The SANT Domain of Ada2 Is Required for Normal Acetylation of Histones by the Yeast SAGA Complex

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Transcription is regulated through chromatin remodeling and histone modification, mediated by large protein complexes. Histone and nucleosome interaction has been shown to be mediated by specific chromatin domains called bromodomains and chromodomains. Here we provide evidence for a similar function of two additional domains within the yeast SAGA complex, containing the histone acetyltransferase Gcn5. We have analyzed deletion and substitution mutations within Gcn5 and Ada2, an interacting protein within SAGA, and have identified substrate recognition functions within the SANT domain of Ada2 and regions of the histone acetyltransferase domain of Gcn5 that are distinct from catalytic function itself. These results suggest that histone and nucleosomal substrate recognition by SAGA involves multiple conserved domains and proteins, beyond those that have been previously identified.

Regulation of messenger RNA transcription is a crucial cellular process. Transcriptional regulation requires activator and repressor proteins and their associated complexes as well as the chromatin structure of the template. Activators bind to specific DNA sequences upstream from promoters and increase transcription by recruiting coactivator, or adaptor, complexes that provide interaction with the basal transcriptional machinery (1). In yeast, a group of adaptor proteins was identified through a genetic screen involving the chimeric activator GAL4-VP16 (2); these proteins included Ada1 (3), Ada2 (2), Ada3 (4), Ada5 (5, 6), and Gcn5 (7).

Chromatin structure (8) correlates with transcriptional repression or activation (9, 10). A variety of mechanisms are believed to influence the state of chromatin, including ATP-dependent remodeling by complexes such as Swi-Snf (11), and histone modifications: acetylation, phosphorylation, methylation, etc. (12–14). The first histone acetyltransferase (HAT) identified was Gcn5 (15), subsequently found to be a subunit of the transcriptional regulatory complex SAGA in yeast (16, 17). Gcn5 associates with Ada2 and Ada3 to form a trimERIC module (18–20), where Ada2 interacts with both Gcn5 and Ada3, but the latter two proteins do not directly interact. At least two yeast native complexes, ADA and SAGA, are known to contain the Ada2-Ada3-Gcn5 trimer. The 1.8-MDa SAGA complex also possesses a number of other transcriptionally relevant subunits (21): Ada1 (22), Ada5, the TATA-binding protein (TBP)-related subgroup of the Spt proteins (Spt3, Spt7, Spt8, and Spt20, identical to Ada5) (23), a subset of the TafIs (Taf17, Taf25, Taf60, Taf78, and Taf90) (24), and Tra1 (25, 26), the yeast homologue of the transcriptional coactivator TRRAP in higher eukaryotes (27). Complexes with analogous composition and containing either PCAF or human GCN5 as the HAT subunit also exist in human cells (28, 29).

The SAGA complex has multiple functions in transcription. The Gcn5 HAT domain is required for transcription and chromatin remodeling at SAGA-dependent promoters (30–32). Additional specific proteins/domains within SAGA promote nucleosome acetylation: mutation of either the bromodomain of Gcn5 (see below) (22) or Taf68 (24) leads to lower levels of acetylation of nucleosomal histones, with little apparent effect on free histone acetylation. Besides possessing HAT activity, SAGA interacts with activation domains (33–35) and TBP (22, 24, 36).

An important question concerning histone modifications is how substrate specificity is achieved. The bromodomain is present in a variety of chromatin-associated proteins (37) and has been implicated in both nucleosome and modification-specific histone tail recognition (38). Gcn5 efficiently acetylates nucleosomal histones only as a component of the SAGA or ADA complex and not as a monomer, and, as mentioned above, this requires the bromodomain of Gcn5. The bromodomain of CBP also specifically targets acetylation to nucleosomal substrates (39). The bromodomains of Gcn5 and TAFII250 interact with acetylated lysine, suggesting that in addition to recognizing nucleosomes, the bromodomain may have a role in recognition of modified histone substrates (40–42). More recently, the bromodomain of heterochromatin protein HP1 was discovered to bind specifically to a methylated lysine residue (Lys9) of histone H3 as part of heterochromatic silencing (43, 44). Another motif with potential transcription- and chromatin-related functions is the SANT domain (45), found in Swi3, Ada2, the co-repressor NCiR, TFIIB, and ISWI. All of these proteins are involved in transcriptional regulation, and most (Swi3, Ada2, NCiR, and ISWI) are present in chromatin-regulatory complexes. SANT-homologous repeats in the Myb oncoprotein have been implicated in DNA binding (46, 47), but the function of the SANT motif itself is still unclear. However, recent reports indicate that a SANT-containing region of several co-repressors can bind (48, 49) and activate (49) histone deacetylases (HDACs), again indicating a chromatin-related role.

