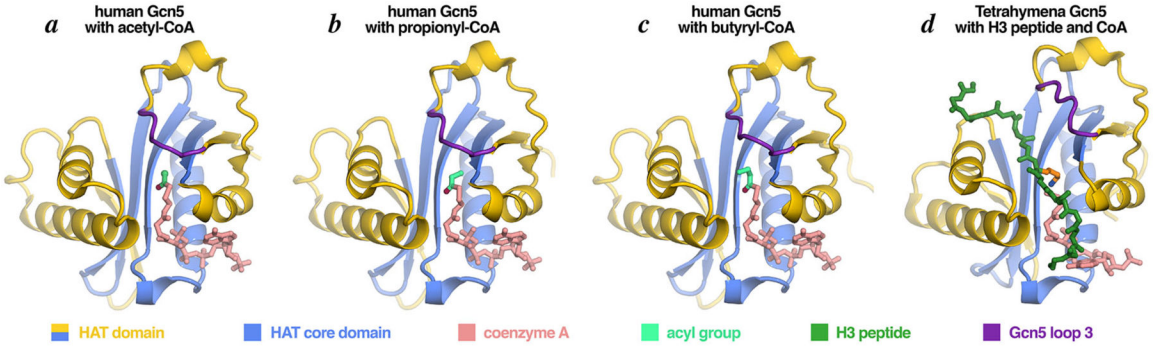

Figure 6: Crystal structures of Gcn5 HAT domains bound to different acyl-CoA cosubstrates and conformational changes associated with substrate binding

The Gcn5 HAT domain can bind to acetyl-CoA and the bulkier propionyl- and butyryl-CoA with modest structural changes (panels a, b, c). Subsequent binding of the histone peptide and catalysis appears to require significant conformational changes in Gcn5 loop 3 (panel d). Loop 3 also moves to make way for the bulky succinyl acyl group of succinyl CoA (panel d) which interacts with Tyr465 in the loop. Molecular graphics prepared in PyMOL using PDB coordinates 1Z4R, 5H84, 5H86, 1PU9.

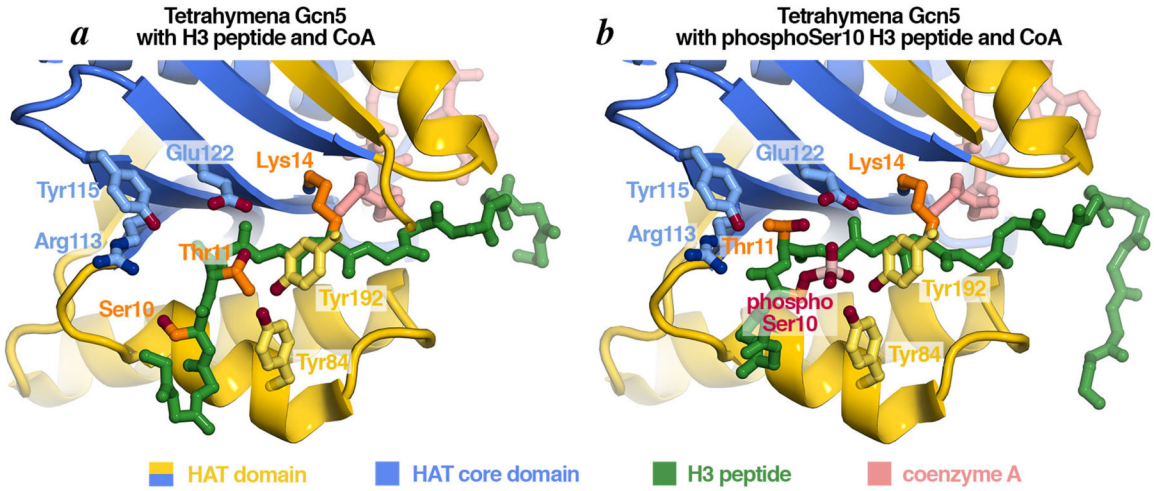

Figure 7: Mechanism of phosphorylation-acetylation epigenetic histone modification crosstalk

Histone H3 S10 phosphorylation induces conformational changes in the H3 peptide that increase its interactions with the catalytic site of *Tetrahymena* Gcn5 (compare H3 Thr11 positions), increasing the binding affinity for the substrate. Key Gcn5 residues that mediate these interactions are shown. Molecular graphics prepared in PyMOL using PDB coordinates 1PU9 and 1PUA.

