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ADA1, a Novel Component of the ADA/GCN5 Complex, Has Broader Effects than GCN5, ADA2, or ADA3

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The *ADA* genes encode factors which are proposed to function as transcriptional coactivators. Here we describe the cloning, sequencing, and initial characterization of a novel *ADA* gene, *ADA1*. Similar to the previously isolated *ada* mutants, *ada1* mutants display decreases in transcription from various reporters. Furthermore, ADA1 interacts with the other ADAs in the ADA/GCN5 complex as demonstrated by partial purification of the complex and immunoprecipitation experiments. We estimate that the complex has a molecular mass of approximately 2 MDa. Previously, it had been demonstrated that *ada5* mutants displayed more severe phenotypic defects than the other *ada* mutants (G. A. Marcus, J. Horiuchi, N. Silverman, and L. Guarente, Mol. Cell. Biol. 16:3197–3205, 1996; S. M. Roberts and F. Winston, Mol. Cell. Biol. 16:3206–3213, 1996). *ada1* mutants display defects similar to those of *ada5* mutants and different from those of the other mutants with respect to promoters affected, inositol auxotrophy, and Spt$^-$ phenotypes. Thus, the ADAs can be separated into two classes, suggesting that the ADA/GCN5 complex may have two separate functions. We present a speculative model on the possible roles of the ADA/GCN5 complex.

RNA polymerase II (Pol II)-dependent transcription in eukaryotes requires general transcription factors and gene-specific transcriptional activators. The general factors include Pol II itself, the TATA binding protein (TBP), TFIIB, and other factors required for preinitiation complex formation at promoters. Activators bind to upstream activating sequences (UAS) or enhancers, which can be hundreds of base pairs upstream from the site of initiation of transcription, and stimulate transcription through their activation domains. In addition to these two types of factors, a third type whose members are referred to as mediators, coactivators, or adaptors has been isolated (3, 32, 44). These have been proposed to function by several mechanisms, including bridging interactions between activator proteins and basal factors and counteracting of the repressive effects on transcription by nucleosomes. Many factors believed to belong to this coactivator group form large heteromeric complexes. For example, the SWI/SNF complex is approximately 2 MDa in size (11, 40, 60) and functions by countering chromatin-mediated repression (14, 27). The suppressor of RNA polymerase B (SRB) complex is required for robust activated and basal transcription and interacts with basal factors to form an RNA Pol II holoenzyme complex (25, 35, 58). The TFIID complex consists of basal transcription factor TBP and associated TAF proteins which are thought to be required for activated transcription (15, 51, 59).

Two other groups of factors which seem to function as adaptors or coactivators, the SPTs and the ADAs, were identified genetically in *Saccharomyces cerevisiae*. The *SPT* genes were isolated as suppressors of the auxotrophies resulting from transposon insertions at the promoters of various biosynthetic genes. The *SPT* genes fall into two classes based upon the insertions that they suppress and upon various other genetic and molecular characteristics (61). One class is the histone class, which includes *SPT4*, *SPT5*, *SPT6*, *SPT11*, and *SPT12*. *SPT11* and *SPT12* encode histones H2A and H2B, respectively. *SPT4*, *SPT5*, and *SPT6* also encode negative regulators of transcription that form a complex and that are thought to be functionally related to H2A and H2B (57). The second class, the TBP class, includes *SPT3*, *SPT7*, *SPT8*, *SPT15*, and *SPT20*. *SPT15* encodes TBP, while the others encode products which are thought to interact with TBP and to play a role in transcriptional-initiation site selection (16, 17, 61).

The *ADA* genes were isolated as suppressors of GAL4-VP16 toxicity (4). When GAL4-VP16 is overexpressed, it is thought to kill yeast cells by sequestering the general transcription factors away from productive transcription complexes. Therefore, mutations that suppress toxicity may be in genes encoding factors that mediate the interaction between the general factors and activation domains. The *ada* mutants fell into five complementation groups, four of which have been characterized, *ADA2*, *ADA3*, *GCN5*, and *ADA5* (4, 37, 38, 43). *GCN5* had been previously isolated as a gene whose product was a factor required for full transcriptional activation by GCN4 (21). *ADA3* has been subsequently isolated as *NGG1*, a gene whose product is required for glucose repression (5, 6).

ADA2, *ADA3*, and *GNC5* are all required for full transcriptional activation by a subset of activators, including GAL4-VP16 and GCN4 (4, 38, 43). This is consistent with a model in which these ADAs function as adaptors for a subset of activators. Also, mutations in the three genes yield similar phenotypes, such as temperature sensitivity and slow growth. Mutants carrying null alleles of any two of the three genes display phenotypes identical to those of single mutants (20, 38). It has been demonstrated that ADA2, ADA3, and GCN5 interact to form a complex (12, 29, 38).

ADA5 is similar to the other *ADA*s except that mutations result in some broader transcriptional defects (37, 45). For example, the *ADH1* promoter is not affected by mutations in *ADA2*, *ADA3*, or *GNC5* but is reduced in *ada5* mutants. Furthermore, *ADA5* has been shown to be identical to *SPT20* linking the *ADA* genes and the *SPT* genes (45). The other *ADA*s have not been isolated as *spt* mutants and do not display

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TABLE 1. Strains used in this study

Strain	Genotype	Source or reference
ada1-1	*MAT***a** *ura3-52 leu2-3,112 his4-519 ade1-100 gal4::HIS4 ada1-1*	4
BWG1-7a	*MAT***a** *ura3-52 leu2-3,112 his4-519 ade1-100*	Laboratory strain
1-7aΔ*ada1*	BWG1-7a Δ*ada1::hisG*	This study
1-7aΔ*ada2*	BWG1-7a Δ*ada2::hisG*	4
PSY316	*MAT*α *ura3-52 leu2-3,112 his3-Δ200 ade2-101 lys2-801*	Laboratory strain
316Δ*ada1*	PSY316 Δ*ada1::hisG*	This study
316Δ*ada2*	PSY316 Δ*ada2::hisG*	4
316Δ*ada3*	PSY316 Δ*ada3::hisG*	34
GMy27	PSY316 Δ*gcn5::hisG*	34
FY56	*MAT*α *ura3-52 LEU2 his4-912*δ *lys2-128*δ	Fred Winston
FY56Δ*ada1*	FY56 Δ*ada1::hisG*	This study
FY56Δ*ada3*	FY56 Δ*ada3::hisG*	This study
FY710	*MAT***a** *ura3-52 leu2Δ1 his4-912*δ *lys2-128*δ Δ*(hta1-htb1)::LEU2*	Fred Winston
FY630	*MAT***a** *ura3-52 leu2Δ1 his4-917*δ *lys2-173R2 trp1Δ63*	Fred Winston
FY630Δ*ada1*	FY630 Δ*ada1::hisG*	This study
FY630Δ*ada3*	FY630 Δ*ada3::hisG*	This study

Spt⁻ phenotypes. Despite these differences, Δ*ada5* Δ*ada3* mutants do not demonstrate any phenotypes more severe than that of a Δ*ADA5* mutant. Furthermore, there is evidence that ADA5 interacts with ADA3 (37), suggesting that it too is part of an ADA/GCN5 complex.

Recently, a functional role for the ADA/GCN5 complex has been suggested. A histone acetyltransferase activity has been purified from *Tetrahymena* macronuclei (7). The gene responsible for this activity was cloned and was determined to be a homolog of *GCN5* (10). Yeast GCN5 has also been shown to have histone acetyltransferase activity. Combined with data that ADA2 and ADA5 interact with activators (2, 37, 39, 55), this suggests that the role of the ADA/GCN5 complex may be to acetylate nucleosomes at transcriptionally active sites (8).

