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ADA5/SPT20 Links the *ADA* and *SPT* Genes, Which Are Involved in Yeast Transcription

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In this report we describe the cloning and characterization of *ADA5*, a gene identified by resistance to GAL4-VP16-mediated toxicity. ADA5 binds directly to the VP16 activation domain but not to a transcriptionally defective VP16 double point mutant. Double mutants with mutations in *ada5* and other genes (*ada2* or *ada3*) isolated by resistance to GAL4-VP16 grow like *ada5* single mutants, suggesting that *ADA5* is in the same pathway as the other *ADA* genes. Further, ADA5 cofractionates and coprecipitates with ADA3. However, an *ada5* deletion mutant exhibits a broader spectrum of phenotypes than mutants with null mutations in the other *ADA* genes. Most interestingly, *ADA5* is identical to *SPT20* (S. M. Roberts and F. Winston, Mol. Cell. Biol. 16: 3206–3213, 1996), showing that it shares phenotypes with the *ADA* and *SPT* family of genes. Of the other *SPT* genes tested, mutants with mutations in *SPT7* and, strikingly, *SPT15* (encoding the TATA-binding protein) show resistance to GAL4-VP16. We present a speculative pathway of transcriptional activation involving the ADA2-ADA3-GCN5-ADA5 complex and the TATA-binding protein.

In eukaryotes, transcriptional activation is a central means of gene regulation. RNA polymerase II, which is responsible for transcribing mRNA, is recruited to transcriptional start sites via its interaction with the general transcription factors. These factors recognize the TATA box or initiator elements found near the start site. Moreover, transcriptional activators, which bind enhancer/UAS elements found hundreds of base pairs away from the TATA box, can greatly stimulate the initiation of transcription. These transcriptional activators are often modular in structure; one domain is responsible for recognizing the DNA, and another domain is responsible for transcriptional activation (18). The mechanism by which these activation domains function remains unknown, although it is the focus of much research.

It is clear that proteins other than the general transcription factors and activators are necessary for activated transcription (41). These factors, termed coactivators or adapters, have been identified by using various genetic and biochemical methods and are thought to function by mediating the interaction between basal factors and activation domains (3, 31, 41). TFIID, a complex of the TATA-binding protein (TBP) and TBP-associated factors (TAFs), is capable of supporting activated transcription, whereas TBP alone cannot (9). Further, different TAF subunits bind to and mediate activation by different classes of activation domains (17, 28, 48, 56). Additionally, other, non-TAF coactivators identified in metazoans or their viruses include PC4/p15 (15, 35), CBP (36), and the X protein from hepatitis B virus (24). These proteins have been shown to have the properties expected of coactivators, including the ability to mediate transcriptional activation.

Yeast genetics has proven to be very useful in identifying potential coactivator genes. For example, the *SRB* genes were isolated as suppressors of truncations in the conserved carboxy-terminal domain of RNA polymerase II (33, 55). These gene products copurify in a complex, called the mediator, that can bind activators (25) and has coactivator activity (25, 32). This mediator complex and the core RNA polymerase II subunits form the RNA polymerase II holoenzyme, which can support activated transcription (32, 34). The products of the *SWI1*, *SWI2/SNF2*, *SWI3*, *SNF5*, and *SNF6* genes, identified as positive regulators of *SUC2* and *HO* transcription (39, 54), are part of a subcomplex of the mediator/holoenzyme (57) that antagonizes histone repression in vivo and in vitro (7, 27).

Other yeast coactivator gene candidates include *ADA2*, *ADA3*, and *GCN5*, isolated in a selection for mutants resistant to GAL4-VP16-mediated toxicity (4, 5, 38). Mutations in these genes relieve toxicity by reducing the ability of the VP16 activation domain to activate transcription without altering the levels of GAL4-VP16 in cells. Moreover, *ada2*, *ada3*, and *gcn5* mutants all have similar phenotypes, including slow growth on minimal medium, temperature sensitivity, and a reduced ability to support activation by certain activation domains in vivo and in vitro (4, 38, 40). ADA2, ADA3, and GCN5 form a complex when translated in vitro (30), and they cofractionate from yeast extracts (53). The fact that double mutants with mutations in these genes have phenotypes indistinguishable from those of single mutants also supports the model that ADA2, ADA3, and GCN5 function together in a complex (38). ADA2 can bind VP16 and GCN4 activation domains (2, 52) and may be is necessary for a TBP-VP16 interaction in yeast extracts (2).

Another set of yeast genes important for proper transcriptional regulation and promoter selection, the *SPT* genes, were identified as suppressors of Ty insertions in yeast promoters (59). These genes may be grouped into two classes, those that affect transcription via chromatin and those that affect transcription in a chromatin-independent manner (58). The latter class includes *SPT15*, which encodes TBP (12, 22), as well as *SPT3*, *SPT7*, and *SPT8* (12). Genetic evidence suggests that SPT3, SPT7, and SPT8 may act as a complex with TBP, and SPT3 can be coimmunoprecipitated with TBP (10, 11, 14). Strains harboring *spt3*, *spt7*, *spt8*, or *spt15* mutations show reduced expression of the Ty element, reduced expression of other yeast genes, and an alteration in promoter selection at Ty loci (58).

Here we report the cloning and initial characterization of *ADA5*. The phenotypes of *ada5* mutants and of double mutants suggest that *ADA5* operates in the same pathway as the other *ADA* genes (*ADA2*, *ADA3*, and *GCN5*). Moreover, ADA5

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cofractionates with ADA3 in yeast extracts. *ADA5* is identical to *SPT20* (45) and is the only *ada* gene to date to display Spt$^-$ phenotypes. We present a speculative pathway of interaction of ADA5 and the other ADA proteins in connecting activators to basal factors at promoters.

MATERIALS AND METHODS

Yeast strains and manipulations. All yeast strains are derivatives of BWG1-7a (*MAT***a** *ade1-100 ura3-52 leu2-3,112 his4-519*) (20), BP1 (*MAT***a** *ade1-100 ura3-52 leu2-3,112 his4-519 gal4*::*HIS4*), or PSy316 (*MAT*α *ura3-52 leu2-3,112 his3*Δ*200 lys2*) (4). GMy37p (*MAT***a** *ura3-52 leu2 his4 gal4*::*HIS4 ada5-1*) was isolated as a strain resistant to GAL4-VP16 toxicity (38). Fy strains, L881, and YSMR strains were gifts from Fred Winston.

Yeast transformations were performed by the lithium acetate method (16). Tetrad analysis and other yeast manipulations were done according to standard techniques (21). Slow-growth phenotypes of *ada* mutants were assayed on synthetic dextrose minimal medium supplemented with only the necessary amino acids and adenine (Difco). Otherwise, strains were grown on synthetic complete (SC) medium containing all amino acids except those needed for plasmid selection.