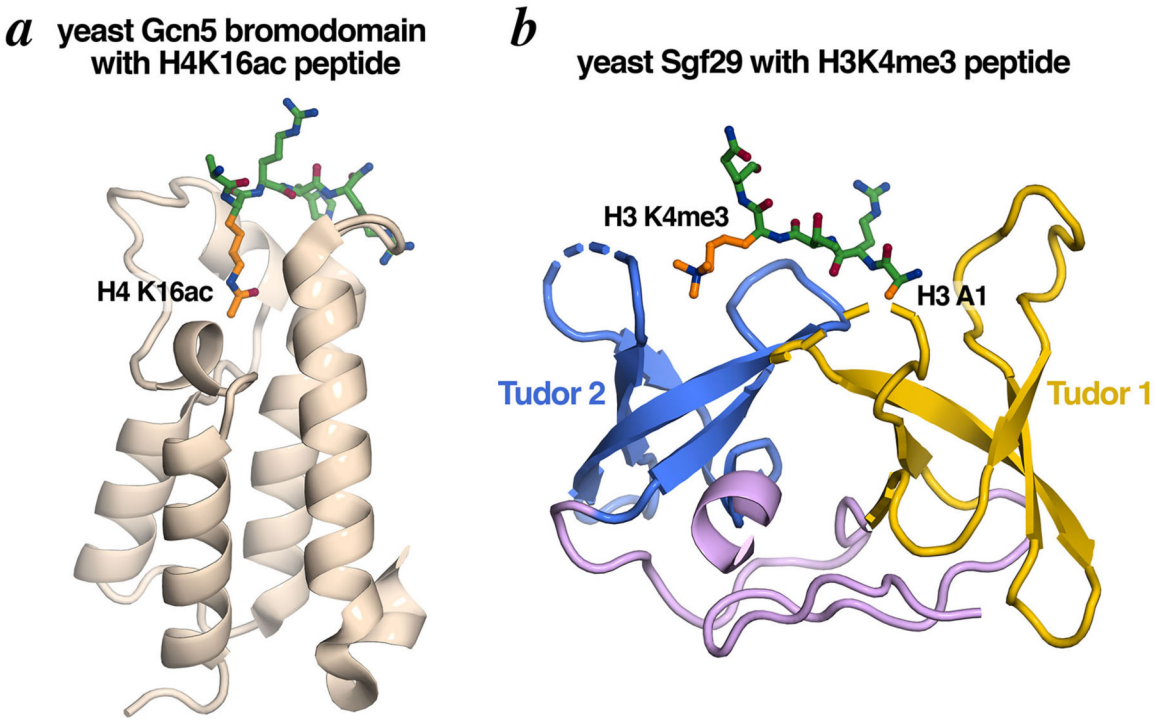

Figure 8: HAT module domains that mediate histone modification crosstalk

(a) The Gcn5 bromodomain serves as a reader of acetylated H4K16. (b) The Sgf29 tandem Tudor domain recognizes methylated H3K4. Molecular graphics prepared in PyMOL using PDB coordinates 1E6I and 3MP1.