Recent structural studies of HATs have revealed how substrate is bound within the catalytic site. The x-ray crystal structures of several HAT domains, including Hat1, Gen5, PCAF, and Esa1, have recently been solved (50). It is known...
that histone tail substrate interacts with the Gcn5 HAT domain within a pronounced cleft created by the N- and C-terminal portions of the domain (51). Previous mutational studies have identified key residues for catalytic function (30, 52–54), and these residues map to contact points with acetyl-CoA and histone substrate. One interesting aspect of the HAT structures is the presence of evolutionarily conserved and solvent-exposed residues in the HAT domain that do not make contact with either acetyl-CoA or histone. Thus, these residues may serve as direct docking sites for certain aspects of nucleosomes or for additional proteins that recognize nucleosomes.

To gain a better understanding of the structure and function of the SAGA complex in relationship to substrate recognition, we have characterized the SAGA complex from a series of deletion and substitution mutants of Ada2 and Gcn5. The Ada2 and Gcn5 deletion mutants chosen for this study and summaries of their previously determined in vivo effects and in vitro interactions are shown in Fig. 1. The results of the present study identify new specific regions of Ada2 and Gcn5 that are important in substrate recognition.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Media—**For ADA2 integrations, ada2Δ strain SB330 (MAT a ada2Δ his3Δ200 leu2Δ1 ura3–52) was produced by transforming FY1369 with an ada2Δ::hisG-URA3-hisG fragment from plasmid pYDA2-RO (2); Ura– transformants were then plated on 5-fluoro-orotic acid media to select for loss of the URA3 marker (55). For integration of GNC5 truncations, gcn5Δ strain FY1370 (MAT a gcn5Δ::HIS3 hisΔ200 leu2Δ1 ura3–52) was used. For integration of GCN5 alanine substitution mutations, gcn5Δ/Ada2-tagged strain SB349 (MAT a gcn5Δ ada2Δ-2FlHAT::TRP1 leu2Δ1 trp1Δ1 ura3–52) was used; it was produced (as described above for SB330) by GCN5 knockout of Ada2-tagged strain SB345 (MAT a ADA2-2Flag::TRP1 leu2Δ1 trp1Δ1 ura3–52), in which strain SB341, FY1369, was genomically tagged at the C terminus of Ada2 with two tandem FLAG epitope tag sequences by the method of Longtine et al. (56), and the hisΔ200 mutation was removed by recombination with a wild-type HIS3 PCR product. YPD and synthetic complete media were used for nonselective and selective growth, respectively (57). Growth assays presented in Table I used minimal (plus leucine), low nitrogen (SLAD), inositol-free synthetic complete, and PEG (glycerol/ethanol) media plates as described by Lo et al. (58). For caffeine and 3-aminotriazole plates, these reagents were included in synthetic complete media at concentrations of 12 and 100 mM, respectively.

**Plasmid Constructs—**To make some of the constructs for plasmid integration, pRS306-PADΔ (22) was digested with Nol1, and forms of ADA2 with 253N (Fig. 1), or the corresponds pSP64 constructs (19). Wild-type or ADA2-nterminal half of Gcn5 (170) was used. For ADA2 alanine substitution mutants, the normalized amounts of SAGA peak fractions were 5.5 m of wild type, 10 m of DEE, and 7.5 m of W-I. For Gcn5 truncations, 10 m of wild type and 1253N and 20 m of Δ169N were used. Proteins were electrobotted to 0.45-μm nitrocellulose membranes, and SAGA subunits were detected with specific antisera as described previously (22). Anti-Ada3 antiserum was provided by L. Guarante’s laboratory and was used at a dilution of 1:5000.