ADA5 **cloning and plasmids.** To clone the wild-type *ADA5* gene, GMy37p, the *ada5-1* mutant, was transformed with a yeast genomic library (55) and colonies that grew as well as the wild type were selected. From these, a clone, p3,1 with an 8.5-kb insert that restored wild-type growth and sensitivity to GAL4-VP16 toxicity was isolated. p3,1 was subjected to partial digestion with *Sau*3AI, and 1- to 3-kb fragments were isolated and ligated into the *Bam*HI site of pRS316 (51) to create a subgenomic library. GMy37p was transformed with this subgenomic library, and colonies that grew as well as the wild type were again selected. Two truncated subclones were isolated for further study: pL1B1 (with a 2.2-kb insert), which partially complements the slow-growth phenotype of GMy37p, and pL1G1 (with a 1.8-kb insert), which fully complements the slow-growth phenotype. Both subclones complement the toxicity resistance phenotype of *ada5-1*. Sequence analysis (see below) revealed that pL1B1 is lacking the upstream sequences of *ADA5* as well as the region encoding the first 10 amino acids. Presumably, the remaining region of *ADA5* is expressed from a cryptic promoter in the vector and an internal methionine in the *ADA5* coding sequence. pL1G1 is a C-terminal *ADA5* truncation, containing approximately 500 bp of upstream sequences and 1,368 bp of the coding region, which encodes residues 1 to 456.

pRS316-ADA5, a subclone with the entire *ADA5* coding region, was created in several stages. The *Bst*XI site in pRS316 (51) was destroyed by digesting, blunting with T4 polymerase, and ligating to create pRS316-*Bst*XI. A 1.9-kb *Eco*RI fragment containing the sequences encoding the first 437 amino acids of ADA5 as well as the upstream sequences was cloned into the *Eco*RI site to create pRS316-$ADA5_{437a}$ and pRS316-$ADA5_{437b}$. pRS316-$ADA5_{437a}$ is oriented such that the *Bst*XI site at the 5′ end of *ADA5* is proximal to the *Sac*I site in the polylinker. pRS316-$ADA5_{437b}$ is in the other orientation. A 1.9-kb *Bst*XI-*Hin*dIII fragment from pL1B1 was cloned into the *Bst*XI-*Hin*dIII sites of pRS316-$ADA5_{437a}$ to create pRS316-ADA5. A 2.6-kb *Xho*I-*Not*I fragment from pRS316-ADA5 was cloned into pRS315 (51) cut with *Xho*I and *Not*I to create pRS315-ADA5.

The *ADA5* coding sequence was amplified by PCR (Perkin-Elmer) with primers ADA5N (CCCGGGAGATCTGCGCCGCAATGAGTGCCAATAGCCCG ACAGG) and ADA5C (CCCGGGGGATCCGCGGCCGCCTAAGATCTTGA CATTGTAGTAGAAGAGGGCG). The resulting fragment was digested with *Not*I and cloned into the *Not*I site of pDB20L (4) to form pDB20L-ADA5. pBluescript-ADA5 was generated by cloning a 2.2-kb *Bam*HI *Hin*dIII fragment from pL1B1 into Bluescript KS+ (Stratagene) cut with *Bam*HI and *Hin*dIII.

To show that the cloned gene corresponds to the *ada5-1* mutation, pRS306-ADA5 was generated by cloning a 1.8-kb *Xba*I fragment from p3,1 into the *Xba*I site of a version of the *URA3* integrating vector pRS306 (51) with the *Eco*RI site destroyed. pRS306-ADA5 was cut with *Eco*RI (a unique site in the 1.8-kb insert) and transformed into PSy316. A colony with an integration at the cloned locus was mated to GMy37p, and the resulting diploid was sporulated. In each of five tetrads dissected, two spores gave rise to slowly growing Ura$^-$ colonies and two spores gave rise to Ura$^+$ colonies with wild-type growth, showing that the cloned gene maps to the *ada5-1* mutant locus.

ADA5 **sequencing.** All sequencing was done by using the Sequenase kit (U.S. Biochemicals). A deletion series from the *Not*I site of pL1B1 was generated with exonucleases III and VII and sequenced with the −20 primer. The sequence of the other strand was obtained by using the T3 primer to sequence a second deletion series from the *Kpn*I site of pL1B1 or from sequence-specific primers. Since pL1B1 does not fully complement the *ada5-1* mutant and since the largest open reading frame on pL1B1 was found to be open at the 5′ end, the other subclone, pL1G1, was partially sequenced. The sequence obtained with the −20 primer in pL1G1 showed that it is lacking sequences after residue 1368 in the putative *ADA5* open reading frame and thus encodes a C-terminal truncation of ADA5. By using a sequence-specific primer, it was determined that the incomplete open reading frame identified in pL1B1 continues for an additional 30 bp in pL1G1. To confirm that the *ADA5* open reading frame is continuous across the truncation junctions present in pL1B1 and pL1G1, the full-length *ADA5* genomic clone (p3,1) was used to sequence across these junctions with sequence-specific primers.

ADA5 **deletion plasmid and strains.** The *ADA5* deletion plasmid was created in several steps. A 550-bp *Xho*I-blunted *Bst*XI fragment from pL1G1 containing sequence encoding the first 12 amino acids of *ADA5* and 5′ flanking sequence was cloned into the *Xho*I and *Eco*RV sites of pBluescript KS+ (Stratagene) to form pBluescript-A5BstX. Next, a 2.4-kb *Bam*HI-*Bgl*II fragment containing the *hisG URA3* cassette from pNKY51 (1) was cloned into the *Bam*HI site of pBluescript-A5BstX. A clone, pADA5nko, was chosen such that the reformed *Bam*HI site was distal to the *ADA5* 5′ sequence. Finally, pBluescript-ADA5 was cut with *Dra*III, ligated to a *Not*I linker (New England Biolabs), and cut with *Not*I and *Bst*YI. The 400-bp *Bst*YI-*Not*I fragment containing sequences encoding the C-terminal 136 amino acids and 3′ flanking sequence was cloned into the *Bam*HI-*Not*I site of pADA5nko to form pADA5KO. This plasmid will delete the coding sequence for 437 amino acids from the N terminus of ADA5, which should produce a null phenotype.

ADA5 deletion strains (Δ*ada5* strains) were generated by transforming yeast cells with pADA5KO cut with *Xho*I-*Not*I. Slowly growing Ura$^+$ transformants were tested for resistance to GAL4-VP16 and mated to previously characterized *ada5* mutant strains of the opposite mating type when these strains were available. Strains that were resistant to GAL4-VP16 were grown on 5-fluoroorotic acid to select for strains that had looped out the *URA3* sequence. Ura$^-$ derivatives were transformed with pRS316-ADA5 to confirm that wild-type growth and sensitivity to GAL4-VP16 were restored by the *ADA5* clone. In this manner, the Ura$^+$ and Ura$^-$ deletion strains GMy29 and GMy30 were generated in the parent strain BWG1-7a, GMy31 and GMy32 were generated in BP1, and GMy33 and GMy34 were generated in PSy316.

The Δ*ada2* Δ*ada5* and Δ*ada3* Δ*ada5* double deletion strains were generated in the following manner. GMy30 containing the plasmid pDB20L-ADA5 was transformed with pADA2KO (4) cut with *Bam*HI and *Xho*I or with pADA3KO cut with *Pvu*II and *Bam*HI (38). *ada2* or *ada3* deletion strains were identified by mating slowly growing transformants to Δ*ada2* or Δ*ada3* strains. Strains that failed to complement the cognate Δ*ada* strain were grown on 5-fluoroorotic acid to select strains that had looped out the *URA3* sequence.

The resulting Ura$^-$ derivatives were grown on yeast extract-peptone-dextrose (YPD) plates and replica plated to identify strains that had lost the *LEU2* plasmid containing the *ADA5* clone. GMy36 (BWG1-7a Δ*ada2* Δ*ada5*) can be restored to wild-type growth only by transformation with both the *ADA5* (pRS315-ADA5) and *ADA2* (pNS3.8 [4]) clones, confirming that it is a double mutant. Similarly, GMy38 (BWG1-7a Δ*ada3* Δ*ada5*) was confirmed by transformation with the *ADA5* and *ADA3* (pADA3HHV [40]) clones.

