In eukaryotes, several factors that are important in the activation of transcription by RNA polymerase II are in large, heteromeric complexes. For example, the yeast SWI2/SNF2, SWI1, SWI3, SNF5, and SNF6 proteins form a large multisubunit complex, which apparently contains repression by chromatin (4, 5, 7, 23). Mutations in SWI2 and SNF5 result in decreased transcription and altered chromatin structure at certain promoters (19). These phenotypes can be suppressed by mutations in histone genes. In another case, the yeast SRB2, SRB4, SRB5, and SRB6 proteins form a holoenzyme complex with RNA polymerase II and certain basal transcription factors (21). The SRB proteins interact with the carboxyl-terminal domain of the largest subunit of RNA polymerase II and are important for both basal and activated transcription in vitro (33). In higher eukaryotes, the TFIIID complex is composed of TATA-binding protein (TBP) and TBP-associated factors (TAFs) (9). While TBP with the other basal factors is sufficient for basal transcription, the TAFs are required for activated transcription (12, 13). Thus, the TAFs are proposed to be coactivators or adaptors required to mediate the stimulatory signal from activators to basal factors. There is also evidence that a family of factors interact with TBP in yeast cells (10, 25, 34).

In addition to the TAFs, other factors, such as the yeast ADA2, ADA3, and GCN5 gene products, have been proposed to be coactivators (3, 22, 24). Mutations in ADA2, ADA3, and GCN5 were isolated in a selection for mutants which confer resistance to toxicity from overexpressed GAL4-VP16. This toxicity is postulated to occur by titration of basal transcription factors away from productive transcription complexes by the strong acidic activation domain of GAL4-VP16 (2). If this titration by GAL4-VP16 requires proteins with coactivator or adaptor function, alterations in these proteins should cause resistance to toxicity. Interestingly, GCN5 had been isolated previously as a gene encoding a transcription factor necessary for full activity of the activator GCN4 (11). ADA2, ADA3, and GCN5 mutants share several phenotypes (3, 22, 24). Strains disrupted for any of the three genes display temperature-sensitive growth as well as a severe growth defect on minimal media. Double mutants between any two of the three genes do not have a more severe slow-growth phenotype than the single mutants. Also, all three genes are required for full transcriptional activity of a similar subset of activators. GAL4-VP16 and GCN4 are dependent on ADA2, ADA3, and GCN5 activity, whereas other activators such as HAP4 and GAL4 are independent or only slightly dependent.

Because of the similar phenotypes between mutations in ADA3, ADA2, and GCN5, we wanted to determine whether their gene products interacted in a complex. In this study, we demonstrate that a trimeric complex is indeed formed by ADA3, ADA2, and GCN5. We further characterize ADA3 and show that it consists of two separable functional domains, both of which are required for function and one of which interacts in the adaptor complex. A model for the structure and function of the ADA complex is proposed.
RESULTS

ADA3 has two functional domains. A strain deleted of ADA3 has a severe slow-growth phenotype on minimal media (24). A wild-type copy of ADA3 or a truncated allele of ADA3, lacking the first 214 codons of the 702-codon gene, fully complements the deletion (Fig. 1A and C). Expression of either the amino-terminal half of ADA3 [ADA3(1–346)] or the carboxy-terminal half of ADA3 [ADA3(364–702)] does not allow complementation. However, when both nonoverlapping clones, ADA3(1–346) and ADA3(364–702), are expressed in a Δada3 strain simultaneously, growth is fully complemented. These results suggest an unusual interaction between two nonoverlapping domains of ADA3. We propose two possible models to explain this observation. First, the amino-terminal domain and the carboxy-terminal domain of ADA3 may independently fold into functional units that do not need to interact with each other, or second, the amino-terminal domain and carboxy-terminal domain may interact with each other without having to be covalently linked.

A Δada3 strain transformed with two plasmids, one expressing a truncated amino-terminal domain, ADA3(214–346), and the other expressing the full carboxy-terminal domain, ADA3(364–702), grows as well as a wild-type strain (Fig. 1B and C). Growth is restored to an intermediate extent when the Δada3 strain is transformed with plasmids expressing a truncated carboxy-terminal domain, ADA3(452–702), and the full-length amino-terminal domain, ADA3(1–346). However, when both the truncated amino-terminal domain and the truncated carboxy-terminal domain are expressed, no complementation is evident. This type of interaction can be thought of as a synthetic phenotype and suggests that both domains of ADA3 interact in the same pathway, i.e., do not have completely separate functions.