**RESULTS**

**Analysis of Ada2 Mutant SAGAs—**The Ada2 deletion mutants fall into three categories with respect to their effects on growth and transcription: normal, impaired, and very impaired (19) (Fig. 1A). We wished to determine the structural and functional basis for these in vivo defects and, in particular, whether HAT activity was altered by any of these mutations in Ada2.

SAGA complexes were prepared by conventional chromatography (16) from integrants of wild-type ADA2 and the deletion mutants. The complexes were analyzed for subunit composition by Western blotting with antibodies against a subset of SAGA components (see below), and salt content was equal made in all assays by way of chromatography buffer. Reaction products were run on SDS-15%–PAGE gels, which were visualized by fluorography (Entensify or Enlightning; PerkinElmer). SAGA immunoprecipitation for HAT assays in Fig. 3 was performed by incubating 40 μl (wild type) or 60 μl (ΔSANT-a) of SAGA Mono Q peak fraction with 2 μl of anti-Taf6,60 or anti-Taf6,145 (negative control) antisera and 30 μl of Protein A-agarose beads (Amersham Biosciences, Inc.) in a total volume of 22 μl with extraction buffer (16) for 1 h at 4 °C. Beads were washed five times with 500 μl of extraction buffer and once with 500 μl of HAT buffer (50 mM Tris, pH 8.0, 5% glycerol, 50 mM KCl, 0.5 mM EDTA, 1 mM dithiorthreitol, 1 mM phenylmethylsulfonyl fluoride). Assays were performed for 45 min at 30 °C with rotation after combining 10 μl of 50% bead slurry (one-sixth of sample) with 50 μl of a mixture containing HAT buffer with 10 mM sodium butyrate, 0.5 μl of [3H]acetyl-CoA (Amersham Biosciences), and 5 μg of nucleosomes or 40 μg of free histones. Of the final 60 μl of slurry, 40 μl was run on SDS-PAGE as described above.

**HAT Assays—**HAT assays in Table I used integrants in an Ada2--2FLAG-tagged strain were grown in 50 ml of YPD to an optical density of 0.8 at the 600-nm wavelength, and extracts were prepared in extraction buffer by glass bead breakage. Amounts of extract containing 1 mg of total protein (Bio-Rad assay) were used for immunoprecipitation with 1.5 μl of anti-FLAG M2 antibody (4.4 mg/ml; Sigma) at 4 °C overnight, followed by 30 μl of Protein G-Sepharose beads (Amersham Biosciences) for several hours and elution with 2.5 μl of 15.8 mg/ml FLAG peptide in 40 μl of extraction buffer for several hours at 4 °C. 5 μl of these elutions were used for HAT assays, and 10 μl were used for Western blots.

**Western Blotting—**For Western blot analysis, SAGA peak fractions from Mono Q were run on SDS-10%–PAGE gels. For Ada2 deletion mutant preparations with lower yields of SAGA (ΔSANT-a and Δ7), more sample was used in each lane; this normalization was based on peak fraction with 2 μl of Taf6,60 and Spt2 Western blots. Amounts used were typically 20 μl for ΔSANT-a, 15 μl for Δ7, and 10 μl for the others. For Ada2 alanine substitution mutants, the normalized amounts of SAGA peak fractions were 5.5 μl of wild type, 10 μl of DEE, and 7.5 μl of W-I. For Gcn5 truncations, 10 μl of wild type and 1253N and 20 μl of Δ169N were used. Proteins were electrobotted to 0.45-μm nitrocellulose membranes, and SAGA subunits were detected with specific antisera as described previously (22). Anti-Ada3 antiserum was provided by L. Guarante’s laboratory and was used at a dilution of 1:5000.
the SANT domain also contained all subunits. (The level of Ada2 appears somewhat low in the upper left panel of Fig. 2, but an antibody study with HA-tagged Ada2(SANT-a) (Fig. 2, right panel) suggests that the Ada2(SANT-a) subunit is present in wild-type amounts but reacts poorly with anti-Ada2 antiserum.) However, despite the finding that all SAGA subunits tested were present in normal amounts, the strain bearing ada2(SANT-a) had impaired functions in vivo, the basis of which is explored below.