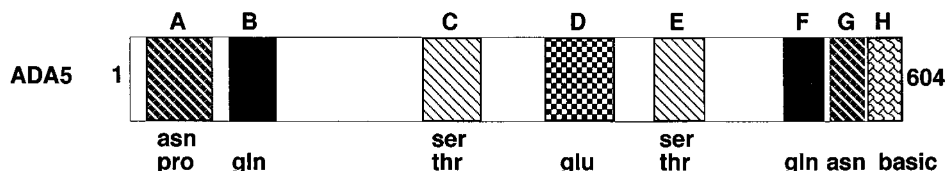
ADA5 expression, antisera, and Western blot (immunoblot) analysis. pET15b-ADA5, an ADA5 expression vector, was created by exploiting the single *Bam*HI site in pL1B1, created when the 2.2-kb *Sau*3AI fragment from p3,1 was cloned into the *Bam*HI site of pRS316. This *Bam*HI site, derived from the *Sau*3AI site at position 28 in the *ADA5* coding sequence, is in frame with the *Bam*HI-site of pET15b (Novagen), a vector that fuses six histidine residues at the N termini of expressed proteins. pL1B1 was digested with *Dra*III, ligated with a *Bam*HI linker (New England Biolabs), and then digested with *Bam*HI. The resulting 2.2-kb fragment was cloned into the *Bam*HI site of pET15b (Novagen) to form pET15b-ADA5. In the bacterial strain BL21(DE3) (Novagen), this plasmid produces insoluble ADA5, which was purified in urea on a nickel column (Qiagen).

Polyclonal rabbit ADA3 or ADA5 antiserum was generated by standard procedures (23). For ADA5, two rabbits were immunized with lyophilized acrylamide slices containing 400 μg of ADA5 resuspended in saline followed by six boosts, three containing 200 μg of ADA5 and three containing 100 μg. Immunoglobulin G was purified from serum on a protein A column (23). Antiserum to ADA3 was generated by injecting rabbits six times with 0.5 mg of a fragment of ADA3, ADA3(580-702), in RIBI adjuvant (RIBI ImmunoChem Research, Inc.). The expression and purification of ADA3(580-702) have been previously described (30). The antiserum was purified by binding and eluting from immunoblots of ADA3(580-702) (23).

Western blotting was performed as follows. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on samples run in the absence of a reducing agent to eliminate a contaminating keratin band that comigrates with ADA5. Proteins were transferred to polyvinylidene difluoride (DuPont) by using a Milliblot graphite electroblotter (Millipore), and filters were probed by standard techniques (23) with affinity-purified anti-ADA3 serum or purified anti-ADA5 immunoglobulin G. Proteins were visualized by using a horseradish peroxidase-conjugated secondary antibody with the ECL system (Amersham).

Fractionation and immunoprecipitation of yeast extracts. BWG1-7a was grown in 5 liters of YPD to an optical density at 600 nm of approximately 4. GMy30 was grown in 10 liters of YPD to an optical density of approximately 2. This yielded a wet weight of 35 g of cells each. Preparation of yeast whole-cell extract and fractionation with a Bio-Rex 70 (Bio-Rad Laboratories) column were performed as described elsewhere (49, 55) with the following modifications. Bio-Rex 70 columns (1.5 by 7 cm) were used, and protein was eluted from the columns successively with buffer A (49) containing 250 mM, 600 mM, and 1.2 M potassium acetate.

Immunoprecipitation experiments were performed with the 600 mM elution fractions from the Bio-Rex 70 columns. Antibodies from crude preimmune



A(4-59): SPTGNDPHVFGIPVNATPSNMGSPGSPVNVPPPMNPAVANVNHPVMRTNSNSNAN

B(69-104): QIQQLQQRQRLLLQQRLLEQQRKQQALQNYEAQFYQ

C(235-269): SSSSPSNNNSTQDNSKIQQPSEPNSGVANTGANT

D(335-382): EHRDMLEETAFSEPHWDSEKKSFIHEHRAESTREGTKGVVGHIEERDE

E(401-446): TTTITNSTFAVSLTKNAMEIASSSSNGVRGASSSTSNSASNTRNNS

F(518-533): QQQLLQRQQQALEQQQ

G(540-559): NANKRSGNNATSNNNNNNNN

H(562-591): KPKVKRPRKNAKKSESGTPAPKKKRMTKKK

FIG. 1. Sequences of *ADA5* domains. The sequence of *ADA5* was determined as described in Materials and Methods and contains an open reading frame encoding 604 amino acids with a predicted molecular mass of 68 kDa. Eight domains with unusual amino acid compositions are listed.

serum and from crude anti-ADA5 serum were cross-linked to protein A-Sepharose beads (Sigma) as previously described (6, 23). Comparison of antibodies eluted from the protein A beads before and after cross-linking by boiling, PAGE, and staining with Coomassie brilliant blue demonstrated that equal amounts of antibody were cross-linked from both sera. The beads were washed several times with 100 mM glycine (pH 2.5) to remove un-cross-linked antibody and then washed several times and equilibrated in IP buffer (6) with 0.1 M potassium acetate. Immunoprecipitations were performed as described previously (6). Briefly, 100 μg of the 600 mM fraction from BWG1-7a or 150 μg of the 600 mM fraction from GMy30 was precleared with protein A-Sepharose beads and then incubated with antibody-cross-linked beads by rotation at 4°C for 3 h. The beads were sedimented and washed four times with 1 ml of IP buffer with 0.1 M potassium acetate. Bound proteins were then eluted from the beads by two successive elutions with 12 μl of 100 mM glycine (pH 2.5). The eluates were pooled and analyzed by Western blotting.

GST-VP16 precipitations. The *ADA5* in vitro transcription-translation plasmid pCITE2b-ADA5 was generated by cloning the 2.2-kb *Bam*HI fragment containing the *ADA5* coding sequence and 3′ sequence from pET15b-ADA5 into the *Bam*HI site of pCITE2b (Novagen). In vitro translations were performed as previously described (30).

Precipitation experiments were performed by loading 10 μg of glutathione *S*-transferase (GST)–VP16(413-490), GST-VP16FA442,FA475, or GST-VP16Δ(413-456) on 10 μl of glutathione-Sepharose beads (Pharmacia) that were preblocked in *Escherichia coli* extract. Ten microliters of in vitro-translated ADA5 and 200 μl of S300–1%T buffer (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.6], 300 mM potassium acetate, 25 mM magnesium acetate, 1% Triton X-100, 20% glycerol) plus 1 mg of *E. coli* extract per ml were added to the GST-VP16 beads and incubated for 1 h at 4°C. Samples were washed four times with 1 ml of S300–1%T buffer, eluted from the beads with 20 mM reduced glutathione (Sigma) in S300 buffer, and electrophoresed on an SDS-polyacrylamide gel. The gel was dried and exposed on XAR film (Kodak). GST-VP16 and GST-VP16Δ expression constructs and purification techniques were described previously (37). GST-VP16FA442,FA475 was made by cloning a *Bam*HI-*Sph*I fragment from pMSVP16FA442/FA475 (42) into the *Bam*HI and *Sph*I sites of pGVP (the GST-VP16 expression plasmid [37]).

Gel shifts and β-galactosidase assays. β-Galactosidase assays were carried out on glass bead yeast extracts (46). The activity of β-galactosidase was normalized to the level of total protein. Gel shift analysis was performed as previously described (4).

Nucleotide sequence accession number. The GenBank accession number for the *ADA5* sequence is U43153.

RESULTS

Properties of the *ada5-1* mutant. A screen for relief of GAL4-VP16-induced toxicity (4) identified multiple alleles of *ADA2*, *ADA3*, and *GCN5* and a single allele of *ADA5*, *ada5-1* (38). The *ada5-1* mutant grows slowly on rich as well as minimal media. This is in contrast to the other *ada* mutants, which have a pronounced slow-growth phenotype only on minimal media (38). This slow-growth phenotype segregated 2 slow:2 wild type in six complete tetrads and cosegregated with resistance to GAL4-VP16 (data not shown).

