The ADA Complex Is a Distinct Histone Acetyltransferase Complex in *Saccharomyces cerevisiae*

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We have identified two Gcn5-dependent histone acetyltransferase (HAT) complexes from *Saccharomyces cerevisiae*, the 0.8-MDa ADA complex and the 1.8-MDa SAGA complex. The SAGA (Spt-Ada-Gcn5-acetyltransferase) complex contains several subunits which also function as part of other protein complexes, including a subset of TATA box binding protein-associated factors (TAFIIs) and Tra1. These observations raise the question of whether the 0.8-MDa ADA complex is a subcomplex of SAGA or whether it is a distinct HAT complex that also shares subunits with SAGA. To address this issue, we sought to determine if the ADA complex contained subunits that are not present in the SAGA complex. In this study, we report the purification of the ADA complex over 10 chromatographic steps. By a combination of mass spectrometry analysis and immunoblotting, we demonstrate that the adapter proteins Ada2, Ada3, and Gcn5 are indeed integral components of ADA. Furthermore, we identify the product of the *S. cerevisiae* gene YOR023C as a novel subunit of the ADA complex and name it Ahc1 for ADA HAT complex component 1. Biochemical functions of YOR023C have not been reported. However, *AHC1* in high copy numbers suppresses the cold sensitivity caused by particular mutations in HTA1 (I. Pinto and F. Winston, personal communication), which encodes histone H2A (J. N. Hirschhorn et al., Mol. Cell. Biol. 15:1999–2009, 1995). Deletion of *AHC1* disrupted the integrity of the ADA complex but did not affect SAGA or give rise to classic Ada− phenotypes. These results indicate that Gcn5, Ada2, and Ada3 function as part of a unique HAT complex (ADA) and represent shared subunits between this complex and SAGA.

Posttranslational modifications of nucleosomal histones have been correlated with the modulation of the structure and function of chromatin (7). One of the most extensively studied modifications is the acetylation of the highly conserved amino-terminal histone tails. The steady-state level of acetylation of histone proteins is accomplished by the action of histone acetyltransferases (HATs) and histone deacetylases (HDACs) (37). Acetylation affects higher-order folding of chromatin fibers (16) and the interaction of nonhistone proteins with histones (14). It also plays an important role in histone deposition and nucleosome assembly during S phase (48) and can increase the affinity of transcription factors for nucleosomal DNA (35, 61). Correlations between transcription and histone acetylation are strengthened by reports showing that active chromosomal domains are hyperacetylated (6, 14, 32). While heterochromatin domains are hypoacetylated (10, 51).

A large number of recent studies have provided a direct molecular link between histone acetylation and transcriptional activation (24, 63). In these reports, it has been shown that several previously identified coactivators-adapters of transcription possess intrinsic HAT activity. Among these coactivators are yeast Gcn5 (11), human Gcn5 (65, 69), p300/Creb-binding protein (CBP)-associated factor (P/CAF) (71), TATA box binding protein (TBP)-associated factor 250 (TAFII250) (41), p300/CBP (2, 43), ACTR (12), and steroid receptor coactivator 1 (SRC-1) (55). Conversely, several transcriptional repressors and/or corepressors have been shown to be associated with HDACs, including Rpd3 (59), Sin3 (27, 33, 34, 73), and N-CoR/SMRT (1, 28). Moreover, human and *Xenopus* complexes containing both HDAC activity and ATP-dependent nucleosome remodeling have been isolated (62, 70, 74).