The other Ada2 mutants resulted in loss of specific subunits from SAGA. SAGA(ΔSANT-b) (with deletion of the C-terminal half of Ada2’s SANT domain) specifically lacked Gcn5, consistent with the previous identification of this region as critical for Gcn5 interaction in vitro (19). SAGA(Δ7) lacks most of SANT and an adjacent region N-terminal to it, and this mutant SAGA also lacked Gcn5; Ada2Δ7 itself may also be absent, since extremely low signal is detected, although this deletion does remove the SANT-a region, which appears to be required for full recognition by the anti-Ada2 antiserum. The Ada2Δ9 deletion removes the C-terminal half of SANT-b, and in this case Gcn5 was still present in SAGA, albeit at a significantly reduced level.

SAGA(Δ12) and SAGA(Δ14) displayed little or no Ada2, Ada3, or Gcn5 and therefore have lost the entire subgroup of SAGA. Previous analysis showed that these deletions destabilized Ada2 and abolished the Ada2-Ada3 interaction in vitro (19); in the context of SAGA, these disruptions have resulted in Ada2/Ada3/Gcn5 failing to be incorporated or maintained in the complex.

Overall, defects in SAGA subunit composition largely correlated with the three categories of in vivo defects. ADA2 mutant strains with normal growth (wild type and ZZ) contained intact SAGA; those with impaired growth (ΔSANT-b and Δ9) lacked the Gcn5 subunit; and those with very impaired growth (Δ7, Δ12, and Δ14) lacked multiple adaptor subunits. These results suggest that Ada2 and Ada3 provide functions in addition to those associated with Gen5/acytlation. However, the one exception to the pattern described above is ΔSANT-a, which had significantly impaired phenotypes (19) despite the fact that the Gen5/Ada2/Ada3 module was present in SAGA at normal levels.

Functional Importance of Ada2 SANT Region—Further functional analysis of Ada2 focused primarily on the SANT-a region, since SAGA bearing this deletion had a full complement of subunits (Fig. 2) yet displayed profound defects in vivo (19). Because of these defects and because the SANT domain is present in other chromatin-interacting proteins, SAGA (ΔSANT-a) was tested for HAT activity in vitro, to determine...
whether the SANT domain influences SAGA’s HAT function (Fig. 3). The three analyzed complexes that contained normal levels of Gcn5 (wild type, SAGA(ΔSANT-a), and SAGA(ΔSANT-a)) were tested for HAT activity on nucleosomal and free histone substrates (Fig. 3A). Activity levels for SAGA(ΔZZ) were only slightly reduced relative to wild type, but SAGA(ΔSANT-a) was much more defective. Nucleosomal HAT activity was particularly low for SAGA(ΔSANT-a), as demonstrated by these direct assays of the Mono Q fractions. In a second HAT experiment (Fig. 3B), normalized amounts of peak SAGA fractions (determined by Western blotting) were assayed with histone H3 N-terminal tail peptide substrate (58). Data from scintillation counting (4C, graph) show that HAT activity of SAGA from ADA2(ΔSANT), the most defective substitution mutant, was almost 2-fold less than wild type (54% of wild type), while ADA2(W-I), a less defective substitution mutant, resulted in SAGA that had 73% of wild-type activity. A fluorographed gel of these assays (4C, bottom) further demonstrates the relative HAT defectiveness of ΔE and W-I SAGA. These results show a correlation between HAT impairment in SAGA and defects in vivo, pointing to a physiological role for this region in chromatin modification.

Effect of Gen5 HAT Domain Deletions on SAGA Composition—Previously, truncations of the bromodomain (22) and alanine substitutions within the HAT domain (30) of Gcn5 were shown to have effects on the HAT activity but not the overall composition of SAGA. Another study (20) demonstrated the effects of large deletions of the HAT domain on growth, in vivo transcription, and in vitro HAT activity, but did not examine SAGA specifically. To address the question of HAT domain deletions on SAGA composition, we analyzed complexes prepared from two N-terminal truncation mutants, gen5Δ169N containing residues 170–439 (deleting region 1 of the Gcn5 HAT domain) or gen5Δ253N containing residues 254–439 (deleting the entire HAT domain) (Fig. 1B).