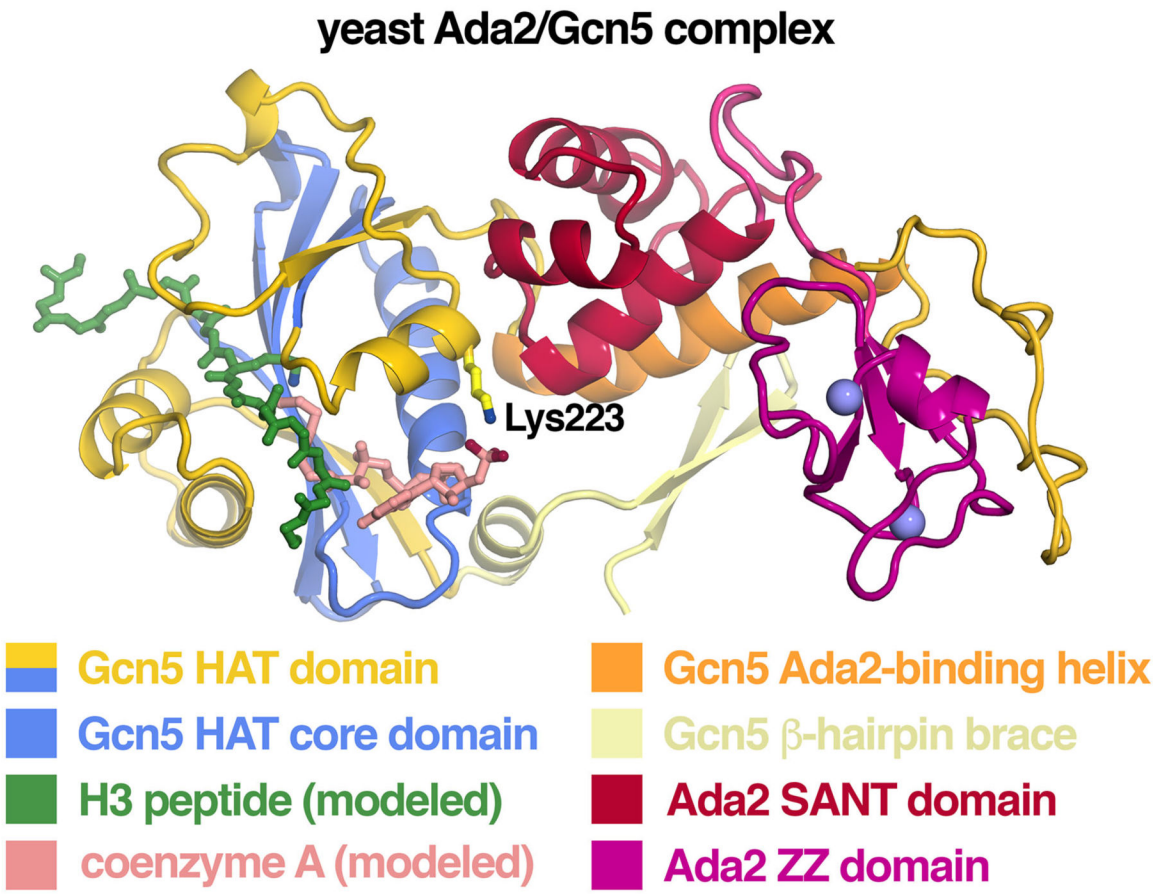

Figure 9: Structure of the Ada2/Gcn5 complex indicates the Ada2 SANT domain helps Gcn5 bind to acetyl-CoA

Crystal structure of the yeast Ada2/Gcn5 complex with modeled H3 peptide and CoA based on the *Tetrahymena* Gcn5/CoA/H3 peptide structure. The Ada2 SANT is unlikely to directly affect histone peptide binding since it is located far away from the peptide binding site. Instead, the SANT domain is positioned by interactions with the Gcn5 β-hairpin brace and the Ada2-binding helix to constrain Gcn5 Lys223 to bind directly to the 3' phosphate of CoA. The Ada2 zinc binding ZZ domain extends even further away from the Gcn5 HAT domain. Molecular graphics prepared in PyMOL using PDB coordinates 6CW2.