ADA2, *ADA3*, and *GCN5* mutants survive GAL4-VP16 toxicity by reducing the ability of GAL4-VP16 to activate transcription, rather than by reducing the level of the toxic protein (4, 38, 40). Since GAL4-VP16 is toxic to wild-type cells, we assayed levels of a less toxic derivative, GAL4-VP16FA, expressed on a low-copy-number plasmid also under control of the *ADH1* promoter, by gel shift (4). Compared with that in the wild type, less of the GAL4-VP16 specific complex was found in the *ada5* mutant (data not shown). Thus, unlike the other *ADA* gene products studied to date, it is possible that cells missing ADA5 escape because of a toxicity reduced level of GAL4-VP16.

Cloning, mapping, and sequencing of *ADA5*. *ADA5* was cloned by complementation of the slow-growth phenotype of the *ada5-1* mutant (see Materials and Methods). The *ADA5* clone has the ability to restore wild-type growth and sensitivity to GAL4-VP16 to the *ada5* mutant strain (data not shown). To confirm that the clone indeed corresponds to the *ADA5* gene, we directed integration of *URA3* in plasmid pRS306-ADA5 to the cloned locus in a wild-type strain (see Materials and Methods). This strain was mated to the *ada5-1* strain, the diploid was sporulated, and tetrads were dissected. In all five tetrads, two spores grew slowly and were Ura^- and two spores grew normally and were Ura^+, showing linkage between the clone and the *ada5-1* mutation.

In order to map *ADA5* to the yeast physical map, a fragment from the gene was radiolabeled and hybridized to a phage grid representing over 90% of the yeast genome (43). Two overlapping clones were identified, showing that *ADA5* maps to the right arm of chromosome XV.

ADA5 was further subcloned and sequenced (see Materials and Methods). The gene encodes a novel protein with 604 amino acids and a predicted molecular mass of 68 kDa. The ADA5 protein contains two glutamine-rich regions, several Ser-Thr-rich regions, a proline-rich region, an acidic region, and a basic region (Fig. 1). As a final confirmation that the open reading frame we identified corresponds to *ADA5*, the open reading frame was amplified by PCR and placed under

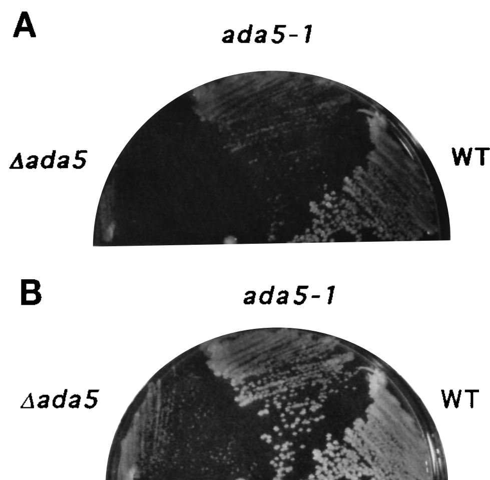


FIG. 2. The *ada5* deletion mutant strain grows more slowly than the *ada5-1* mutant strain. GMy30 (Δ*ada5*), GMy37p (*ada5-1*), and BWG1-7a (isogenic wild-type [WT] strain) were streaked on rich (YPD) medium. Growth was scored after 2 days (A) and 3 days (B).

the control of the *ADH1* promoter (see Materials and Methods). This plasmid complements the *ada5-1* mutation as well as the genomic clone.

Characterization of *ADA5* deletion mutants. *ada5* deletion mutants (Δ*ada5* mutants) were constructed by homologous recombination as described in Materials and Methods. The deletion mutant is viable but grows more slowly than the *ada5-1* mutant (Fig. 2) and more slowly than *ada2*, *ada3*, or *gcn5* deletion mutants (not shown). Like other *ada* mutants, the *ada5* mutant is temperature sensitive for growth and completely resistant to GAL4-VP16. However, unlike the other *ada* mutants, the *ada5* mutant is auxotrophic for inositol (data not shown).

Analysis of an amino-terminal fragment of ADA5 for complementation of cell growth and toxicity by GAL4-VP16. In the process of subcloning *ADA5*, we discovered that a portion of the *ADA5* coding sequence could be deleted without loss, or with only partial loss, of the ability to complement an *ada5* mutant strain (see Materials and Methods). As shown in Fig. 3A and B, $ADA5_{437}$, encoding the first 437 amino acids of ADA5, can complement a Δ*ada5* strain for growth on rich medium and partially complement for growth on minimal medium. However, $ADA5_{437}$ strains remain resistant to GAL4-VP16 (Fig. 3C). These findings show that the ADA fragment containing amino acids 1 to 437 complements the growth phenotype but does not restore the toxicity of GAL4-VP16. It is thus possible that the deletion in *ADA5* separates two functions of the gene product. Alternatively, the observed properties of the deletion may result from a quantitative reduction in ADA5 activity.

In vivo activation defects of *ada5* strains. The lower levels of GAL4-VP16FA in *ada5* mutants may indicate that *ADA5* is required for activation at the *ADH1* promoter. To determine whether the *ADH1* promoter or other yeast promoters require *ADA5* for activation, we introduced β-galactosidase reporter plasmids under the control of eight different yeast UAS sequences into the wild-type, the *ada5-1* mutant, and the *ada5* deletion strains.

The activities of these reporters, as measured by β-galactosidase assays, are shown in Table 1. The activities of the *HO* and *INO1* promoters are the most drastically reduced. The activities of the GCN4-responsive HIS66 and 14x2 promoters, which are known to require *ADA2*/*ADA3*/*GCN5* (4, 38, 40), are also severely reduced in the *ada5* mutant and deletion strains. The *CYC1* UAS1 promoter, which is known not to require *ADA2*, *ADA3*, and *GCN5*, is reduced 3-fold in the *ada5-1* mutant and 10-fold in the *ada5* deletion strain. Interestingly,