Many of these chromatin-modifying activities have been found within large multisubunit protein complexes that also contain several components with homology or identity to known transcriptional regulators (25, 58). Indeed, the coactivator-adaptor protein Gcn5 is part of large multisubunit complexes in *Saccharomyces cerevisiae*, which enhances its ability to acetylate nucleosomal histones (20, 30, 46, 50, 51). In yeast, Gcn5 is involved in the regulation of a variety of genes (9, 18, 39, 51, 66). The largest of the Gcn5-dependent HAT complexes is the 1.8-MDa SAGA complex. SAGA comprises at least four distinct classes of gene products (22, 23). First, there are the Ada proteins Ada1, Ada2, Ada3, Gcn5 (Ada4), and Ada5 (Spt20), which have been isolated as proteins interacting functionally with the yeast activator Gcn4 and the herpes simplex virus activation domain VP16 (3, 4). The second group comprises Spt3, Spt7, Spt8, and Spt20 (Ada5). These proteins are all members of the TBP-related set of Spt proteins, initially identified as suppressors of transcription initiation defects caused by promoter insertions of the transposable element Ty (68). The third group of proteins found to be part of SAGA are a subset of TAFIIs, including TAFII20/17, TAFII25/23, TAFII60, TAFII68/61, and TAFII90 (22). Finally, the product of the essential gene *TRA1* has been shown to be a component...
of SAGA (23, 52). Apparent counterparts of the SAGA complex have been isolated from mammalian cells (40, 42, 67).

The second Gcn5-dependent HAT complex is the 0.8-MDa ADA complex, which differs from SAGA in many aspects. While the ADA complex is also dependent on and cofractionates with Ada2, it is not dependent on Ada1, Ada5 (Spt20), or the other Spt proteins found in SAGA (20, 57). Both the ADA and SAGA complexes can stimulate in vitro transcription from nucleosome templates in an acetyl coenzyme A-dependent reaction (56). However, the SAGA complex has been shown to physically interact with the acidic activators Gcn4 and VP16, whereas ADA failed to do so (60). In addition, we recently demonstrated that the ADA and SAGA HAT complexes generate overlapping, yet distinct, patterns of lysine acetylation on histone H3. While ADA can acetylate lysine residues 14 and 18 in histone H3, SAGA acetylates to some extent all four lysines in H3 (21).

Despite these differences between the two Gcn5-dependent HAT complexes, it remained unclear whether the smaller ADA is a subcomplex of the larger SAGA or functions as a distinct HAT complex in yeast. Fourteen subunits contained in the SAGA complex have been identified so far (22, 23). On the other hand, the proteins contained in ADA, other than the three adapter proteins (i.e., Ada2, Ada3, and Gcn5), were unknown. The best way to address whether ADA is distinct from SAGA is through the identification of ADA specific components. We therefore purified the native ADA HAT complex from yeast. Mass spectrometry and immunoblotting analysis of the purified complex demonstrated that the yeast adapter proteins Ada2, Ada3, and Gcn5 are indeed components of the ADA complex. Importantly, we demonstrate by

### TABLE 1. Purification of the ADA HAT complex

| Purification step | Total protein (µg) | Total units (µU) | Sp Act (µU/µg) | Purification (fold) |
|------------------|--------------------|-----------------|----------------|---------------------|
| NiAg eluate      | 580,000            | 3,000           | 5.2            | 1                   |
| 20 ml Mono Q     | 19,500             | 1,620           | 83             | 16                  |
| 1 ml Mono Q      | 4,000              | 4,800           | 1,200          | 214                 |
| Mono             | 186                | 4,350           | 23,387         | 4,498               |
| DNA cellulose    | 52,5               | 4,125           | 78,572         | 14,030              |
| Superose 6       | 16.8               | 3,090           | 184,000        | 34,783              |
| Histone agarose  | 7.9                | 1,680           | 212,658        | 40,580              |
| Superose 6       | 2.52               | 1,305           | 517,875        | 98,550              |
| Mini Q           | 0.82               | 620             | 755,854        | 147,826             |
| Superose 6 PC 3.2/30 | 0.34 | 525 | 1.54 × 10⁶ | 298,550 |

* Purification was done with whole-cell extract prepared from 90 liters of yeast cell culture.