In this study, we describe the cloning and characterization of a novel *ADA* gene, *ADA1*. We present evidence that ADA1 is part of a large ADA/GCN5 complex and that *ada1* mutants display phenotypes similar to that of *ada5* and broader than those of *ada2*, *ada3*, and *gcn5* mutants. Thus, although the ADA proteins bind to form a complex, they can be separated into two classes, suggesting that the ADA/GCN5 complex may have a second function in addition to histone acetylation.

MATERIALS AND METHODS

Strains and media. Strains used in this study are shown in Table 1. BWG1-7a, 1-7aΔ*ada1*, and 1-7aΔ*ada2* were used for purification and immunoprecipitation experiments. BWG1-7a, 1-7aΔ*ada1*, PSY316, and 316Δ*ada1* were used for β-galactosidase assays. Inositol studies were performed with PSY316 and PSY316 in which the appropriate *ADA* gene was disrupted. Strains FY56, FY710, and FY630, used to determine Spt phenotypes, were gifts from S. Roberts and F. Winston. Disruptions of *ADA1* in yeast cells were generated by transforming the appropriate strains with pADA1KO (described below) that had been linearized with *Xho*I. *ADA3* was deleted with pADA3KO (38). Disruptions were verified by Southern analysis.

Rich, synthetic complete, and synthetic minimal media were prepared as previously described (53).

Cloning and sequencing of *ADA1*. Three original clones complementing the slow-growth, morphological-defect, and GAL4-VP16-resistance phenotypes of the *ada1-1* mutant (4) were originally isolated. One clone, YCP50ADA1, had an 11-kb insert. Two others had identical 16-kb inserts. Physical mapping with restriction enzymes revealed that these clones overlapped over a region of 5.7 kb. This region had a convenient *Hin*dIII site. By using a second *Hin*dIII site in the vector (YCP50) and a third in a nonoverlapping region in YCP50ADA1, the 5.7-kb region was split into two fragments which were separately subcloned into YCP50. Neither subclone complemented the slow-growth phenotype of the *ada1-1* mutant, suggesting that the relevant gene had been cut in half. The *Hin*dIII fragments were subcloned into pBluescript (Stratagene) and sequenced from the internal *Hin*dIII site. Sequencing was performed by the dideoxy chain termination method (49). Further sequence was obtained by either subcloning convenient restriction fragments into pBluescript or using custom synthetic oligonucleotide primers. This revealed an open reading frame of 488 codons (later verified as *ADA1*) with an internal *Hin*dIII site.

Plasmid constructions. PCR primers were designed such that the coding region of *ADA1* could be amplified with *Bam*HI and *Afl*III sites on the 5′ end and a six-histidine tag followed by a stop codon and a *Bam*HI site on the 3′ end of the gene. The PCR product was digested with *Bam*HI and ligated into the *Bam*HI site of pBluescript to generate BluescriptADA1. The BluescriptADA1 *Bam*HI fragment was cloned into the *Bgl*II site of DB20LeuBgl (4) to generate DB20LADA1.

To verify that the cloned *ADA1* gene actually corresponded to the gene that was mutated in the *ada1-1* strain, it was necessary to mark the genomic locus of the cloned gene. The *Bam*HI fragment from DB20LADA1 which contained the cloned gene under control of the *ADH1* promoter was inserted into the *Bam*HI site in pRS306 (54), a *URA3*-marked integrating plasmid. This construct was digested with *Bcl*I to direct integration to the cloned locus and was used as described in Results.

The disruption construct of *ADA1*, pADA1KO, was generated in several steps. Vector pUC19Xho-Hind was constructed by inserting an *Xho*I linker into the *Sma*I site of pUC19 and destroying the *Hin*dIII site by cutting, filling in with Klenow fragment, and ligating. A 5.4-kb *Xho*I fragment containing the coding and flanking sequences of *ADA1* was digested from YCP50ADA1 and ligated into the *Xho*I site of pUC19Xho-Hind. This was subsequently digested with *Hin*dIII and *Eco*RV, filled-in with Klenow fragment, and ligated to a *Bgl*II linker. The *hisG-URA3* cassette from pNKY51 (1) was ligated into the *Bgl*II site. This construct disrupts *ADA1* between the codons for amino acids 113 and 408.

For recombinant expression of *ADA1*, the bacterial expression vector pUHE24.2ΔCAT (38) was used. The *ADA1* coding region fused to a six-histidine tag was digested from BluescriptADA1 with *Afl*III and *Bam*HI. This fragment was cloned into the *Nco*I and *Bam*HI sites of pUHE24.2ΔCAT to generate pUHE24.2ADA1.

For recombinant expression of *GCN5*, the *GCN5* coding sequence was amplified by PCR with primers GCN5N and GCN5C (38). The product was digested with *Eco*RI and *Not*I and cloned into the *Eco*RI and *Not*I sites of pET28a (Novagen). The resulting plasmid, pET28a-EcoGCN5, could be used to express a carboxyl-terminal fragment of GCN5 with a six-histidine tag at its amino-terminal end.

To make plasmid pPADA26HIS, pDB200CADA26HIS, an ARS-CEN derivative of pADA26HIS (55), was digested with *Spe*I, filled in with T4 DNA polymerase, and then digested with *Afl*II. The fragment that contained the C-terminal one-third of *ADA2* fused to a six-histidine tag and that was followed by the *ADH1* terminator was purified and cloned into pNS3.8 (4) that had been digested with *Afl*II and *Msc*I. This created a plasmid which contained the *ADA2* promoter expressing the *ADA2*-six-histidine fusion gene followed by the *ADH1* terminator.

Yeast manipulations. Transformations were performed by the lithium acetate method (53). For β-galactosidase assays, cells were grown to an optical density at 600 nm of approximately 1.0 and activity was measured in glass bead extracts as previously described (46). Cells were grown in synthetic complete medium lacking uracil. For HIS(2)14x2 and HIS(1)66 activity, cells were induced in minimal media complemented only for the auxotrophies of the yeast for 8 h. β-Galactosidase activity was measured as nanomoles per minute per milligram of protein. Reporters used were pLGΔAluXho (22) for CYC1 UAS1, which binds the activator HAP1; pLG265UP1 (18) for CYC1 UAS2, which binds the HAP2/3/4/5 heterotetrameric activator; HIS(1)66 (26) and HIS(2)14x2 (26), which bind GCN4; pCP0 (50), which contains the *ADH1* promoter; and pdAT2 (36), which contains the thymidine-rich UAS of the *DED1* gene. Other general yeast techniques were performed according to standard protocols (23).

Generation of antisera. pUHE24.2ADA1 was transformed into AG115 (30) cells. Cells were grown to an optical density of 0.5, induced with 2 mM isopropyl-β-D-thiogalactopyranoside, and grown for another 2 h. Cells were lysed by sonication, and the insoluble material, which contained recombinant ADA1 fused to a six-histidine tag, was resuspended in 8 M urea. Recombinant ADA1 was purified on a Ni-nitrilotriacetic acid (NTA) agarose column (Qiagen) as recommended by the manufacturer.

To obtain polyclonal antisera to ADA1, 1 mg of recombinant ADA1 was dialyzed into 2 ml of phosphate-buffered saline solution, mixed with adjuvant (RIBI ImmunoChem Research, Inc.), and injected into two rabbits according to standard protocols (24). After several boosts, antibodies to ADA1 were purified from crude serum by binding to immunoblots of recombinant ADA1 and eluting at pH 2.5 or by binding and eluting from recombinant ADA1 cross-linked to cyanogen bromide-activated Sepharose beads.

In order to generate antibodies to GCN5, a carboxyl-terminal fragment of GCN5, tagged with six histidines, was purified in a manner similar to that for ADA1 above, except that plasmid pET28a-EcoGCN5 and strain BL21:DE3 (Novagen) were used. The eluate from the Ni-NTA agarose column was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and acrylamide slices containing the GCN5 protein fragment were

lyophilized and resuspended in phosphate-buffered saline. Antibodies to GCN5 were generated as described for ADA1 except that 400 μg of protein was injected into each rabbit. Antibodies to ADA2 (38), ADA3 (37), ADA5 (37), TBP, and TFIIB (33) have been described previously.