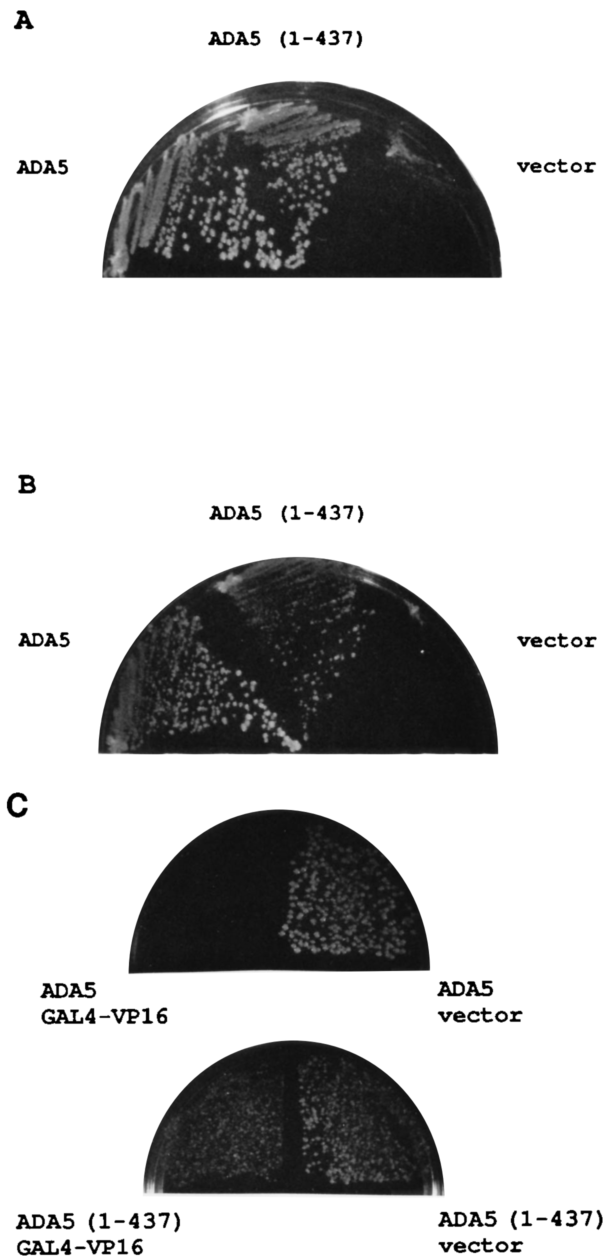


FIG. 3. The sequence encoding the first 437 amino acids of ADA5 is sufficient to complement the slow-growth phenotype of Δ*ada5* mutants on rich medium but only partially complements on minimal medium. (A) GMy30, a Δ*ada5* mutant, was transformed with the full-length *ADA5* clone, the $ADA5_{437}$ fragment (containing sequence encoding the first 437 amino acids of ADA5), or a vector control. Transformants were either restreaked on SC medium and scored after 2 days (A) or restreaked on synthetic dextrose medium and scored after 3 days (B). (C) $ADA5_{437}$ does not restore sensitivity to GAL4-VP16 to an *ada5* deletion strain. GMy30 complemented by full-length *ADA5* or $ADA5_{437}$ was transformed with pSB201, a 2μm plasmid expressing GAL4-VP16 from the *ADH1* promoter, or a vector control and plated on SC medium. Transformation plates were scored for growth after 3 days.

TABLE 1. Requirement of *ADA5* for promoter activation[a]

Promoter	β-Galactosidase activity in:		
	WT	*ada5-1* mutant	Δ*ada5* mutant
UAS1	989	282	92
UAS2	347	251	71
HIS66	217	19	23
14x2	20	3	4
HO	206	23	10
INO	101	4	<1
SD5	9,588	ND[b]	3,027
ADH1	8,948	3,245	1,600

[a] BWG1-7a (wild-type strain [WT]) and *ada5-1* and Δ*ada5* mutants were transformed with the following β-galactosidase reporter plasmids: pLG312ΔAluXho, which contains the *CYC1* UAS1 (19); p265UP1, which contains the *CYC1* UAS2 (13); pHIS66, which contains the *HIS4* UAS (26); p14x2, which contains two synthetic GCN4 binding sites (26); pCP8 (gift of C. Peterson), which contains the *HO* URS1 (position −1516 to −901); p*INO1* (50); and pLGSD5, which contains the *GAL1-10* promoter, and pCP0, which contain the *ADH1* promoter (47). β-Galactosidase gene activities are means of results from three assays differing by less than 20% and are normalized to the level of total protein. The activity of pLGSD5 was measured after induction with galactose, and the activity of the *INO1* reporter was assayed after 5 h of induction in inositol-free medium (50).

[b] ND, not determined.

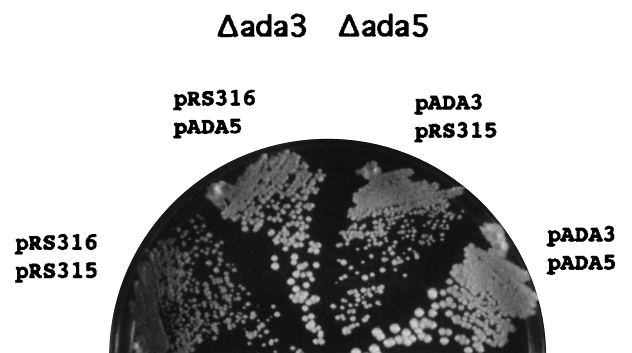


FIG. 4. Δ*ada3* Δ*ada5* double deletion mutants grow as well as Δ*ada5* deletion mutants. GMy38, a Δ*ada5* Δ*ada3* double deletion mutant, was transformed with all pairwise combinations of pRS315-ADA5 or pRS315 (a *LEU2* vector) and pADA3HHV or pRS316 (a *URA3* vector). Transformants indicated by plasmid-borne genes were restreaked on dropout medium and scored after 3 days. Note that the Δ*ada3* Δ*ada5* transformant (pRS316 pRS315) grows comparably to the Δ*ada5* transformant (pADA3 pRS315). Growth of the Δ*ada3* transformant (pRS316 pADA5) is intermediate between those of the double deletion strain and the wild type (pADA3 pADA5).

the activities of the *CYC1* UAS2, *ADH1*, and *GAL1-10* promoters, which are not affected by *ada2* mutations, are reduced only three- to fivefold in the *ada5* deletion strain.

In summary, the *ada5* deletion strain has a broad defect in transcription affecting all of the promoters tested. However, there may be a relationship between the spectra of effects in *ada5* and *ada2* mutants. In particular, the SD5, *ADH1*, and UAS2 promoters, which are the least affected in the *ada5* mutants, are not affected at all in *ada2* mutants (4, 40). The reduction in activation of the *ADH1* and *INO1* promoters explains the novel phenotypes of *ada5* mutants, i.e., a small reduction in GAL4-VP16 levels and inositol auxotrophy.

Double mutants with mutations in *ADA5* and other *ADA* genes. The above-described findings raise the question of whether *ADA5* functions in the same pathway as the other *ADA* genes. To begin to address this issue, we constructed double mutants carrying *ada5* and *ada2* or *ada3* mutations (see Materials and Methods). We found that both Δ*ada2* Δ*ada5* and Δ*ada3* Δ*ada5* double deletion mutants are viable and grow no more slowly than Δ*ada5* single mutants (Fig. 4 and data not shown). These findings suggest that *ADA5* may operate in the same pathway as the other *ADA* genes. In Discussion, we speculate on how *ADA5* may operate in the same pathway as the other *ADA* genes yet have a more general function in transcription.

Specific binding of ADA5 to functional VP16 activation domains. We began a biochemical analysis of ADA5 by studying whether the protein bound to the activation domain of VP16. ADA5 was translated in vitro, radiolabeled, and added to glutathione-Sepharose beads coupled to GST-VP16. After extensive washing, the GST fusion was eluted from the beads with glutathione, and the eluent was electrophoresed on an SDS-polyacrylamide gel. Autoradiography of the gel indicated a band the size of the onput ADA5 protein, showing that the protein bound to the beads (Fig. 5). Two mutant VP16 proteins were also fused to GST and used as controls. The first contained a deletion of residues 457 to 490 of VP16, leaving intact residues 413 to 456. This VP16 derivative shows greatly reduced activity in yeast cells (52). The second mutation changes only the Phe residues at positions 442 and 475 to Ala. This mutant shows a large reduction in activity in mammalian cells (42). In both cases, the mutations abolished the binding of ADA5. Following autoradiography, Coomassie staining of the gel showed that the same amounts of all three GST fusion proteins were bound to and eluted from the beads (data not shown). This experiment shows that ADA5 binds to the activation domain of VP16 but not to nonfunctional mutant domains. Models for ADA5 function must take into account this activity.

Cofractionation of ADA5 and ADA3. The genetic and biochemical findings summarized in the introduction indicate that ADA2, ADA3, and GCN5 function together in a complex (30, 38). Because the growth phenotypes of Δ*ada5* Δ*ada2* and Δ*ada5* Δ*ada3* double mutants suggest that *ADA5* may be acting in the same pathway as *ADA2*, *ADA3*, and *GCN5*, we examined whether ADA5 copurifies with a member of the ADA complex, ADA3.