* One microunit is defined as the activity required to transfer 1 fmol of acetyl residues to histones under standard histone acetyltransferase assay conditions (see Materials and Methods).
several criteria that the gene product of the open reading frame \( YOR023C \) is a novel component of ADA and is not present in SAGA. \( YOR023C \) in high copy numbers suppresses the cold sensitivity caused by particular mutations in HTA1 (45a), which encodes histone H2A (29). We named this protein Ahc1 for ADA HAT complex component 1. The presence of Ahc1 in the ADA complex indicates that it is a unique HAT complex in yeast that shares a subset of Ada proteins (Ada2, Ada3, and Gcn5) with the SAGA complex.

**MATERIALS AND METHODS**

**Yeast strains.** ADA was purified from yeast strain CY396 (swi2::HIS3, HO-lacZ, SWI2-HA-6HIS::URA3) and was described previously (44). Construction of a complete disruption of the \( YOR023C \) gene was carried out applying the one-step gene disruption (49) method with \( LEU2 \) as the disrupting marker.
Transformation into yeast strain YJW 104 (MATa ade2-1 his3-11 leu2-3,112 trp1-1 ura3-1 can1-100) created YJW 103 (MATa ade2-1 his3-11 ada1Δ::LEU2 trp1-1 ura3-1 can1-100). We verified the correct integration by PCR with two

**RESULTS**

**Purification of the ADA complex.** To investigate its relationship to SAGA, we purified the ADA complex by two criteria. Column fractions indicated fractions was separated after Mono Q chromatography by SDS–10% polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. Membranes were incubated with antibodies (α) against Ada1, Ada2, Ada3, and Sp8.

For purification of the ADA complex, we

For plate growth experiments, wild-type, ada1Δ, and ada2Δ yeast strains were transformed with high-copy-number Gal4-VPl6 plasmid (4) or empty vector (pDB20L-BgIII) bearing the LEU2 selective marker and plated directly onto synthetic dextrose minimal medium. Plates were grown for 3 days at 30°C; Gal4-VPl6 plates were incubated for an additional day at room temperature. For in vivo transcription assays, wild-type, ada1Δ, and ada2Δ double transformants, containing pGDS5 reporter plasmid (26) and low-copy-number Gal4-VPl6 (4) or Gal4-VPl6-MCS plasmid or empty vector (pRS315) (54), were grown to an optical density of 0.8 at 600 nm in selective synthetic complete medium. Extracts, prepared by breaking cells with glass beads, were tested for β-galactosidase activity and protein concentration as described previously (47). Reported values are the averages of the results from two to four independent transformants for each strain-plasmid combination.

**HAT assays, Western blotting, antibodies, and immunoprecipitation.** HAT assays were performed as previously described (33). For each chromatography, equivalent amounts of fractionated samples were subjected to electrophoresis with sodium dodecyl sulfate (SDS)–10% polyacrylamide gels, transferred to nitrocellulose membranes, and processed for immunoblotting. Anti-Ada1 antibodies were raised in rabbits against a synthetic peptide spanning the amino terminal 16 amino acids of Yor023C (MSPMAQDKLQHQHNHPN) by Research Genetics. A monoclonal anti-HA antibody was purchased from Boehringer Mannheim (Mannheim, Germany). Immunoblotting procedures and an enhanced chemiluminescence kit from Amersham according to the manufacturer’s protocol. For immunoprecipitation experiments, equivalent titers of anti-Ada2 or anti-Ahc1 antibodies were incubated with 20 μl of preequilibrated protein A-Sepharose resin (Pharmacia) for 1 h at room temperature. Beads were washed five times with immunoprecipitation (IP) buffer (50 mM HEPEs [pH 7.8], 150 mM NaCl, 10% glycerol, 0.1% Tween 20, 2 μg of leupeptin per ml, 2 μg of pepstatin A per ml, 1 mM PMSF), and purified ADA and SAGA fractions were added and incubated in immunoprecipitation (IP) buffer for 4 h at 4°C on a rotating wheel. After incubation supernatants were collected, the beads were washed five times with IP buffer. Input material, supernatants, and beads were directly assayed for HAT activity with free-core histones as a substrate. Protein concentrations were determined according to the method described by Bradford (8).