Extracts and fractionation. The purification of the ADA/GCN5 complex was performed in 1-7aΔ*ada2* (4) carrying pPADA26HIS. Cells were grown in minimal media to yield an approximate wet weight of 500 g. Preparation and fractionation of whole-cell extract on a Bio-Rex 70 column (5 by 10 cm) were performed as described by others (52) except elutions were performed with 250, 600, and 1,200 mM potassium acetate. Elutions were analyzed for the presence of ADA2 and ADA3 proteins by Western blotting. The relevant fractions from the 600 mM potassium acetate elutions were pooled and dialyzed against a buffer containing 20 mM HEPES (pH 7.6), 10% glycerol, 300 mM potassium acetate, 10 mM β-mercaptoethanol, and protease inhibitors. This material was then bound to an Ni-NTA agarose column (1.5 by 10 cm) and eluted with an imidazole gradient. Fractions enriched in ADA2 and ADA3 were pooled and diluted into buffer H (20 mM HEPES [pH 7.6], 10% glycerol, 1 mM dithiothreitol, 1 mM EDTA, protease inhibitors) to a final salt concentration of 100 mM potassium acetate. This material was then fractionated over a Poros HQ-20 column (4.6 by 100 mm) (PerSeptive Biosystems) with an elution gradient from 100 to 1,500 mM potassium acetate of 10 column volumes by using the BioCAD Sprint apparatus (PerSeptive Biosystems). Fractions that contained the peak of ADA proteins were pooled and loaded onto a Superose 6 HR 10/30 gel filtration column (Pharmacia Biotech). The column was then washed with buffer H containing 300 mM potassium acetate, and fractions were collected. As a control for determining the approximate molecular weight of the ADA/GCN5 complex, protein size standards (Bio-Rad) were fractionated on the Superose 6 column under identical conditions.

For quantifying purification, a densitometer (LKB) was used to measure the relative band intensities on immunoblots of ADA2, ADA3, and ADA1.

Immunoprecipitations. Preparation and fractionation of extracts of BWG1-7a and 1-7aΔ*ada1* were performed as described previously (37). Cross-linking of antisera to protein A-Sepharose beads and immunoprecipitation experiments were also performed according to published methods (11) except that 1 ml of the 600 mM eluate from the Bio-Rex 70 column was used for the precipitations and except that bound material was eluted off the antisera-coated beads with 20 μl of 50 mM glycine (pH 2.5), neutralized with 3 μl of 1 M Tris (pH 7.5), and mixed with 4× SDS-PAGE loading buffer.

Nucleotide sequence accession number. The sequence of *ADA1* has been assigned GenBank accession no. U76735; that of the sequence of *HFI1*, a gene identical to *ADA1*, is U41324 (19).

RESULTS

Cloning and sequencing of *ADA1*. *ada* mutants were isolated from a screen for GAL4-VP16-resistant mutants as previously described (4), with the exception that the first *ada1* allele, *ada1-1*, was isolated from nonmutagenized cells. The *ada1* mutants isolated have severe slow-growth defects and morphological changes (28). These phenotypes are recessive. We therefore used the slow-growth phenotype of *ada1-1* to isolate complementing clones from a yeast genomic library on an ARS-CEN-containing vector (4, 42). Three overlapping clones which not only restored wild-type growth but also restored wild-type morphology and sensitivity to overexpressed GAL4-VP16 were isolated. By analyzing the complementation of subclones and sequencing, a putative open reading frame of 488 codons was identified (see Materials and Methods). The putative open reading frame was amplified by PCR and cloned into an episome under the control of the *ADH1* promoter (see Materials and Methods). This construct also complemented the slow-growth phenotype, restored wild-type morphology, and restored sensitivity to GAL4-VP16 overexpression. To verify that the cloned gene corresponded to *ADA1*, the *URA3* marker was integrated at the cloned locus in a wild-type strain (see Materials and Methods) and mated to *ada1-1*. The resultant diploid was sporulated, and in six of six four-spore tetrads tested, resistance to GAL4-VP16 toxicity segregated 2:2 in opposition to the Ura^+ phenotype. It was determined through database searches that a gene identical to *ADA1*, termed *HFI1*, had been cloned (19). There are no other genes with any significant homologies to *ADA1*.

ADA1 was deleted in yeast cells as described in Materials and Methods. The deletion mutant was similar to *ada1* other mutants with respect to slow growth and resistance to GAL4-VP16 toxicity. Both phenotypes were fully complemented by the cloned gene. The morphological changes for *ada1* mutants mentioned above were determined to be strain specific. Disrupting *ADA1* in BWG1-7a resulted in extremely elongated cells, similar to the morphology of the original *ada1-1* mutant, which was in a BWG1-7a background. Disrupting *ADA1* in PSY316 resulted in a slight change in cell morphology which was much less apparent than that in BWG1-7a.

TABLE 2. β-Galactosidase assays[a] of wild-type and Δ*ada1* strains

Reporter	Activity (nmol/min/mg of protein) of strain:	
	wt[b]	Δ*ada1*
CYC1 UAS1	144	6.7
CYC1 UAS2up1	265	28
HIS(1)66	8160	547
HIS(2)14x2	2990	89
ADH1	12800	2400
dAdT	319	627

[a] Assays were performed as described in Materials and Methods. Measurements are the averages of assays performed on four individual transformants. Standard errors were less than 20% of the means.
[b] wt, wild type.

***ada1* mutants display transcriptional defects.** Consistent with a model in which the ADA proteins function as transcriptional coactivators or adaptors, the *ADA*s isolated previously have specific defects in transcription (4, 37, 38, 43). In order to determine whether Δ*ada1* strains were also defective for transcription from certain activators, β-galactosidase assays were performed on wild-type and Δ*ada1* strains transformed with various reporters. As demonstrated in Table 2, Δ*ada1* strains show decreased activity from several reporters. The activities of reporters which bind activators HAP1 (*CYC1* UAS1) and HAP2/3/4/5 (*CYC1* UAS2UP1) were reduced 21- and 9-fold, respectively, and those of reporters which bind the activator GCN4, HIS(1)66 and HIS(2)14x2, were reduced 15- and 34-fold, respectively. The activity of the *ADH1* reporter was also reduced fivefold in a Δ*ada1* strain. This is of particular interest because the Δ*ada2*, Δ*ada3*, and Δ*gnc5* strains do not show this decrease (4, 38), while Δ*ada5* strains do (37). These results suggest that the *ADA* genes can be separated into two classes based upon transcriptional differences. The activity of a reporter containing the thymidine-rich sequences of the *DED1* gene as a UAS, dAdT, was not reduced in a Δ*ada1* strain. In fact, activity may have been slightly elevated. This argues that it is not a general defect in transcription that occurs in Δ*ada1* strains but rather specific defects that affect only a subset of promoters.

***ada1* mutants are auxotrophic for inositol and display Spt^- phenotypes.** Mutations in many factors that are thought to be coactivators result in inositol auxotrophy. These include the *SRB* genes (34), the *SWI/SNF* genes (41), and some *SPT* genes, including *ADA5/SPT20* (45). In order to determine whether deleting *ADA1* resulted in inositol auxotrophy, various strains were grown on plates lacking inositol. As shown in Fig. 1, a Δ*ada1* strain does not grow on media lacking inositol, whereas Δ*ada2*, Δ*ada3*, and Δ*gcn5* strains do. This further links *ADA1* to *ADA5* phenotypically and separates them from *ADA2*, *ADA3*, and *GCN5*.