Both the amino-terminal and carboxy-terminal domains of ADA3 are required to complement the defect in transcription of a Δada3 strain. We assayed two lacZ reporters in wild-type and Δada3 strains expressing various ADA3 constructs. p14×2His contains lacZ under the control of two synthetic GCN4 binding sites upstream of a minimal CYC1 promoter. pRBH1 contains a single LexA binding site upstream of a CYC1 minimal promoter and was transformed in combination with plexA-GCN4, which expresses a fusion consisting of residues 1 to 202 of LexA fused to residues 9 to 172 of GCN4, or plexA202, which expresses residues 1 to 202 of LexA alone. As shown in Table 1, the activity measured from the p14×2His reporter is reduced 6-fold in a Δada3 strain compared with the wild type, and the activity of LexA-GCN4 is reduced 18-fold. Expressing only the amino-terminal domain or only the carboxy-terminal domain has no effect on this transcription defect, whereas expressing both domains together restores levels to that observed in the presence of full-length ADA3. Thus, GCN4 and LexA-GCN4 seem to require both domains of ADA3 for full activity.

LexA-ADA3 activity is ADA2 dependent. When ADA2 is fused to a LexA moiety, it can activate transcription from reporters containing LexA binding sites (29; also see Table 3 controls). This activity is reduced 3.5-fold in a Δada3 strain. Furthermore, LexA-ADA2 activity can be hyperstimulated when ADA3 is overexpressed. One explanation for this hyperstimulation is that ADA2 and ADA3 interact in a heteromeric complex. lexA-ADA2 is expressed from the strong ADH1 promoter on a high-copy-number plasmid. Thus, there is a large excess of LexA-ADA2 compared with ADA3. If a complex of LexA-ADA2 and ADA3 is required for activity from a lexA operator, overexpression of ADA3 should lead to more complexes and a hyperstimulation of activity.
The dependence of LexA-ADA2 upon ADA3 for activity from LexA sites prompted us to determine whether LexA-ADA3 can also activate transcription and, if so, whether this activation requires ADA2. As shown in Table 2, LexA-ADA3 activates transcription to a similar level as LexA-ADA2. This activation is dependent upon ADA2 to the same extent as LexA-ADA2 activation is dependent upon ADA3. Finally, LexA-ADA3 activity can be hyperstimulated by overexpressing ADA2 in a manner identical to LexA-ADA2 activity being hyperstimulated by excess ADA3. This mutual dependence that each ADA has for the other in terms of LexA activity is consistent with a model in which ADA2 and ADA3 form a heteromeric complex. Combined with the results described later that ADA3 interacts with ADA2 in vitro, this finding provides supporting evidence that a ADA3-ADA2 heteromeric complex exists in vivo.

The carboxyl-terminal domain of ADA3 alone can activate LexA-ADA2. We wanted to determine whether the amino-terminal domain, the carboxyl-terminal domain, or both together were required for the stimulation of LexA-ADA2 activity by ADA3. As shown in Table 3, overexpressing the amino-terminal domain of ADA3 alone has no effect on LexA-ADA2 activity in both wild-type and Δada3 strains. However, overexpressing the carboxyl-terminal domain of ADA3 alone stimulates LexA-ADA2 activity in wild-type and Δada3 strains seven- and fourfold, respectively. In wild-type cells, this stimulation is even greater than that by full-length ADA3. In Δada3 cells, stimulation by the carboxyl-terminal domain is observed but is not as great as with full-length ADA3. These data support the model that the carboxyl-terminal domain is the region of ADA3 responsible for complexing with ADA2 in vivo.