**Mass spectrometry analysis.** ADA peak fractions after the final Mini Q column were concentrated in Microconcentrator-30 apparatus (Amicon), loaded onto a SDS–10% polyacrylamide gel, and stained with Coomassie blue. After destaining, the bands were excised and digested in gel with trypsin, according to the method of Shevchenko et al. (53). Identification of proteins was accomplished by microcolumn high-performance liquid chromatography coupled to electrospray ionization tandem mass spectrometry and database searching. A 100-μg gel band was electroblotted onto a nitrocellulose membrane, and the membrane was cut into 10-cm sections. For each section, amino acid sequence data were acquired during the entire gradient run (36). Tandem mass spectra were searched against the Saccharomyces genome database (Saccharomyces Genome Database at Stanford University with the SEQUEST program (15). Every sequence with high scores that matched a tandem mass spectrum was manually verified. To facilitate the identification of potential contaminants, sequences for human keratin and bovine fibrinogen were included.

**β-Galactosidase assays and overexpression of Gal4-VPl6.** For plate growth experiments, wild-type, ada1Δ, and ada2Δ yeast strains were transformed with high-copy-number Gal4-VPl6 plasmid (4) or empty vector (pDB20L-BgIII) bearing the LEU2 selective marker and plated directly onto synthetic dextrose minimal medium. Plates were grown for 3 days at 30°C; Gal4-VPl6 plates were incubated for an additional day at room temperature. For in vivo transcription assays, wild-type, ada1Δ, and ada2Δ double transformants, containing pGDS5 reporter plasmid (26) and low-copy-number Gal4-VPl6 (4) or Gal4-VPl6-MCS plasmid or empty vector (pRS315) (54), were grown to an optical density of 0.8 at 600 nm in selective synthetic complete medium. Extracts, prepared by breaking cells with glass beads, were tested for β-galactosidase activity and protein concentration as described previously (47). Reported values are the averages of the results from two to four independent transformants for each strain-plasmid combination.
substrate specificity, since the ADA complex preferentially acetylates nucleosomal histones H3 and H2B (20). We also examined column fractions by Western blot analysis with antibodies to Ada2 and Gcn5. Only the peak fractions containing ADA complex activity from each column were used for subsequent chromatographic steps. The results of the purification are presented in Table 1. Aliquots of fractions from the 10th column, a Superose 6 PC 3.2/30 size exclusion column, were run on an SDS–7.5% polyacrylamide gel and analyzed by silver staining (Fig. 1B). Eight proteins with approximate molecular masses of 50, 55, 65, 90, 97, 110, 180, and 250 kDa coeluted with the ADA HAT activity (Fig. 1B, lanes 17 and 18). These protein bands were excised and analyzed by mass spectrometry.

The proteins migrating with molecular masses of 50, 55, and 97 kDa were identified as the adapter proteins Ada2, Gcn5, and Ada3, respectively (Fig. 2). Sixteen peptides from p97 identified this protein as Ada3. Ada2 and Gcn5 were identified by nine and five peptides, respectively. These findings confirmed previous genetic and biochemical studies with less-purified material (20). Mass spectrometry also tentatively identified the remaining subunits of the ADA complex. However, to
confirm that each putative subunit is not a contaminant in the final fraction, it is necessary to generate antibodies against peptides from the protein. This allows an examination of the copurification and immunoprecipitation of the putative subunit with the ADA complex. Moreover, by generating a yeast strain where the corresponding open reading frame is deleted, its importance for the activity and integrity of the ADA complex can be examined. Using these criteria, we have thus far confirmed the presence of one novel subunit of the ADA complex (see below).