To determine whether *ada1* mutants displayed other phenotypes similar to that of *ada5/spt20* mutants, we tested whether Δ*ada1* strains displayed an Spt^- phenotype. *ADA1* was disrupted in strain FY56, which contains δ element insertions at

FIG. 1. Inositol phenotype of a Δ*ada1* strain. 316Δ*ada1*, 316Δ*ada2*, 316Δ*ada3*, GMy27 (316Δ*gnc5*), and, as a positive control, 316Δ*ada1* transformed with YCP50ADA1 (Δ*ada1*+*ADA1*) were grown on synthetic complete media lacking inositol (−Inositol) and on an identical plate on which 200 μl of 200 mM inositol had previously been spread (+Inositol).

the *HIS4* and *LYS2* loci. As demonstrated in Fig. 2A, a Δ*ADA1* mutant was indeed able to suppress the *his4-912*δ and *lys2-128*δ alleles present in yeast strain FY56. In contrast, a Δ*ADA3* mutant was unable to suppress these alleles, further delineating the two classes of *ADA* genes. As a positive control, strain FY710 (*hta1 htb1*Δ) was also able to suppress both the *his4-912*δ and *lys2-128*δ alleles.

As mentioned previously, the *SPT* genes can be separated into two classes: the histone class and the TBP class. Paradoxically, *ADA5* had been isolated as an *SPT* of the TBP class (45), while GCN5 has been shown to acetylate histones (10). Thus, we were interested in knowing whether *ADA1* is of the TBP or histone class of *SPT*s. Since the *his4-912*δ and *lys2-128*δ alleles do not distinguish between the two classes, *ADA1* and *ADA3* were disrupted in strain FY630, which contains *his4-917*δ, an allele specifically suppressed by the TBP class but not the histone class. As demonstrated in Fig. 2B, a mutant with a deletion of *ADA1* was able to suppress the *his4-917*δ allele while a mutant with a deletion of *ADA3* was not. This demonstrates that *ADA1* is of the TBP class of *SPT* genes.

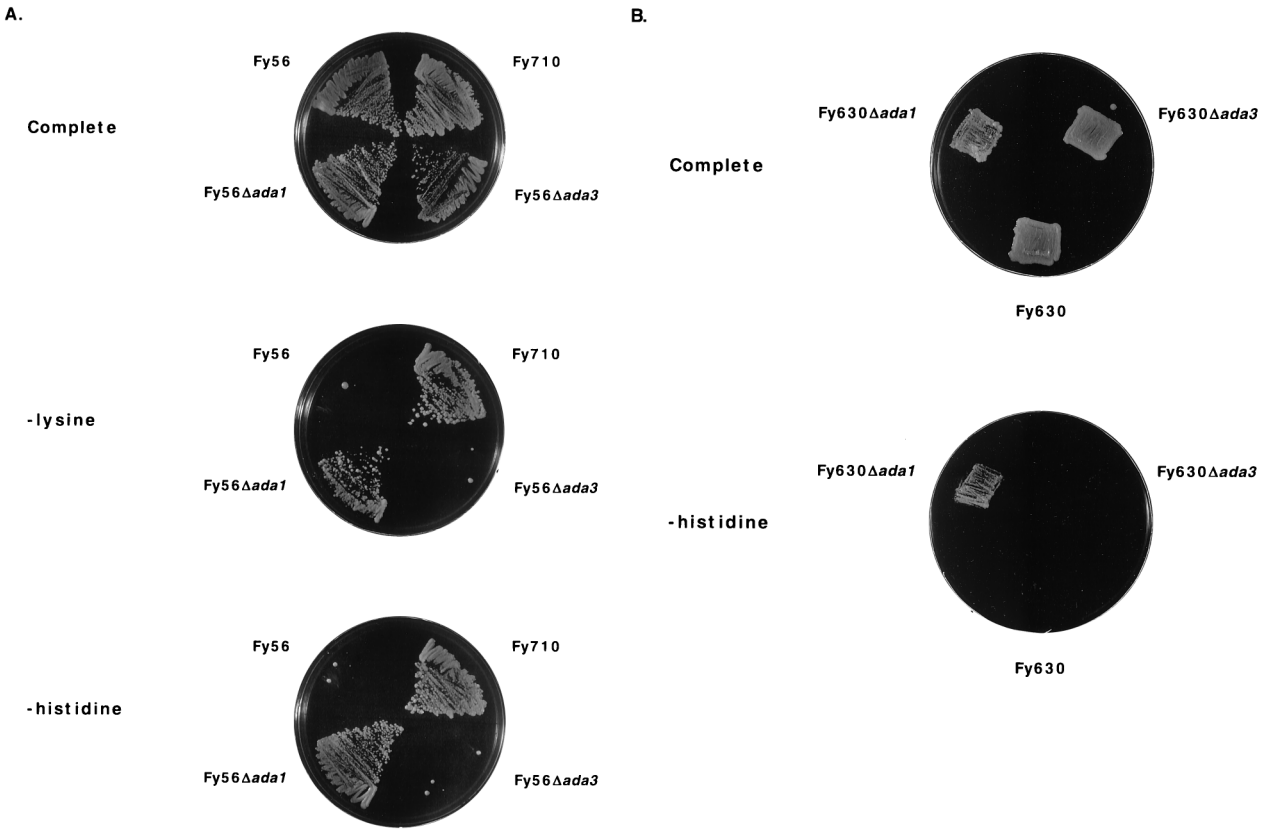

FIG. 2. Spt⁻ phenotype of a Δ*ada1* strain. (A) The indicated strains were streaked on synthetic complete media (Complete) or media lacking lysine (−lysine) or histidine (−histidine) to determine whether they suppressed the *his4-912*δ and *lys2-128*δ alleles present. FY710, for which the histone *hta1-htb1* locus is disrupted, was used as a positive control. (B) The indicated strains were patched on synthetic complete media (Complete) or on media lacking histidine (−histidine) to determine whether they suppressed the *his4-917*δ allele, which is specifically suppressed by mutants in the TBP class of *SPT*s.

TABLE 3. Partial purification of the ADA/GCN5 complex

Fraction	Protein (mg) in:		Yield (%)	Fold purification[a]	
	Onput	Pooled fractions		Column	Total
Crude extract		2975	100		
Bio-Rex 70	2975	179.5	60	10	10
Ni-NTA agarose	149.5	72	38	1.6	16
Poros HQ-20	72	3.6	19	10	160
Superose 6 HR[b]	0.23	0.0004	0.03	18	2880

[a] The fold purification for a column is the ratio of the yield divided by the protein in the pooled fractions of the column to that of the previous column. The total fold purification at a particular column step is the product of that column's fold purification and the total fold purification for the previous column.

[b] The Superose 6 HR column was an analytical column with a low capacity and yield.

Partial copurification of an ADA complex. 1-7aΔ*ada2* was transformed with pPADA26His, a plasmid which expresses a six-histidine-tagged version of ADA2 from its own promoter and which fully complements a deletion of the gene (56). The resulting strain was grown in minimal media and was used to make a whole-cell extract which was fractionated over a Bio-Rex 70 column (see Materials and Methods). After step elution with potassium acetate, the ADA proteins were detected by Western blot analysis in two fractions: the 600 mM fraction and the 1,200 mM fraction. The 600 mM fraction contained about 60% of the ADA proteins, and the 1,200 mM fraction accounted for the remainder (56). We estimate that both the 600 and 1,200 mM eluent fractions represent a 10-fold purification of the ADA/GCN5 complex (Table 3).

Next, the 600 mM eluent was bound to a Ni-NTA agarose column. The Ni-NTA column was eluted with a gradient of imidazole, and the ADA proteins were quantitatively recovered in the peak fractions of the eluent. This step represented only a 1.6-fold purification of the ADA/GCN5 complex (56) because a relatively large fraction of proteins in the yeast extract bound to the column under the conditions we used.