The first step in purification of the ADA complex (which will be described in detail elsewhere) was to chromatograph a yeast whole-cell extract on a Bio-Rex 70 cation-exchange column. We assayed the flowthrough and three salt elution fractions from this column by Western blot analysis for ADA5 and ADA3. As shown in Fig. 6A, ADA3 and ADA5 eluted primarily in the 600 and 1,200 mM potassium acetate fractions in very similar proportions. ADA2 and GCN5 also elute in the 600 and 1,200 mM fractions with a profile identical to those of ADA3 and ADA5 (53). As a control, an extract from a Δ*ada5*

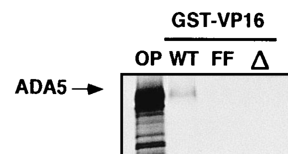


FIG. 5. ADA5 binds to VP16. ADA5 was translated in a reticulocyte lysate and labeled with [^{35}S]Met. GST-VP16 (WT) and, as controls, GST fused to two mutant VP16 derivatives were coupled to glutathione-Sepharose beads. The experiment for lane FF utilized an activation domain which changes Phe-442 and Phe-475 to Ala, while the experiment for lane Δ utilized an activation domain that has residues 457 to 490 removed. GST-VP16 beads were incubated with ^{35}S-ADA5, washed extensively, and eluted with glutathione. The eluent was electrophoresed on an SDS-polyacrylamide gel and autoradiographed. Lane OP, 1/10 the onput of translated ADA5 used in these experiments.

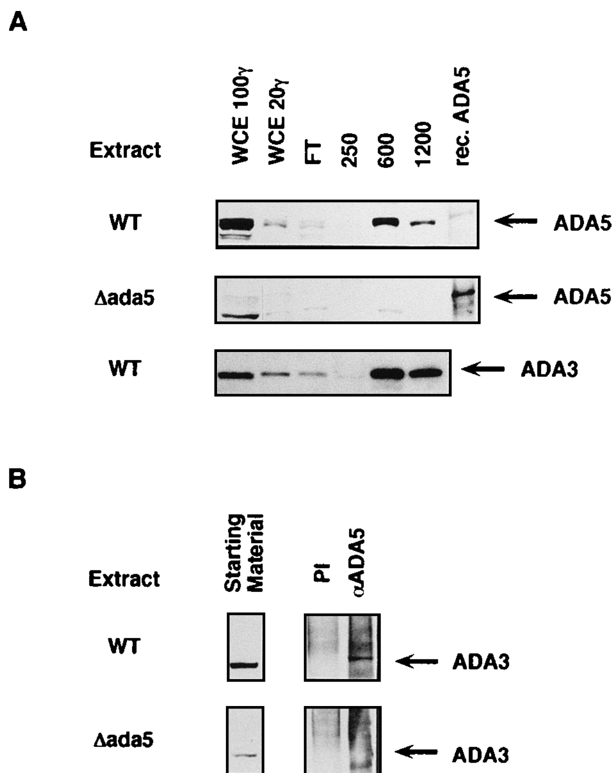


FIG. 6. ADA5 and ADA3 are physically bound to each other in a yeast extract. (A) Whole-cell yeast extracts (WCE) from a wild-type (WT) or a Δ*ada5* strain were fractionated on a Bio-Rex 70 column. Proteins were stepwise eluted in 250, 600, and 1,200 mM potassium acetate. One hundred and 20 μg (γ) of the whole-cell extract, 20 μg of flowthrough (FT), and 20 μg of each elution were assayed for ADA5 and ADA3 by Western blot analysis. ADA5 and ADA3 cofractionate over this column. Samples from a Δ*ada5* extract fractionated similarly are shown, demonstrating that the indicated band is ADA5. Recombinant ADA5 produced in bacteria (see Materials and Methods) was loaded as a control (rec. ADA5). (B) Protein from the 600 mM fraction of the Bio-Rex 70 column (100 μg from the wild-type extract and 150 μg from the Δ*ada5* extract) was immunoprecipitated with anti-ADA5 (αADA5) or preimmune (PI) serum. The precipitate was assayed by Western blotting for ADA3. ADA3 is precipitated with the anti-ADA5 serum but not the preimmune serum. Moreover, ADA3 is not precipitated from a Δ*ada5* extract. Also shown are Western blots with 20 μg of starting material for each extract.

strain was also fractionated, clearly showing that the indicated band is ADA5 because it was absent in the mutant.

To obtain more direct evidence for the association between ADA3 and ADA5, the 600 mM fraction from the Bio-Rex column was immunoprecipitated with anti-ADA5 or preimmune serum. As a control, this immunoprecipitation was also performed with the 600 mM fraction of the *ada5* deletion extract. Western blot analysis of the precipitates with anti-ADA3 (Fig. 6B) shows that ADA3 was precipitated with anti-ADA5 but not preimmune serum. Moreover, ADA3 was not precipitated from the extract missing ADA5. (Note that the lanes labeled starting material were loaded with the same amount of total protein.) The observation that less ADA3 is detected in the Δ*ada5* extract may indicate that less ADA3 is present in the absence of ADA5. We conclude from these findings that ADA5 and ADA3 are physically bound to one another in a complex in yeast extracts.

Identification of *ADA5* as *SPT20*. By comparing sequences, it was determined that *ADA5* is identical to *SPT20* (45), a newly isolated *SPT* gene in the TBP class (12, 22). *spt20* mutants, like the other *spt* mutants of this class, have pleiotropic mating, growth, and sporulation defects. Furthermore, transcription of Ty1 and other yeast genes is compromised. Thus, unlike the other *ada* mutants (29, 45), *ada5* mutants also have an Spt^- phenotype.

Ada^- phenotype in two *SPT15* (TBP) mutants. We examined *spt3*, *-7*, *-8*, *-15*, and *-20* mutants (all in the TBP class), as well as *spt5*, *-6*, and *-4* mutants, for resistance to GAL4-VP16-mediated toxicity (the Ada^- phenotype). As shown in Table 2, the *spt20* mutant (as expected) and the *spt7* mutant were completely resistant to GAL4-VP16. Like *ada5* mutants, *spt7* mutants show reduced activation of the *ADH1* promoter (44), and we cannot rule out the possibility that this is the basis of the resistance. Interestingly, other *spt* mutants, including *spt3* and *spt8* mutants in the TBP class, were sensitive to GAL4-VP16. Roberts and Winston have found that *spt7* and *spt20* have very similar phenotypes, including inositol auxotrophy and slow growth on rich media, that are not shared by *spt8* or *spt3* (45).

Two *spt15* (TBP) alleles, *spt15-21* and *spt15-122*, caused partial resistance to GAL4-VP16 (Table 2). Interestingly, *spt15-21*, the *spt15* allele causing the most resistance, also causes inositol auxotrophy (45). Because of the possible pleiotropic nature of *spt15* mutations, resistance to GAL4-VP16 could be due to reduced levels of the toxic chimera in the mutant strains. To address this issue, levels of GAL4-VP16 in both the *spt15-21* and *spt15-122* strains were determined by gel shift analysis (Fig. 7). Levels in the *spt15-122* strain were indistinguishable from those in the wild type, while levels in the *spt15-21* strain were somewhat higher than those in the wild type. In addition, for reasons that are not clear, the form of the chimera which corresponds to full length (i.e., the unproteolytically cleaved form) is favored in the *spt15-21* extract. Thus, the Ada^- phenotype of these mutants is not due to a reduction in levels of GAL4-VP16. These findings provide the first genetic clue that the ADA functional pathway includes a basal factor, TBP, and they are consistent with in vitro findings (2) (see Discussion).