**Identification of a novel component of the ADA complex.**

Mass spectrometry analysis of the p65 band revealed the presence of three peptides from the same open reading frame, YOR023C (Fig. 2). This previously uncharacterized open reading frame has been renamed AHC1 for ADA HAT complex component 1. The amino acid sequence of the entire protein is shown in Fig. 2B. We generated antibodies against the peptide spanning the first 16 N-terminal amino acid residues (Fig. 2B) and used this antiserum to follow this protein during the course of purification. Fractions from the initial Mono Q column were tested with the Ahc1 antiserum, since this column separates the ADA, NuA4, NuA3, and SAGA complexes (13). The Western blot results in Fig. 3 demonstrate that Ada2 and Ada3 cofractionated, as expected, with the ADA and SAGA complexes. Spt8 cofractionated only with SAGA and was not detected in fractions containing ADA. By contrast, Ahc1 cofractionated only with the ADA complex and was not found in the fractions containing the SAGA complex. Thus, while Ada2,
Ada3, and Gcn5 are contained in both complexes, each also appears to have unique subunits not found in the other, i.e., Spt8 in SAGA and Ahc1 in ADA.

To confirm the copurification of Ahc1 with the ADA complex, we performed Western blotting on highly purified ADA fractions from further chromatographic steps. Figure 4 shows Western blots with antibodies against ADA subunits and Ahc1 on fractions from columns that were used very late in the purification process, Superose 6 and Mini Q. As shown in Fig. 4A, Gcn5, Ada2, and Ahc1 coeluted with ADA HAT activity on the Superose 6 column that represented the eighth chromatographic step. Similarly, Ada2, Ada3, Gcn5, and Ahc1 coeluted with ADA complex HAT activity on the Mini Q column, the ninth column. Thus, Ahc1 copurifies with the other subunits and the HAT activity of the ADA complex through multiple chromatographic steps, suggesting that it is a bona fide subunit of the complex.

To confirm the physical association of Ahc1 with the ADA complex, we tested whether the anti-Ahc1 antisera were able to immunoprecipitate the ADA HAT complex. As demonstrated in Fig. 5, partially purified ADA prepared from the wild-type strain was eluted in fractions 18 to 22 (identified by H3-H2B HAT activity and Ada2 and Gcn5 Western blotting). In addition, the other previously identified HATs, NuA4, NuA3, and SAGA, were eluted as predicted (13). Figure 5B shows the fractionation of complexes from extract prepared from the strain bearing a disruption in AHC1. In this instance, we found that extracts from ahc1Δ cells specifically lacked the ADA complex (fractions 18 to 22), while the SAGA, NuA4, and NuA3 complexes were unaffected. There was neither detectable nucleo-
somal ADA HAT activity (Fig. 5) nor free-core histone HAT activity for ADA (results not shown). Furthermore, immunoblotting analysis with antibodies against Ada2 and Gcn5 indicated that, in addition to the loss of activity, the ADA complex itself was lost in the \textit{ahc1} preparation. Importantly, SAGA was unaffected (fractions 36 to 38). Therefore, Ahc1 behaved like Ada2 and Ada3 (20) in that it was required for the overall structural integrity of the ADA complex (19a).

To further substantiate the importance of Ahc1 for ADA integrity, we wished to address the possibility that the complex would be restored when \textit{AHC1} is expressed on a low-copy-number plasmid in \textit{ahc1} cells. To this end, we cloned \textit{AHC1} bearing a triple HA epitope tag at the C terminus into the ARS-CEN vector pRS314 (54) and expressed it under its endogenous promoter in the \textit{AHC1} deletion strain. Whole-cell extracts were prepared from yeast strains YJW 103 (\textit{ahc1}D) and YJW 104 (\textit{ahc1}D \textit{pAHC1-HA3}), and ADA was fractionated as described (see Materials and Methods). While ADA was missing in YJW 103 (Fig. 6A), we found that ADA HAT activity was present in Mono Q fractions 16 to 20 in YJW 104 (Fig. 6B). Immunodetection with antibodies against Ada2 and a monoclonal anti-HA antibody to detect epitope-tagged Ahc1 confirmed the presence of ADA in this preparation. The ADA complex was apparently fully restored in YJW 104 cells as demonstrated by size exclusion chromatography (Fig. 6C and D). The complex eluted in fractions 22 to 24 from a Superose 6 column, giving it a size of about 800 kDa. Again, no ADA complex was detectable in \textit{ahc1}D cells; however, the ADA complex was present in \textit{ahc1}D-pAHC1-HA as detected by its H3 HAT activity (Fig. 6C) and by Western blotting (Fig. 6D). Note that the slight histone H4 activity in fraction 24 on Superose 6 is from a slightly smaller contaminating HAT complex which peaks in Mono Q fractions 14 and 15 (Fig. 5) and is unrelated to ADA (12a).