The peak fractions eluted from the Ni-NTA column were fractionated on an HQ-20 anion-exchange column and were eluted with a gradient of potassium acetate. As shown in Fig. 3, cofractionation of ADA1, ADA2, ADA3, GCN5, and ADA5 was observed. The peak of the ADA/GCN5 complex eluted from the column at approximately 1.1 M potassium acetate. The ADA proteins were purified approximately 160-fold in the peak fractions from this column (Table 3). The fractions eluted from the HQ-20 column were also probed for the basal transcription factor, TBP. While TBP was present in the material loaded onto the column, it did not cofractionate with the ADA complex (Fig. 3) and, in fact, flowed through the column. Fractions from the HQ-20 column were also probed with antibodies to other transcription factors. TFIIB and TFIIE behaved similarly to TBP and flowed through the column, while SRB4, SRB5, SWI3, and TFIIH fractionated together, eluting prior to the ADA/GCN5 complex (56).

Finally, the fractions from the HQ-20 column containing the peak amounts of ADA proteins were pooled and subjected to fractionation on a Superose 6 gel filtration column. Again, all ADA proteins cofractionated over this column (Fig. 4). A comparison of the elution profiles of the ADA proteins with those of known protein standards indicated that the ADA proteins eluted at an approximate molecular mass of 2 MDa. Use of the sizing column resulted in an 18-fold purification of the ADA proteins, bringing the total purification to approximately 2,880-fold (Table 3). These data suggest that the ADA gene products, including GCN5, form a complex in yeast.

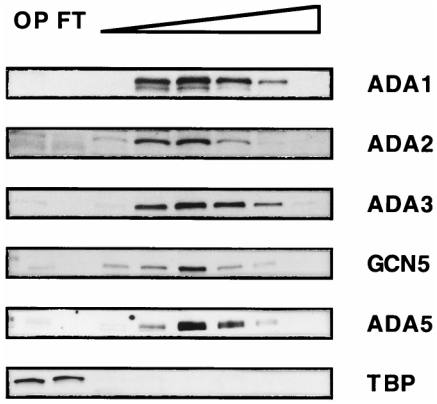

FIG. 3. Cofractionation of ADA1, ADA2, ADA3, GCN5, and ADA5 over an HQ-20 anion-exchange column. All panels show immunoblots of fractions from the HQ-20 column eluted with a potassium acetate gradient. The onput (OP) and flowthrough (FT) and every odd-numbered fraction between fractions 13 and 23 were probed with antibodies to the indicated proteins. The band recognized by the GCN5 antibody ran slightly faster than expected. It is possible that GCN5 was slightly degraded in our extracts.

Fractionation of the 1,200 mM potassium acetate elution from the Bio-Rex 70 column on either an HQ-20 column or a Superose 6 column also resulted in cofractionation of all five ADA proteins (56).

ADA1 interacts with the ADA complex by immunoprecipitation. In order to verify that ADA1 interacts with the other ADAs, whole-cell extracts were made from wild-type and Δ*ada1* strains grown in rich media. The extracts were then passed over a Bio-Rex 70 column, and the flowthrough and 250, 600, and 1,200 mM potassium acetate elutions were examined by Western blotting. As demonstrated in Fig. 5A, ADA1 eluted from this column in a profile identical to that of ADA3. The band that is seen in the 600 mM fraction of the Δ*ada1* strain is a background band that migrated at a slightly higher molecular weight than that of ADA1 (compare wt and Δada1 START lanes in the ADA1 Western blot of Fig. 5B). In contrast to the partial purification of the ADA/GCN5 complex

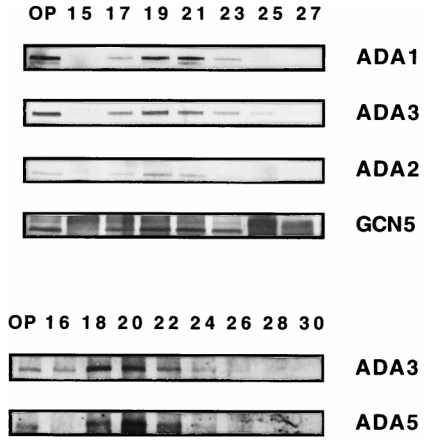

FIG. 4. Cofractionation of the ADA proteins over a Superose 6 gel filtration column. Aliquots of the onput (OP) to the column and the indicated fractions were trichloroacetic acid precipitated and analyzed by Western blotting. Antibodies to the indicated proteins were used to probe the blots. Aliquots corresponded to 3% of the onput and 25% of the fractions.