SAGA was prepared from these mutants and analyzed for subunit content by Western blotting (Fig. 5). Interestingly, the mutant Gcn5 proteins were absent from SAGA. SAGA subunits other than Gcn5, including Ada2 and Ada3, were found to be present in wild-type amounts in both mutants. The Gcn5 mutant proteins are likely to be stable in vivo because a LexA fusion of the more severe truncation (Gen5Δ253N) could be successfully overexpressed in yeast. In addition, both mutants are structurally competent, since they bind to Ada2 when expressed by coupled in vitro transcription/translation (19). Furthermore, E. coli-expressed versions of the truncated proteins were detectable by the anti-Gcn5 antisera (data not shown). It should be noted that a less dramatic Gcn5 N-terminal truncation mutant (gen5Δ43N) displayed wild-type growth and in vivo transcription previously (20), indicating that it is incorpo-

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2. R. Candau, unpublished data.
rated into SAGA. Thus, the results in Fig. 4 implicate HAT domain region I and/or its surrounding regions as critical to the association of Gcn5 with the rest of the SAGA complex, despite the considerable distance of this region of Gcn5 from the region that had previously been shown to interact with Ada2 (Gcn5 residues 254–280) (20).

Mutation of Potential Interaction Residues in the Gcn5 HAT Domain—The HAT domain contains multiple residues that are highly conserved but are not apparently involved in HAT function (i.e. they do not make histone or CoA contacts), nor are they catalytic or required for structural integrity (51). A subset of these conserved residues are solvent-exposed and therefore could be required for Gcn5’s interaction with the SAGA complex (as shown above) or for establishing HAT substrate specificity. To investigate this, we made a series of substitution mutations (KE-YD, Y-KD-E, K-TKE, I-R-H-N, and K-T-K) combining multiple alanine substitutions of these evolutionarily conserved and solvent-exposed residues (Fig. 6 in aqua) on four predicted potential interaction surfaces of the HAT domain (Fig. 6B). Avoided were residues shown by mutagenesis (30, 52) or predicted by structure determination (51, 54) to be specifically involved in HAT catalytic mechanism or interaction with its substrates (histone tail or acetyl-CoA) (Fig. 6A in red).

As summarized in Table I, integrants of these Gcn5 mutants were tested for growth along with those of wild-type Gcn5 and empty vector (gcn5/H9004) to determine their in vivo effects. Conditions used were three different temperatures (23, 30, and 37 °C) and various plate media on which gcn5/H9004 cells show a significant phenotype (22, 58): minimal, inositol-free, low nitrogen, YPGE (glycerol and ethanol carbon sources), 12 mM caffeine, and 100 mM 3-aminotriazole. Two mutants, KE-YD and I-R-H-N, displayed wild type-like growth under all conditions. Two other, overlapping mutants, K-TKE and K-T-K, showed subtle growth defects, displaying partial growth impairment under two specific conditions (YPGE at 23 °C and 3-aminotriazole at 30 °C) but growing like wild-type under all others tested. One mutant, Y-KD-E, was very defective and grew similarly to gcn5/H9004 cells.
These substitutions could be in either substrate recognition surfaces or SAGA interaction surfaces within the HAT domain of Gcn5 (as in Fig. 5). To determine whether normal amounts of Gcn5 (relative to SAGA) were present, complexes were isolated by Ada2-FLAG immunoprecipitation. Anti-TafII60 immunoblot analysis indicated that a similar amount of the SAGA complex was present in each immunoprecipitate (Fig. 6). All of the mutant strains were shown to contain wild-type amounts of Gcn5 (Fig. 6), indicating that all of the mutant proteins interacted with SAGA. In fact, a combined mutant of all 16 potential interaction residues (KE-YD/I-R-H-N/K-TKE/Y-KD-E) still resulted in wild-type levels of Gcn5 in the complexes, indicating that the alanine substitutions, even altogether, were not sufficient to dislodge Gcn5 from SAGA. If the indicated HAT domain surfaces are in fact critical for Gcn5-SAGA interaction, more dramatic substitutions (e.g. substituting bulky or oppositely charged residues instead of alanine) may be required to effect loss of Gcn5.