An \textit{AHC1} deletion does not display an Ada phenotype. Mutations in \textit{ADA2}, \textit{ADA3}, and \textit{GCN5} (\textit{ADA4}) were isolated in a selection for mutants that confer resistance to toxicity from overexpressed Gal4-VP16. In addition, mutations in any of these genes reduced transcriptional activation by the acidic activators VP16 and GCN4 (4, 38, 45). Therefore, we wished to ask whether a mutation in \textit{AHC1} is resistant to overexpression of Gal4-VP16 and shows reduced Gal-VP16-mediated transcription levels. Overexpression of Gal4-VP16 and in vivo transcription assays were performed as described in Materials and Methods. As demonstrated in Fig. 7, \textit{AHC1} deletion did not exert the typical adapter (Ada\textsuperscript{2}) phenotypes as described for deletions of \textit{ADA2}, \textit{ADA3}, or \textit{GCN5} (4, 38, 45). First, we found that the \textit{ahc1}D strain did not have a growth defect on minimal medium (Fig. 7A, upper panels). The wild type and the \textit{AHC1} deletion strain showed similar growth on minimal media while the adapter \textit{ada2}D mutant grew more poorly and resulted in smaller colonies. Second, the \textit{AHC1} deletion strain showed similar growth on minimal media while the adapter \textit{ada2}D mutant grew more poorly and resulted in smaller colonies. Second, the \textit{AHC1} deletion was unable to relieve the toxicity of overexpressed Gal4-VP16 (Fig. 7A, lower panels). No significant growth was seen for either the wild type.

![FIG. 7. An \textit{AHC1} deletion does not display a classic Ada phenotype. (A) Transformants of wild-type, \textit{ahc1}D, and \textit{ada2}D (adapter control) cells containing high-copy-number empty vector were plated on minimal medium to assess overall growth phenotype (upper panels). To test for adapter phenotype (Ada\textsuperscript{2}; relief of toxicity of overexpressed chimeric activator Gal4-VP16), cells were transformed with high-copy-number activator plasmid and plated on minimal medium (lower panels). (B) Quantitation of acidic-activator-mediated in vivo transcription is presented. Wild-type, \textit{ahc1}D, and \textit{ada2}D cells were transformed with pLGSD5 reporter plasmid and low-copy-number empty vector, Gal4-VP16, or Gal4-VP16\textit{HA3}, and extracts were assayed for \textit{b}-galactosidase activity.](https://mcb.asm.org/content/12/5/6628/F1.large.jpg)
or the \( \text{ahc1}\Delta \) strain, whereas \( \text{ada}\Delta \) cells were able to grow. Third, \( \text{Gal4}\cdot\text{VP16} \)-mediated transcription levels were similar in the wild type and the mutant (Fig. 7B). Thus, results from these three assays measuring \( \text{Ada}^- \) phenotypes indicate that a strain lacking Ahc1 does not exhibit these defects.

**DISCUSSION**

The discovery that several transcriptional coactivator proteins are HATs provided a direct molecular link between histone acetylation and activation of transcription. One of these coactivators possessing HAT activity, the yeast protein Gen5, functions as the catalytic subunit in several native high-molecular-weight complexes (20, 46, 50, 51). The largest of these Gen5-dependent native HAT complexes, the 1.8-MDa SAGA complex, has recently been purified and characterized (22, 23). SAGA contains Tra1, several Ada proteins, the TBP class of Spt proteins, and a subset of TAFII proteins. Members from each of these classes of proteins have been demonstrated to be essential for structural integrity (20, 57), transcriptional stimulation (64), or nucleosomal histone acetylation (22) by SAGA.