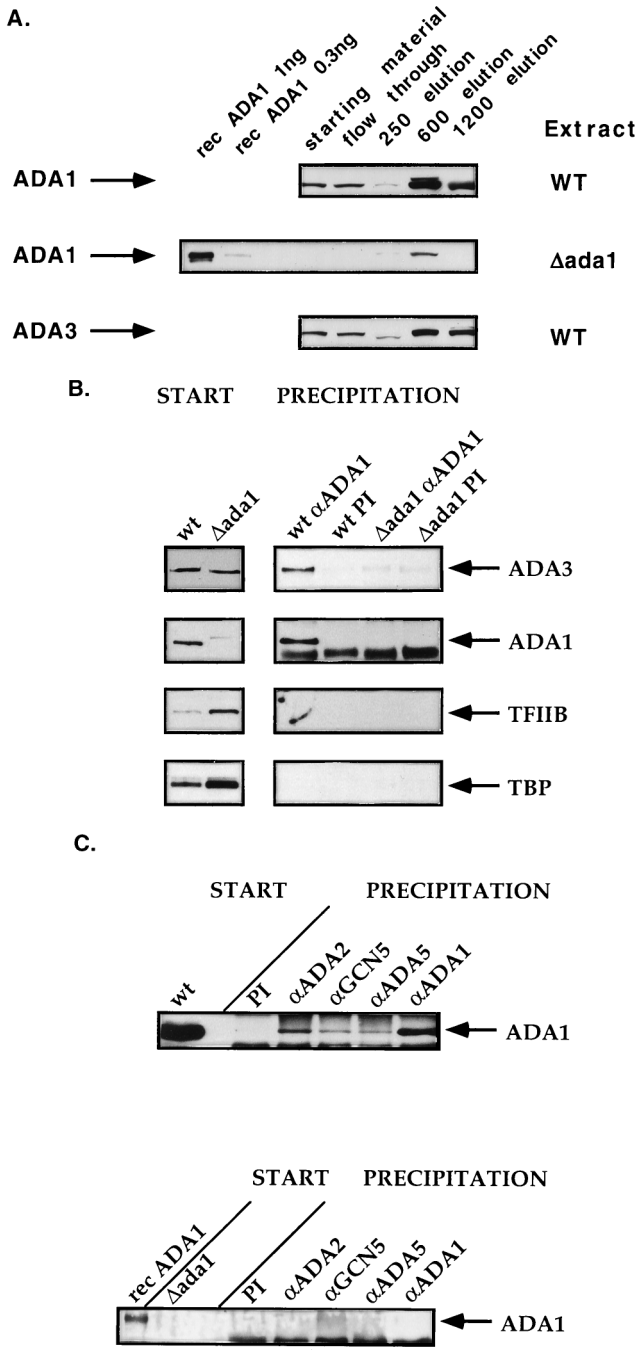

FIG. 5. Coprecipitation experiments between ADA1 and other ADA proteins. (A) Whole-cell extracts from BWG1-7a (WT) or 1-7aΔ*ada1* (Δada1) strains were fractionated on a Bio-Rex 70 column as described in Materials and Methods. Proteins were eluted stepwise in 250, 600 and 1,200 mM potassium acetate. Then, 20 μg each of whole-cell extract (starting material), the flow-through, and each of the elutions were assayed for ADA1 and ADA3 by Western blot analysis. Recombinant ADA1 (rec ADA1) was used as a control in the Δ*ada1* blot to determine the migration of ADA1 by SDS-PAGE. The band in the 600 mM elution in the Δ*ada1* extract is a background band that migrates slightly above that of ADA1. (B) Immunoprecipitations were performed on 1 ml of the 600 mM elution from the wild-type (wt) and Δ*ada1* extracts shown in panel A by using either protein A-Sepharose beads cross-linked to ADA1 antibody (αADA1) or preimmune serum (PI). Then, 100 μl of the 600 mM elution (START) of wild-type and Δ*ada1* extracts as well as the precipitations was subjected to SDS-PAGE and immunoblotted with the antibodies to the indicated proteins (ADA3, ADA1, TFIIB, and TBP). The band below ADA1 corresponds to an uncross-linked immunoglobulin G heavy chain which was eluted off the beads and which cross-reacts with the secondary antibody. (C) Immunoprecipitations were performed on 1 ml of the 600 mM elution from the wild-type (wt) and the Δ*ada1* extracts shown in panel A. Protein A-Sepharose beads cross-linked to preimmune serum (PI) and antibodies to ADA2 (αADA2), GCN5 (αGCN5), ADA5 (αADA5), and ADA1 (αADA1) were used for immunoprecipitations. Then, 250 μl of the 600 mM elution from the extracts (START) as well as from each of the precipitations was subjected to SDS-PAGE and analyzed by Western blotting for the presence of ADA1. As in panel B, the band below ADA1 in the precipitation lanes corresponds to an immunoglobulin G heavy chain. Rec ADA1 refers to 5 ng of recombinant ADA1.

described above, some ADA1 and ADA3 was observed in the flowthrough fraction. The flowthrough fraction was run over a Superose 6 column, and all five ADA proteins cofractionated, as determined by Western blotting (28), suggesting that the column residue may have been limiting in this second preparation.

ADA1 was immunoprecipitated from the 600 mM potassium acetate elutions from the Bio-Rex 70 column with ADA1 antibodies cross-linked to protein A-Sepharose beads. As expected, antibodies to ADA1 were able to precipitate ADA1 from wild-type but not from Δ*ada1* extracts and preimmune antisera were unable to precipitate ADA1 from either extract (Fig. 5B, ADA1 Western blot). As shown in the top portion of Fig. 5B, ADA3 coimmunoprecipitated with ADA1, implying that ADA1 and ADA3 interact. Importantly, ADA3 was not precipitated in a Δ*ada1* extract or by preimmune serum, demonstrating that this interaction is specific and dependent on ADA1. Two other proteins probed in this experiment, TBP and TFIIB, did not coprecipitate with ADA1, indicating that they either do not interact with ADA1 or that an interaction is too weak to be detected.

To demonstrate that the other ADA gene products also interact either directly or indirectly with ADA1, antibodies to ADA2, GCN5, ADA5, and, as controls, to ADA1 and preimmune serum were cross-linked to protein A-Sepharose beads and used in immunoprecipitation experiments. As shown in Fig. 5C, antibodies to ADA2, GCN5, ADA5, and ADA1 but not to preimmune serum were able to precipitate ADA1 from wild-type extracts, demonstrating that there is an interaction between all of the ADA gene products. As verification that the protein coprecipitated by these antibodies is ADA1, the lower blot of Fig. 5C demonstrates that the protein is not precipitated from a Δ*ada1* extract. These results suggest that ADA1, ADA2, ADA3, GCN5, and ADA5 are all found in a single complex.

DISCUSSION

We report here the cloning, sequencing, and characterization of *ADA1*, which encodes a novel member of the ADA/GCN5 complex. *ADA1* encodes a 488-amino-acid protein. *ada1* mutants display defects in transcription similar to those of *ada5* mutants and broader than those found in *ada2*, *ada3*, and *gcn5* mutants. Notably, mutations in *ADA2*, *ADA3*, and *GCN5* do not affect transcription from the *ADH1* promoter, while mutations in *ADA1* and *ADA5* decrease the activity of this reporter approximately fivefold. Indeed, since the activator that was used in the ADA screen, GAL4-VP16, was overexpressed by using the *ADH1* promoter, it was initially thought that *ADA1* was totally unrelated to *ADA2*, *ADA3*, and *GCN5* because resistance could arise from a reduction in the amount of GAL4-VP16 produced. On the other hand, all *ada* mutants display similar decreases in transcription from GCN4-dependent promoters. Importantly, activity from the dAdT reporter was not reduced in a Δ*ada1* strain, demonstrating that not all transcription is dependent on *ADA1*. It has been proposed that

the dAdT UAS activates transcription because this DNA sequence by itself disrupts nucleosomes (31). It is possible that disruption of chromatin by dAdT bypasses the need for the histone acetyl transferase activity of the ADA/GCN5 complex in transcription.

Two classes of ADA genes. The similarity in transcription defects between Δ*ada1* and Δ*ada5* strains led us to test whether the strains had other phenotypes in common. We have determined that Δ*ada1* strains are auxotrophic for inositol and are Spt$^-$, two phenotypes described for *ada5* mutants (45). Furthermore, both *ADA1* and *ADA5* are in the TBP class of *SPT*s, not the histone class. This is in agreement with previous genetic and biochemical data and suggests a link between the functions of the ADA/GCN5 complex and TBP (2, 37). Controls consisting of representative mutants of the *ADA2*, *ADA3*, and *GCN5* class of *ADA*s are neither auxotrophic for inositol nor Spt$^-$. Thus, the *ADA* genes can be separated into two distinct classes based on three criteria: transcriptional reporters that are affected, inositol auxotrophy, and Spt phenotype.

The five ADA gene products form a complex. With two different classes of *ADA* genes, it seemed possible that two different complexes may be formed. Previously, it has been shown that ADA2, GCN5, and ADA3 form a complex in vitro (2, 29). Also, various individual interactions between these three ADA proteins have been demonstrated in vivo (29, 38). Therefore, it is clear that components of one of the classes form a complex. Interestingly, an interaction between ADA3 and ADA5 has also been demonstrated (37), indicating a possible interaction between members of different classes of ADAs. Here we present evidence that all five ADA proteins bind together in a complex by two methods. First, we demonstrate that the five proteins remain together throughout an approximately 2,880-fold purification over four successive columns. In each of these columns, the ADA proteins eluted with identical profiles (Fig. 3 and 4) (56). Second, we demonstrate, using immunoprecipitation experiments, specific interactions between ADA1 and each of the other ADA proteins (Fig. 5). These two lines of evidence, taken together, strongly support a model in which all five ADA proteins form a complex in vivo.

The elution profile of the ADA proteins through the Superose 6 column indicates that the complex migrates with a molecular mass of approximately 2 MDa. This is much larger than the sum of the molecular masses of the five known individual subunits, suggesting that there may be other components that have yet to be identified or that the known components are present as multimers. Many other transcription factors have been shown to be associated in large-molecular-weight complexes, such as the TFIID complex, the SWI/SNF complex, and the holoenzyme complex. One possibility is that the ADA proteins could be part of one of these complexes. However, probing the partially purified ADA fractions with antibodies to TBP, Pol II, SWI3, and various SRBs demonstrated that these proteins do not peak with the ADA proteins (56). Thus, the ADA/GCN5 complex may be distinct and novel.

In all the purification steps performed, all five ADA proteins cofractionate. This cofractionation is not limited to the ADAs in the 600 mM potassium acetate elution of the initial Bio-Rex 70 column but extends also to the ADAs in the flowthrough and 1,200 mM potassium acetate elutions (28, 56). Thus, although we cannot rule out the formal possibility that ADA1 and ADA5 may form an alternate complex without the other ADAs, we have not found any evidence of this, and it seems more likely that all five ADA proteins are always associated.

It has recently been shown that a *Tetrahymena* GCN5 complex contains a protein that cross-reacts with antibody to ADA1 (9). Also, human homologs of ADA2 and GCN5 have been isolated, and these homologs have been demonstrated to interact with each other (13). These data suggest that the ADA/GCN5 complex is evolutionarily conserved.