These results suggested that the defective phenotypes may instead be due to an effect on HAT activity. To test this, HAT assays were performed on immunoprecipitates from all of the mutant groups, using both free histone and nucleosomal substrates (Table I). All of the mutant complexes displayed HAT activities significantly lower than those of wild type. The lowest HAT activities overall were seen in mutants that displayed phenotypic defects (Y-KD-E, K-TKE, and K-T-K), suggesting...
that the \textit{in vivo} effects could be due to changes in HAT activity due to loss of an interaction required for HAT function. Y-KD-E was completely defective \textit{in vivo} and had the lowest function of all of the substitution mutants on nucleosomal histones, in fact as low as SAGA(Gcn5Δ). It is interesting that predictions from the crystal structure suggest that these residues are not required for binding the histone substrate (51). The severe HAT defects of the K-TKE and K-T-K proteins \textit{in vitro} come despite the fact that the phenotypes of these mutants are subtle, suggesting that these substitutions are compensated for \textit{in vivo}. Furthermore, S1 nuclease analysis (36) of HIS23 and HO RNA from the K-TKE mutant (as well as the wild type-like KE-YD and H-N, a subset of I-R-H-N) showed that transcription of these genes was unaffected by these particular GCN5 mutations (data not shown).

Mutants that displayed no defective phenotypes \textit{in vivo} (KE-YD and I-R-H-N) also displayed lowered HAT activities \textit{in vitro}. KE-YD had the most striking effect of the mutants; free histone HAT was reduced 2–3 fold relative to wild-type, while nucleosomal HAT was reduced just 2–3-fold, suggesting a modest effect of these alterations on recognition of both histone and nucleosomal substrates. Taken overall in context of all of the mutants, the HAT results for KE-YD may indicate a specific defect in interaction with nucleosomes.

Further support for a significant role for Gen5's KE-YD, I-R-H-N, and K-TKE residues \textit{in vivo} (despite their nonexistent or weak phenotypes) is shown by combined mutations of these groups (Fig. 6D). As shown on plates of three different media, a KE-YD/I-R-H-N combined mutant was partially impaired for growth at 30 °C, with colony size intermediate between wild-type and gen5Δ. Furthermore, when the subtle phenotype K-TKE substitutions were added to this combination, cells grew as poorly as gen5Δ under all conditions tested. These results demonstrate that in the case of these three residue groups, mutations in multiple surfaces are required to cause major functional defects \textit{in vivo}.

**DISCUSSION**

There are a large number of transcriptional regulatory proteins that interact with and alter chromatin, and these display considerable specificity in their action with respect to histone selectivity and ability to recognize nucleosomes. How proteins that modify chromatin achieve specificity in substrate recognition is an important mechanistic issue, and, given the role of chromatin in cancer (63), relevant to possible development of specific agents to treat disease states. One relevant feature of both ATP-dependent remodeling proteins and histone modification proteins is the fact that they are components of large complexes, and it is clear that specificity is established in part by this assembly. Two aspects of complex association establish specificity. First, domains within the enzymes themselves take on new roles, as exemplified by the bromodomain of Gen5, which is required for normal nucleosome acetylation by Gen5 when in the SAGA complex (22) but cannot on its own in the monomeric enzyme confer the ability to target nucleosomes. The second attribute of the complexes in setting specificity is the presence of additional interacting proteins that have a role in establishing specificity. For example, Taf68 in the SAGA complex confers nucleosome specificity to Gen5 acetylation. In this report, we have identified two new interaction modules, one of each type discussed above, that allow the SAGA complex to interact with nucleosomal and histone substrates. The first is via a region in the N-terminal portion of Gen5's HAT domain, and the second is via the SANT domain in Ada2.