DISCUSSION

Here we report the cloning and initial characterization of *ADA5*, a novel gene selected by resistance to GAL4-VP16-mediated toxicity. Strains with mutations in *ADA5* display

TABLE 2. Resistance of *spt* mutants to GAL4-VP16-mediated toxicity[a]

Strain	*SPT* genotype	Resistance to GAL4-VP16[b]
Fy3	Wild type	−
Fy363	*spt5-194*	−
Fy137	*spt6-140*	−
L881	*spt3-401*	−
Fy51	*spt3*Δ*203*::*TRP1*	−
Fy963	*spt7*Δ::*LEU2*	++++
Fy463	*spt8-302*::*LEU2*	−
Fy383	*spt15-21*	++
Fy508	*spt15-122*	+
YSMR191	*spt20-61*	++++
YSMR210	*spt20*Δ::*URA3*	++++
YSMR247	*spt4*Δ::*URA3*	−

[a] Strains were transformed with pGAL4-VP16Ura (38) and matching pRS426 vector, plated on SC medium, and scored for growth after 3 days. L881, Fy191, Fy210, and Fy247 were transformed with the *LEU2*-marked plasmids pSB202 (4) and pRS425 because these strains are Ura^+ Leu^-. GAL4-VP16 plasmids with either marker are equally toxic to yeast cells (unpublished data).

[b] −, only pinpoint colonies form, ++++, the size of the colonies expressing GAL4-VP16 is the same as that of the vector controls.

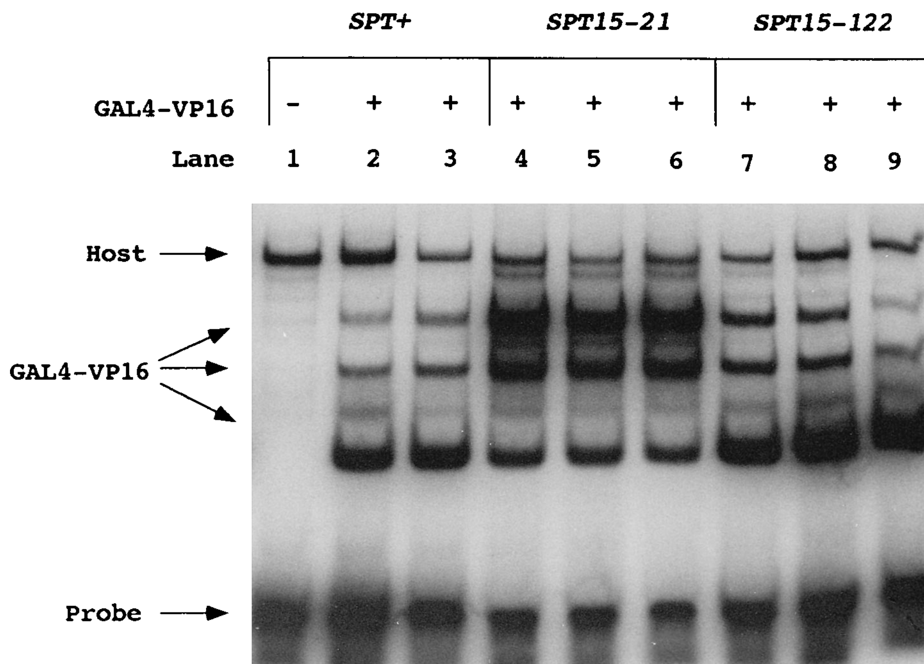


FIG. 7. Levels of GAL4-VP16 are not reduced in *spt15-21* or *spt15-122* mutants. The indicated *SPT*$^+$ or *spt15* strains were transformed with pGAL4-VP16FA a/c, an ARS-CEN plasmid that expresses GAL4-VP16FA from the *ADH1* promoter. Fifty-microgram portions of whole-cell protein extracts were mixed with a radiolabeled GAL4 oligonucleotide probe. Lane 1, which utilized an extract from a strain without GAL4-VP16, shows complexes due to host proteins (host). GAL4-VP16 complexes are indicated (4).

some phenotypes that are distinct from those of strains with mutations in the previously described genes *ADA2*, *ADA3*, and *GCN5*, which arose from the same selection. First, Δ*ada5* strains show reduced levels of GAL4-VP16, unlike *ada2*, *ada3*, or *gcn5* deletion strains. Second, *ada5* mutants grow more slowly on rich and minimal media than other *ada* mutants. Third, *ada5* strains are inositol auxotrophs and have Spt$^-$ phenotypes. Fourth, promoters that function independently of *ADA2*, such as UAS1 and *ADH1*, are dependent on *ADA5*. The effects on the *ADH1* promoter account for the reduction in GAL4-VP16 levels.

Although *ADA5* appears to be distinct from the other *ADA* genes, some phenotypes suggest they may all function in the same pathway. The activators that require *ADA2* for activation are also dependent on *ADA5*. GCN4-mediated activation, which is highly *ADA2* dependent (40), is also *ADA5* dependent. HAP4- and GAL4-mediated activation, which are only slightly *ADA2* dependent (40), are less *ADA5* dependent than most of the other activators tested (Table 1, activities of UAS2 and SD5). Moreover, double mutants with mutations in *ada5* and either *ada2* or *ada3* have growth phenotypes no more severe than those of *ada5* single mutants. If the ADA2 complex and ADA5 work through different activation pathways, then we would expect these double mutants to have a more severe growth defect than either of the single mutants. These genetic data suggest that *ADA5* and *ADA2-ADA3-GCN5* function in the same pathway of transcriptional activation in cells.

Biochemical studies provide direct evidence that ADA5 is a part of the ADA complex. Fractionation of a yeast extract on a Bio-Rex 70 column shows cofractionation of ADA5 and ADA3. Further, immunoprecipitation of ADA5 coprecipitates ADA3. If an extract from a Δ*ada5* strain is used as a control in this experiment, no ADA3 is precipitated.

***ADA5* as an *SPT* gene.** The more general effects of *ada5* mutations on transcription are underscored by its identity with *SPT20* (45). It is intriguing that this gene influences promoter selection when two promoters are in competition, as well as transcriptional activation (as do other *ADA* genes). The identity of *ADA5* and *SPT20* prompted us to examine a panel of *SPT* mutants for the Ada$^-$ phenotype, i.e., resistance to toxicity by overexpressed GAL4-VP16. *spt7* mutants were also resistant, and, like *spt20* mutants, their resistance was complete. Other *spt* mutants, including *spt3* and *spt8* mutants, in the TBP phenotypic class were sensitive. These findings suggest the possibility that SPT7 and SPT20 function together in the transcription process, a surmise also indicated by the findings of Roberts and Winston (45).

***SPT15* (TBP) mutants show resistance to GAL4-VP16.** We also observed that two *spt15* mutants were partially resistant to GAL4-VP16. This result provided the first possible genetic link between the ADA pathway of transcriptional activation and a basal factor, namely, TBP. To further characterize this finding, we showed that the resistance of these *spt15* mutants did not arise from a reduction in levels of the toxic chimera. The similarity in phenotypes between *spt7*, *ada5*, and *spt15* alleles, including inositol auxotrophy and toxicity resistance, suggests that the target of the ADA activation pathway could be TBP. Our genetic findings are consistent with the earlier biochemical findings of Barlev et al. (2), who also proposed that ADA2-ADA3-GCN5 may contact the basal machinery by binding to TBP. They found that TBP in yeast extracts from a wild-type strain bound to a VP16 column, while TBP from *ada2* mutant extracts did not.