One complex with two separate functions? As mentioned before, it has been determined that GCN5 functions as a histone acetyltransferase (10) and can acetylate free histone H3 in vitro. However, it is unable to acetylate whole nucleosomes. Thus, one function proposed for the other ADAs in the complex is to alter the conformation of either GCN5 or of nucleosomes, allowing the acetylation of whole nucleosomes (47). We have demonstrated here that there are at least five ADA proteins in the ADA/GCN5 complex and that they can be separated into two distinct classes. We propose that the ADA proteins of one class, ADA2, ADA3, and GCN5, function together to acetylate nucleosomes, resulting in the clearing away of repressive nucleosomes from some promoters. The ADAs of the other class, ADA1 and ADA5, have an additional function. This is supported by the observation that *ada1* and *ada5* mutants have more pronounced phenotypic defects than *ada2*, *ada3*, and *gcn5* mutants. If GCN5-dependent acetylation of histones was the sole function of the ADA/GCN5 complex, one would not expect to observe any *ada* mutants with phenotypes more severe than those of Δ*gcn5* mutants.

The proposed second function of the ADA/GCN5 complex has not been defined yet. One possibility is that GCN5 is not the only histone acetyltransferase in the ADA complex and that there is a second one that interacts with ADA1 and ADA5. On the other hand, it is fascinating that both *ADA1* and *ADA5*, but not the other *ADA*s, are in the TBP class of *SPT*s. Recently, it has been demonstrated that some strains with mutations in *SPT15*, which encodes TBP, are resistant to GAL4-VP16 toxicity (37). It is extremely interesting to speculate, therefore, that the function of this second class of ADAs may be to interact with basal factors, possibly TBP. The ADA/GCN5 complex may be brought to promoters through interactions between activators and ADA2 (2, 39, 55) and ADA5 (37). Once there, the ADA2, ADA3, and GCN5 class of ADAs may function to acetylate nucleosomes, opening up the promoter region, while the ADA1 and ADA5 class of ADAs recruit TBP and possibly other basal factors to bind to the TATA box. The observation that Δ*ada1* or Δ*ada5* strains display broader defects than Δ*ada2*, Δ*ada3*, or Δ*gcn5* strains, combined with the observation that Δ*ada3* and Δ*ada5* strains do not display phenotypes more severe than Δ*ada5* strains (37), suggests the following. Disrupting a member of the ADA2, ADA3, and GCN5 class of ADA will not disrupt the activity of the ADA1 and ADA5 class, whereas disrupting ADA1 or ADA5 may disrupt the activity of the entire ADA/GCN5 complex.

It should be mentioned that TBP is not present in our partially purified ADA fractions and is not immunoprecipitated by ADA antibodies (Fig. 3 and 4). However, other groups have demonstrated such interactions (2, 48), and it is possible that TBP is not a stably bound component of the ADA/GCN5 complex but rather is only transiently associated, i.e., only when bound to DNA or when activators are bound to the ADA complex. Alternatively, it is possible that the ADAs in the 1,200 mM potassium acetate fraction from the Bio-Rex 70 column have different properties from the ADAs in the 600 mM fraction which we have been studying. Indeed, it has recently been reported that several ADA/GCN5 complexes may exist in yeast (48). It will be interesting to determine whether the ADAs in the 1,200 mM potassium acetate fraction may be bound to TBP or other basal factors.