**Substitution Mutations in Gen5 Suggest New Interaction Points with Histones and with Nucleosomes**—Previous evidence from the x-ray crystal structure of the HAT domain of Gen5 revealed the identity of residues that are involved in histone binding, cofactor acetyl-CoA interaction, and maintenance of structure. However, there were a number of evolutionarily conserved and solvent-exposed residues that did not have a role in these interactions. Because of the possibility that some of these residues might have a role in substrate specificity, we mutagenized groups of residues. The groups were based on potential binding surfaces as predicted from the crystallographic structure (Fig. 6). The results of this approach led to identification of the N-terminal portion of the HAT domain as potentially involved in establishing specificity for nucleosomal substrate interaction. This region is composed of two α-helices and a β-sheet, and the conserved residues KE-YD occur on the external surface. Immunoprecipitated complexes bearing the KE-YD substitution mutations were particularly defective for nucleosome acetylation compared with wild-type or I-R-H-N substitution mutant complexes, indicating that these residues may play a role in nucleosome substrate recognition. This region may directly interact with nucleosomes (i.e. when Gen5 is stably incorporated into the complex) or may interact through another protein, such as Taf68, since it has been shown to be important in nucleosome interaction. However, we do not think this protein is Taf68, because we do not see any loss of Taf68 in either the deletion mutant of Gen5 HAT domain (Fig. 5) or in the substitution mutation KE-YD (data not shown). The fact that certain substitutions in Gen5 (K-TKE and K-T-K) show...
low and high temperature defects is consistent with effects on protein/protein interaction rather than disrupting the structure. We have not detected a phenotype for the KE-YD substitution mutant in growth assays (except in combination with other mutations such as I-R-H-N). In this regard, it should be noted that alanine substitution of physiologically relevant residues of histone H3, for example, also result in no detectable growth phenotype but cause lowered transcription of certain genes (58). Interestingly, this N-terminal portion of the HAT domain is also required for Gcn5’s interaction with the SAGA complex (Fig. 5; see mutant A169N). However, the substitution mutations in this region did not lower the amount of Gcn5 in the complex. The difference between these results may be due to the existence of multiple additional contact points within this region. The contact points could be direct ones that define a surface of interaction with another protein in the complex that is required for association and may, in addition, recognize a unique aspect of nucleosomes. It is also possible that the association with the complex and association with nucleosomes are separate functions of this region.

A second interesting mutant is Y-KD-E, in the C-terminal portion of the HAT domain. Y-KD-E was extremely defective for growth and had very low activity on both free histone and nucleosomal substrates. Careful examination of the x-ray crystal structure of the Gcn5 HAT domain in complex with the histone-tail peptide and acetyl-CoA (51) indicates that these residues are unlikely to make histone substrate contacts. It is possible that this C-terminal region makes contact with Ada2 in the complex. Thus, taken together with the data on KE-YD in the N-terminal portion of the HAT domain, one model is that the C-terminal region provides crucial interaction with Ada2 to allow an appropriate conformation of the domain for HAT activity in the context of the SAGA complex, and the N-terminal region provides interactions with nucleosomal substrates (either direct or through another protein).

Substitutions in Ada2 Indicate That the SANT Domain Is Important in Nucleosome Interaction—Mutant analysis of Ada2 revealed apparent dual functions of the SANT domain within SAGA, such that SANT can be roughly divided into two linked subdomains, SANT-a and SANT-b. The primary function of SANT-b, the second half of SANT, is specific interaction with the acetylase Gcn5, since SAGA from a ΔSANT-b mutant lacked Gcn5 but no other observed subunits. Through results of the Δ7 and Δ9 mutations, the most critical region for this interaction can be further mapped to the N-terminal half of SANT-b (i.e. the third quarter of SANT). It is interesting to note that SANT domains in several corepressors (CoREST, SMRT, and NCoR) have been shown to have a similar function. CoREST has recently been shown to be in a stable repression complex with HDACs, and the SANT domain of CoREST is the interaction surface with the HDACs (48). Thus, the interaction of both a HAT and an HDAC with their respective complexes requires the SANT-b domain. In the case of SMRT and NCoR, their SANT domains not only interact with HDAC3 but also activate its deacetylase function (49). This raises the interesting possibility that the SANT-a domain within these co-repressors may be required for histone interaction for deacetylation, parallel to the situation for acetylation through Ada2 interaction with Gcn5.