We suggest a pathway which leads from the activation domain of transcriptional activators to a complex, ADA2-ADA3-GCN5-ADA5, and to TBP. Several findings are consistent with this model. First, ADA2 has been shown to bind to the activation domains of VP16 (2, 52), GCN4 (2), and ADR1 (8). We show here that ADA5 binds to VP16 and that amino acid substitutions in VP16 can abolish this binding. Second, *ada5*

(and *spt7*) mutations can affect promoter selection (i.e., the mutants have Spt$^-$ phenotypes), suggesting that TBP may be a target of the ADA complex. Third, mutations in *SPT15* (TBP) can give rise to partial resistance to GAL4-VP16. This finding, along with the earlier biochemical data (2), also indicates that TBP might be the ultimate target of ADA-mediated activation.

How can *ada5* mutants share some phenotypes with *ada2*, *ada3*, and *gcn5* mutants yet exert a broader range of effects? We suggest the possibility that ADA5 (and possibly SPT7) retains a partial activity in the absence of ADA2, ADA3, and GCN5. This activity may suffice in *ada2* mutants for transcription of *ADH1*, *INO1*, and perhaps other genes. Deletion of *ADA5*, in this model, would destroy all activity of the complex and give rise to a broader spectrum of phenotypes (i.e., Spt$^-$, Ino$^-$, and slower growth).

In summary, we have presented the analysis of a novel gene, *ADA5*, that shares phenotypes of two broad classes of yeast genes affecting transcription, the *ADA* genes and the *SPT* genes. It will be interesting to see how this genetic connection between these different classes of factors reflects the physical arrangement of the coactivators at promoters.

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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. **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.
7. **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.
8. **Denis, C.** 1996. Personal communication.
9. **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.
10. **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.
11. **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.
12. **Eisenmann, D. M., C. Dollard, and F. Winston.** 1989. *SPT15*, the gene encoding the yeast TATA binding factor TFIID, is required for normal transcription initiation *in vivo*. Cell **58:**1183–1191.
13. **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.
14. **Gansheroff, L., C. Dollard, P. Tan, and F. Winston.** 1995. The *Saccharomyces cerevisiae SPT7* gene encodes a very acidic protein important for transcription *in vivo*. Genetics **139:**523–536.
15. **Ge, H., and R. Roeder.** 1994. Purification, cloning, and characterization of a human coactivator, PC-4, that mediates transcriptional activation of class II genes. Cell **78:**513–523.
16. **Gietz, D., A. St. Jean, R. A. Woods, and R. H. Schiesti.** 1992. Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:**1425.
17. **Goodrich, J. A., T. Hoey, C. J. Thut, A. Admon, and R. Tjian.** 1993. Drosophila TafII 40 interacts with both a VP16 activation domain and the basal transcription factor TFIIB. Cell **75:**519–530.
18. **Guarente, L.** 1992. Mechanism and regulation of transcriptional activation in eukaryotes: conserved features from yeast to humans, p. 1007–1036. *In* S. L. McKnight and K. R. Yamamoto (ed.), Transcriptional regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
19. **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.
20. **Guarente, L., and T. Mason.** 1983. Heme regulates transcription of the *CYC1* gene of S. cerevisiae via an upstream activation site. Cell **32:**1279–1286.
21. **Guthrie, C., and G. R. Fink (ed.).** 1991. Methods in enzymology, vol. 194. Guide to yeast genetics and molecular biology. Academic Press, Inc., San Diego, Calif.
22. **Hahn, S., S. Buratowski, P. A. Sharp, and L. Guarente.** 1989. Isolation of the gene encoding the yeast TATA binding protein TFIID: a gene identical to the SPT15 suppressor of Ty element insertions. Cell **58:**1173–1181.
23. **Harlow, E., and D. Lane.** 1988. Antibodies: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Haviv, I., D. Vaizel, and Y. Shaul.** 1995. The X protein of hepatitis B virus coactivates potent activation domains. Mol. Cell. Biol. **15:**1079–1085.
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. 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:**498–502.
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. **Hoey, T., B. D. Dynlacht, M. G. Peterson, B. F. Pugh, and R. Tjian.** 1993. Molecular cloning and functional analysis of Drosophila TAF110 reveal properties expected of coactivators. Cell **72:**247–260.
29. **Horiuchi, J., and L. Guarente.** 1996. Unpublished data.
30. **Horiuchi, J., N. Silverman, G. 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.
31. **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.
32. **Kim, Y. J., S. Björklund, Y. Li, M. H. Sayre, and R. D. Kornberg.** 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:**599–608.
33. **Koleske, A. J., S. Buratwoski, 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.
34. **Koleske, A. J., and R. A. Young.** 1994. An RNA polymerase II holoenzyme responsive to activators. Nature (London) **368:**466–469.
35. **Kretzschmar, M., K. Kaiser, F. Lottspeich, and M. Meisterernst.** 1994. A novel mediator of class II gene transcription with homology to viral immediate-early transcriptional regulators. Cell **78:**525–534.
36. **Kwok, R. P. S., J. R. Lundblad, J. C. Chrivia, J. P. Richards, H. P. Bachinger, R. G. Brennan, S. G. E. Roberts, M. R. Green, and R. H. Goodman.** 1994. Nuclear protein CBP is a coactivator for the transcription factor CREB. Nature (London) **370:**223–226.
37. **Lin, Y. S., and M. R. Green.** 1991. Mechanism of action of an acidic transcriptional activator *in vitro*. Cell **64:**971–981.
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. **Neigeborn, L., and M. Carlson.** 1984. Genes affecting the regulation of *SUC2* gene expression by glucose repression in *Saccharomyces cerevisiae*. Genetics **108:**845–858.
40. **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.
41. **Pugh, B. F., and R. Tjian.** 1990. Mechanism of transcriptional activation by Sptl: evidence for coactivators. Cell **61:**1187–1197.
42. **Regier, J. L., F. Shen, and S. J. Triezenberg.** 1993. Pattern of aromatic and

hydrophobic amino acids critical for one of two subdomains of the VP16 transcriptional activator. Proc. Nat. Acad. Sci. USA **90:**883–887.

43. **Riles, L., J. E. Dutchik, A. Baktha, B. K. McCauley, E. C. Thayer, M. P. Leckie, V. V. Braden, J. E. Depke, and M. V. Olson.** 1993. Physical maps of the six smallest chromosomes of *Saccharomyces cerevisiae* at a resolution of 2.6 kilobase pairs. Genetics **134:**81–150.
44. **Roberts, S., and F. Winston.** 1996. Personal communication.
45. **Roberts, S., 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 β-galactosidase from overlapping coding sequences in yeast. J. Mol. Biol. **170:**883–904.
47. **Santangelo, G. M., J. Tornow, C. S. McLaughlin, and K. Moldave.** 1988. Properties of promoters cloned randomly from the *Saccharomyces cerevisaie* genome. Mol. Cell. Biol. **8:**4217–4224.
48. **Sauer, F., S. K. Hansen, and R. Tjian.** 1995. Multiple TAF_{II}s directing synergistic activation of transcription. Science **270:**1783–1788.
49. **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.
50. **Scafe, C., D. Chao, J. Lopes, J. P. Hirsch, S. Henry, and R. Young.** 1990. RNA polymerase II C-terminal repeat influences response to transcriptional enhancer signals. Nature (London) **347:**491–494.
51. **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.
52. **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.
53. **Silverman, N., and L. Guarente.** 1996. Unpublished data.
54. **Stern, M. J., R. E. Jensen, and I. Herskowitz.** 1984. Five *SWI* genes are required for expression of the *HO* gene in yeast. J. Mol. Biol. **178:**853–868.
55. **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.
56. **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.
57. **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.
58. **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.
59. **Winston, F., D. T. Chaleff, B. Valent, and G. R. Fink.** 1984. Mutations affecting Ty-mediated expression of the *HIS4* gene of *Saccharomyces cerevisiae*. Genetics **107:**179–197.