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REFERENCES

1. **Alani, E., L. Cao, and N. Kleckner.** 1987. A method for gene disruption that allows repeated use of *URA3* selection in the construction of multiply disrupted yeast strains. Genetics **116:**541–545.
2. **Barlev, N. A., R. Candau, L. Wang, P. Darpino, N. Silverman, and S. L. Berger.** 1995. Characterization of physical interactions of the putative transcriptional adaptor, ADA2, with acidic activation domains and TATA-binding protein. J. Biol. Chem. **270:**19337–19344.
3. **Berger, S. L., W. D. Cress, A. Cress, S. J. Triezenberg, and L. Guarente.** 1990. Selective inhibition of activated but not basal transcription by the acidic activation domain of VP16: evidence for transcriptional adaptors. Cell **61:**1199–1208.
4. **Berger, S. L., B. Piña, N. Silverman, G. A. Marcus, J. Agapite, J. L. Regier, S. J. Triezenberg, and L. Guarente.** 1992. Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. Cell **70:**251–265.
5. **Brandl, C. J., A. M. Furlanetto, J. A. Martens, and K. S. Hamilton.** 1993. Characterization of *NGG1*, a novel yeast gene required for glucose repression of GAL4p-regulated transcription. EMBO J. **12:**5255–5265.
6. **Brandl, C. J., J. A. Martens, A. Margaliot, D. Stenning, A. M. Furlanetto, A. Saleh, K. S. Hamilton, and J. Genereaux.** 1996. Structure/functional properties of the yeast dual regulator protein NGG1 that are required for glucose repression. J. Biol. Chem. **271:**9298–9306.
7. **Brownell, J. E., and C. D. Allis.** 1995. An activity gel assay detects a single, catalytically active histone acetyltransferase subunit in *Tetrahymena* macronuclei. Proc. Natl. Acad. Sci. USA **92:**6364–6368.
8. **Brownell, J. E., and C. D. Allis.** 1996. Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. Curr. Opin. Genet. Dev. **6:**176–184.
9. **Brownell, J. E., T. A. Ranalli, and C. D. Allis.** Personal communication.
10. **Brownell, J. E., J. Zhou, T. Ranalli, R. Kobayashi, D. G. Edmondson, S. Y. Roth, and C. D. Allis.** 1996. *Tetrahymena* histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell **84:**843–851.
11. **Cairns, B. R., Y.-J. Kim, M. H. Sayre, B. C. Laurent, and R. D. Kornberg.** 1994. A multisubunit complex containing the *SWI1/ADR6*, *SWI2/SNF2*, *SWI3*, *SNF5*, and *SNF6* gene products isolated from yeast. Proc. Natl. Acad. Sci. USA **91:**1950–1954.
12. **Candau, R., and S. L. Berger.** 1996. Structural and functional analysis of yeast putative adaptors. Evidence for an adaptor complex in vivo. J. Biol. Chem. **271:**5237–5245.
13. **Candau, R., P. A. Moore, L. Wang, N. Barlev, C. Y. Ying, C. A. Rosen, and S. L. Berger.** 1996. Identification of human proteins functionally conserved with the yeast putative adaptors ADA2 and GCN5. Mol. Cell. Biol. **16:**593–602.
14. **Cote, J., J. Quinn, J. L. Workman, and C. L. Peterson.** 1994. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science **265:**53–60.
15. **Dynlacht, B. D., T. Hoey, and R. Tjian.** 1991. Isolation of coactivators associated with the TATA-binding protein that mediate transcriptional activation. Cell **66:**563–576.
16. **Eisenmann, D. M., K. M. Arndt, S. L. Ricupero, J. W. Rooney, and F. Winston.** 1992. SPT3 interacts with TFIID to allow normal transcription in *Saccharomyces cerevisiae*. Genes Dev. **6:**1319–1331.
17. **Eisenmann, D. M., C. Chapon, S. M. Roberts, C. Dollard, and F. Winston.** 1994. The Saccharomyces cerevisiae *SPT8* gene encodes a very acidic protein that is functionally related to SPT3 and TATA-binding protein. Genetics **137:**647–657.
18. **Forsberg, S. L., and L. Guarente.** 1989. Identification and characterization of HAP4: a third component in the CCAAT-bound HAP2/HAP3 heteromer. Genes Dev. **3:**1166–1178.
19. **Fox, T. D.** Personal communication.
20. **Georgakopoulos, T., N. Gounalaki, and G. Thireos.** 1995. Genetics evidence for the interaction of the yeast transcriptional coactivator proteins GCN5 and ADA2. Mol. Gen. Genet. **246:**723–728.
21. **Georgakopoulos, T., and G. Thireos.** 1992. Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. EMBO J. **11:**4145–4152.
22. **Guarente, L., B. Lalonde, P. Gifford, and E. Alani.** 1984. Distinctly regulated tandem upstream activation sites mediate catabolite repression of the *CYC1* gene. Cell **36:**503–511.
23. **Guthrie, C., and G. R. Fink.** 1991. Guide to yeast genetics and molecular biology. Methods Enzymol. **194:**835.
24. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Hengartner, C. J., C. M. Thompson, J. Zhang, D. M. Chao, S. M. Liao, A. J. Koleske, S. Okamura, and R. A. Young.** 1995. Association of an activator with an RNA polymerase II holoenzyme. Genes Dev. **9:**897–910.
26. **Hinnebusch, A. G., G. Lucchini, and G. R. Fink.** 1985. A synthetic *HIS4* regulatory element confers general amino acid control on the cytochrome c gene (*CYC1*) of yeast. Proc. Natl. Acad. Sci. USA **82:**495–502.
27. **Hirschhorn, J. N., S. A. Brown, C. D. Clark, and F. Winston.** 1992. Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. Genes Dev. **6:**2288–2298.
28. **Horiuchi, J., and L. Guarente.** Unpublished results.
29. **Horiuchi, J., N. Silverman, G. A. Marcus, and L. Guarente.** 1995. ADA3, a putative transcriptional adaptor, consists of two separable domains and interacts with ADA2 and GCN5 in a trimeric complex. Mol. Cell. Biol. **15:**1203–1209.
30. **Ireton, K., D. Z. Rudner, K. J. Siranosian, and A. D. Grossman.** 1993. Integration of multiple developmental signals in *Bacillus subtilis* through the Spo0A transcription factor. Genes Dev. **7:**283–294.
31. **Iyer, V., and K. Struhl.** 1995. Poly(dA:dT), a ubiquitous promoter element that stimulates transcription via its intrinsic DNA structure. EMBO J. **14:**2570–2579.
32. **Kelleher, R. J. I., P. M. Flanagan, and R. D. Kornberg.** 1990. A novel mediator between activator proteins and the RNA polymerase II transcription apparatus. Cell **61:**1209–1215.
33. **Knaus, R., R. Pollock, and L. Guarente.** 1996. Yeast SUB1 is a suppressor of TFIIB mutations and has homology to the human coactivator PC4. EMBO J. **15:**101–108.
34. **Koleske, A. J., S. Buratowski, M. Nonet, and R. A. Young.** 1992. A novel transcription factor reveals a functional link between the RNA polymerase II CTD and TFIID. Cell **69:**883–894.
35. **Koleske, A. J., and R. A. Young.** 1994. An RNA polymerase II holoenzyme responsive to activators. Nature **368:**466–469.
36. **Lue, N. F., A. R. Buchman, and R. D. Kornberg.** 1989. Activation of yeast RNA polymerase II promoters by a thymidine-rich upstream element in vitro. Proc. Natl. Acad. Sci. USA **86:**486–490.
37. **Marcus, G. A., J. Horiuchi, N. Silverman, and L. Guarente.** 1996. *ADA5/SPT20* links the *ADA* and *SPT* genes, which are involved in yeast transcription. Mol. Cell. Biol. **16:**3197–3205.
38. **Marcus, G. A., N. Silverman, S. L. Berger, J. Horiuchi, and L. Guarente.** 1994. Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors. EMBO J. **13:**4807–4815.
39. **Melcher, K., and S. A. Johnston.** 1995. GAL4 interacts with TATA-binding protein and coactivators. Mol. Cell. Biol. **15:**2839–2848.
40. **Peterson, C. L., A. Dingwall, and M. P. Scott.** 1994. Five SWI/SNF gene products are components of a large multisubunit complex required for transcriptional enhancement. Proc. Natl. Acad. Sci. USA **91:**2905–2908.
41. **Peterson, C. L., W. Kruger, and I. Herskowitz.** 1991. A functional interaction between the C-terminal domain of RNA polymerase II and the negative regulator SIN1. Cell **64:**1135–1143.
42. **Pfeifer, K., B. Arcangioli, and L. Guarente.** 1987. Yeast HAP1 activator competes with the factor RC2 for binding to the upstream activation site UAS1 of the *CYC1* gene. Cell **49:**9–18.
43. **Piña, B., S. Berger, G. A. Marcus, N. Silverman, J. Agapite, and L. Guarente.** 1993. *ADA3*: a gene, identified by resistance to GAL4-VP16, with properties similar to and different from those of *ADA2*. Mol. Cell. Biol. **13:**5981–5989.
44. **Pugh, B. F., and R. Tjian.** 1990. Mechanism of transcriptional activation by Sp1: evidence for coactivators. Cell **61:**1187–1197.
45. **Roberts, S. M., and F. Winston.** 1996. *SPT20/ADA5* encodes a novel protein functionally related to the TATA-binding protein and important for transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **16:**3206–3213.
46. **Rose, M., and D. Botstein.** 1983. Structure and function of the yeast *URA3* gene. Differentially regulated expression of hybrid beta-galactosidase from overlapping coding sequences in yeast. J. Mol. Biol. **170:**883–904.
47. **Roth, S. Y., and C. D. Allis.** 1996. The subunit exchange model of histone acetylation. Trends Cell Biol. **6:**371–375.
48. **Saleh, A., V. Lang, R. Cook, and C. Brandl.** 1997. Identification of native complexes containing the yeast coactivator/repressor proteins NGG1/ADA3 and ADA2. J. Biol. Chem. **272:**5571–5578.
49. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
50. **Santangelo, G. M., J. Tornow, C. S. McLaughlin, and K. Moldave.** 1988. Properties of promoters cloned randomly from the *Saccharomyces cerevisiae* genome. Mol. Cell. Biol. **8:**4217–4224.
51. **Sauer, F., S. K. Hansen, and R. Tjian.** 1995. Multiple TAF_{II}s directing synergistic activation of transcription. Science **270:**1783–1788.
52. **Sayre, M. H., H. Tschochner, and R. D. Kornberg.** 1992. Reconstitution of

transcription with five purified initiation factors and RNA polymerase II from *Saccharomyces cerevisiae*. J. Biol. Chem. **267:**23376–23382.
53. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
54. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122:**19–27.
55. **Silverman, N., J. Agapite, and L. Guarente.** 1994. Yeast ADA2 protein binds to the VP16 protein activation domain and activates transcription. Proc. Natl. Acad. Sci. USA **91:**11665–11668.
56. **Silverman, N., and L. Guarente.** Unpublished results.
57. **Swanson, M. S., and F. Winston.** 1992. SPT4, SPT5 and SPT6 interactions: effects on transcription and viability in *Saccharomyces cerevisiae*. Genetics **132:**325–336.
58. **Thompson, C. M., A. J. Koleske, D. M. Chao, and R. A. Young.** 1993. A multisubunit complex associated with the RNA polymerase II CTD and TATA-binding protein in yeast. Cell **73:**1361–1375.
59. **Thut, C. J., J. L. Chen, R. Klemm, and R. Tjian.** 1995. p53 transcriptional activation mediated by coactivators TAFII40 and TAFII60. Science **267:**100–104.
60. **Wilson, C. J., D. M. Chao, A. N. Imbalzano, G. R. Schnitzler, R. E. Kingston, and R. A. Young.** 1996. RNA polymerase II holoenzyme contains SWI/SNF regulators involved in chromatin remodeling. Cell **84:**235–244.
61. **Winston, F.** 1992. Analysis of *SPT* genes: a genetic approach toward analysis of TFIID, histones, and other transcription factors of yeast, p. 1271–1293. *In* S. L. McKnight and K. R. Yamamoto (ed.), Transcriptional regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.