The function of SANT-a, the first half of SANT, appears to be directly related to interaction with or recognition of chromatin. SANT-a deletion led to no observed changes in SAGA composition, and purified complexes contained a full complement of Gcn5. Despite this, it was clear that the SANT-a region must have a significant role in vivo, since ΔSANT-a mutant cells are defective in growth and transcription (19). In vitro studies with SAGA have now revealed a likely functional reason for these phenotypes: defects in histone acetylation by the SAGA complex. The Ada2 SANT-a region is needed for full HAT activity of SAGA, particularly on nucleosomal substrates, indicating that the mutant’s in vivo defects may be due to Ada2’s influence on chromatin modification. Alanine substitution mutations have further characterized SANT-a’s function and the importance of certain conserved residues in the motif to chromatin utilization by SAGA. The sensitivity of this domain to mutation is demonstrated by the fact that several sets of point mutations in SANT-a had major in vivo effects nearly comparable with those of the SANT-a deletion. And in at least two cases, the substitutions caused growth defects that correlated with defective HAT activity in purified SAGA, further suggesting a chromatin-related role for SANT in transcriptional regulation.

There are several possible models for the involvement of Ada2’s SANT domain in chromatin modification. One possibility is that SANT-a interacts directly with histones, perhaps stabilizing Gcn5’s substrate binding and allowing generally more efficient acetylation. But a more complex scenario with relevance to circumstances in vivo involves the recognition by SANT of specific forms of histones or unique aspects of nucleosomes. Following the “histone code” hypothesis, the SANT domain might recognize a specific modification on a specific histone tail in the same way that two other long recognized, transcription-related motifs were recently found to function: the bromodomain binds acetyl-lysine, and the chromodomain binds methyl-lysine on histone tails. Another possibility is that SANT confers a specificity for nucleosomes as a substrate, causing preferential interaction with nucleosomal structures rather than free histones. Ada2 SANT may accomplish this either by binding substrate directly or by way of its close associations with Gcn5, affecting the acetylase’s substrate interactions, perhaps by causing conformational changes. Gcn5 without Ada2 is unable to modify nucleosomes, and the present study demonstrates that ΔSANT-a SAGA is particularly defective for nucleosome acetylation. This defect was also seen previously upon deletion of the Gcn5 bromodomain (22), although the bromodomain is not as critical to function as SANT. Interestingly, both SANT and bromodomains are found in other chromatin-remodeling complexes such as Swi-Snf, suggesting that these motifs may in general play a role in nucleosome interaction/recognition by regulatory complexes. Another possible target of SANT is nucleosomal DNA; since Myb domains have been shown to have DNA binding function, especially on promoter sequences (64), it is conceivable that the Myb-related SANT region could help stabilize histone substrate interaction by interacting with DNA within or adjacent to nucleosomes. Furthermore, if Ada2 SANT had any specificity toward SAGA-dependent promoter sequences, this would provide another level of promoter targeting by SAGA, in addition to activator interaction. However, in the above cases, it should be noted that SANT-mediated substrate interactions would not be strictly nucleosome-specific, since SANT-a mutant SAGA complexes are also significantly impaired for free histone acetylation. Finally, another possibility is that the ΔSANT-a defects are due to loss of an unknown subunit from SAGA, since SAGA’s ability to acetylate nucleosomes is determined by factor(s) other than just Gcn5 and Ada2 (16).

Thus, there are now four distinct proteins/sequence elements that have been observed to have a role in nucleosome interaction/recognition by SAGA: the bromodomain of Gcn5 (22), Taf68 (16), the SANT homology region of Ada2 (this study), and potentially the external surface of the N-terminal portion of the Gcn5 HAT domain. Whether these proteins/regions interact with each other, directly interact with nucleosomes, or
interact with one or more additional components that specifically recognize nucleosomes is a question for future studies. This could be an important general issue in chromatin remodeling, because Tat68 is a component of TFIIID (which contains the acetyltransferase Tat145), and the bromodomain and SANT domain are present in Swi2 and Swi3, respectively, in the Swi-Snf nucleosome remodeling complex. Therefore, it appears that there are multiple conserved domains in chromatin remodeling complexes that promote interaction with histone nucleosomal substrates.

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The SANT Domain of Ada2 Is Required for Normal Acetylation of Histones by the Yeast SAGA Complex

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