While many subunits of the SAGA complex have been identified, proteins other than Ada2, Ada3, and Gen5 comprising the 0.8-MDa ADA complex were not known. A long-standing question to address was whether ADA functions as a distinct complex in \( S. cerevisiae \) or is a subcomplex of the larger SAGA complex. There are several lines of evidence which suggested that the ADA complex may be distinct from SAGA. First, ADA is capable of acetylating nucleosomal histones although lacking TAFII68. A depletion of TAFII68 from SAGA resulted in the loss of both nucleosomal acetylation and transcriptional stimulation for SAGA (22). Second, ADA and SAGA show different lysine specificities within histone H3 (21). One can speculate that there are distinct proteins within either complex that are required for this specificity. Third, in contrast to SAGA, ADA fails to interact with the acidic activators Gen4 and VP16 in vitro (60), even though it contains Ada2, which has been shown to physically interact with these activation domains (3). Fourth, a recent study has demonstrated that a deletion of the bromodomain within Gen5 significantly reduced nucleosomal HAT activity of SAGA, while the ability of ADA to acetylate nucleosomes was unchanged (57). All of these observations suggest distinct activities of the ADA complex relative to the SAGA complex. However, these differences could arise from the fact that ADA lacks many subunits found in SAGA and/or that the ADA complex may contain distinct subunits not found in the SAGA complex.

In this study, we have purified the ADA HAT complex and have identified a novel subunit of this complex. Indeed, we find that a protein of previously unknown function, Yor023C, herein named Ahc1, is a unique subunit of the ADA complex. Several lines of evidence demonstrate that Ahc1 is an integral component of the ADA complex. First, Ahc1 was identified by mass spectrometry analysis as a protein in the highly purified ADA complex (Fig. 2). Second, Western blot experiments using an anti-Ahc1 antiserum confirmed the copurification of Ahc1 with the HAT activity of ADA. Third, anti-Ahc1 antibodies immunoprecipitated the ADA complex, demonstrating that Ahc1 is a stably interacting component of the purified complex (Fig. 4A and B). Fourth, an \( \text{AHCI} \) deletion strain specifically lacked the ADA complex, while the larger SAGA complex was unaffected by this deletion. Thus, the structural integrity of the ADA complex was dependent on the presence of the \( \text{AHCI} \) gene product. Fifth, reintroducing Ahc1 on a plasmid restored the ADA complex, as shown in Fig. 6.

The finding that the ADA complex contains unique subunits not found in the SAGA complex illustrates that it is a distinct complex and not merely a subcomplex of SAGA. While the functions of the ADA complex remain under investigation, it is clear that it is not responsible for the classic \( \text{Ada}^- \) phenotypes (e.g., the lower Gal4-VP16 function in vivo) (4). While the deletion of \( \text{AHCI} \) disrupted the ADA complex, it did not result in an \( \text{Ada}^- \) phenotype (Fig. 7). It is therefore likely that ADA is not involved in transcriptional activation mediated by acidic activators in the same manner as SAGA is. Consistent with this is the observation that SAGA, but not ADA, interacts with the acidic activators VP16 and Gen4 (60). Moreover, deletion of the SAGA components Ada1, Spt20, and Spt7, which are not in the ADA complex, disrupts the SAGA complex and also results in \( \text{Ada}^- \) phenotypes (57). Thus, the functions of Ada2, Ada3, and Gen5 which give rise to the \( \text{Ada}^- \) phenotypes are most likely attributable to the functions of the SAGA complex. However, a genetic link between the ADA complex and histone function is suggested by the fact that AHCI in high copy numbers suppresses the cold sensitivity mediated by particular mutations in histone H2A (29, 45a). The presence of the adapter proteins Ada2, Ada3, and Gen5 in two unique complexes indicates that these proteins may perform important roles in complexes with distinct functions. In this regard, the Ada proteins are similar to other proteins that are involved in transcriptional regulation (e.g., several TAFIIIs and Tra1 [22, 23]).

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