The carboxyl-terminal domain of ADA3 interacts with ADA2 via far-Western analysis. To demonstrate an interaction between the ADA3 carboxyl-terminal domain and ADA2 biochemically, far-Western experiments were performed. In Fig. 2A, purified recombinant DHFR, ADA3(452–702), and ADA3(364–702), as well as an extract from E. coli expressing GST, were run on denaturing gels and either stained with Coomassie blue or transferred to nitrocellulose. The nitrocel-
The carboxyl-terminal domain of ADA3 can coimmunoprecipitate ADA2 in vitro. In a further attempt to demonstrate the interaction between the ADA3 carboxyl-terminal domain and ADA2, communoprecipitation experiments with in vitro-translated products were performed (Fig. 3). Two forms of ADA3[452–702], one tagged with an HA epitope and one lacking the epitope, were cotranslated with either ADA2 or a negative control protein, luciferase. The translation products were immunoprecipitated with a monoclonal antibody to the HA epitope. Lanes 1 to 4 show the cotranslation products, and lanes 5 to 8 show the immunoprecipitation products. As seen in lane 7, ADA2 clearly coprecipitates with ADA3, whereas luciferase does not (lanes 3 and 6). Neither ADA2 nor luciferase is recognized nonspecifically by the HA antibody (lanes 4 and 5), and neither ADA2 nor ADA3[452–702] is precipitated when ADA3 is untagged (lanes 1 and 8). This result in combination with the far-Western results argues strongly for a specific interaction between ADA2 and the carboxyl-terminal domain of ADA3.

GCN5 binds to the ADA3-ADA2 complex. As mentioned earlier, a third gene, GCN5, was also isolated from the adaptor screen. We wanted to determine whether GCN5 might also bind to the ADA2-ADA3 complex. Thus, the three proteins, ADA3[214–702], ADA2, and GCN5, were cotranslated in vitro. As described above, two forms of ADA3 were used, one tagged with the HA epitope and one untagged (Fig. 4, lanes 3 and 4). When immunoprecipitated with a monoclonal antibody directed against the HA epitope, ADA2 and GCN5 were coprecipitated with tagged ADA3, while none of the proteins were precipitated when ADA3 was untagged (lanes 1 and 2). Furthermore, antibody directed against ADA2 could immunoprecipitate both GCN5 and both forms of ADA3 (lanes 5 and 7). Preimmune serum failed to precipitate any of the three proteins (lane 6). Thus, GCN5 binds to either ADA2 or ADA3 or both.

Both the ADA3 carboxyl-terminal domain and GCN5 bind to ADA2 to form a trimeric complex. Figure 4 demonstrates an interaction among ADA2, ADA3[214–702], and GCN5. However, it does not address the question of which proteins make direct contact or whether all three comprise a single complex. To address this issue, all three proteins, ADA3[452–702]HA, ADA2, and GCN5, as well as each combination of two proteins were cotranslated. ADA3[452–702]HA was also translated alone as a negative control. As shown in Fig. 5, lane 7, when all three proteins are cotranslated, all three are precipitated with an anti-HA epitope monoclonal antibody. When ADA2 and ADA3[452–702]HA are cotranslated, ADA2 is coimmunopre-
precipitated with ADA3. However, when GCN5 is cotranslated with ADA3(452–702)HA in the absence of ADA2, only ADA3 is precipitated. Further, GCN5 is not precipitated with the amino-terminal domain, ADA3(1–346) (data not shown). This result indicates that there is no direct interaction between ADA3 and GCN5. These findings suggest that ADA2, ADA3, and GCN5 form a trimeric complex, with ADA2 serving as a linchpin. Consistent with this view, it has been shown that ADA2 and GCN5 can form a complex in the absence of ADA3 (22).

**DISCUSSION**

In this report, we demonstrate the formation of an ADA2-ADA3-GCN5 complex in vitro and begin a structural dissection of this complex. It is now emerging that several factors that are generally important in transcription, such as TFIID (9), the SRBs (21), and the SWI/SNF complex, are heteromeric complexes (23). Also, the similarity in phenotypes of mutations in several SPT genes suggests that their products might exist in a complex (31). Two domains in ADA3. Two nonoverlapping segments of the ADA3 gene, one amino terminal and the other carboxyl terminal, work together to complement defects in a Δada3 strain. Expression of both domains of the protein restores wild-type

**FIG. 2.** Far-Western experiments of ADA2 and ADA3. (A) An extract from *E. coli* expressing GST as well as purified recombinant ADA3(580–702), ADA3(452–702), and DHFR were subjected to SDS-PAGE and either stained with Coomassie blue or transferred to nitrocellulose, denatured, stepwise renatured, and probed with [35S]methionine-labeled ADA2. The 37-kDa band in the lane labeled ADA3(452–702) represents full-length ADA3(452–702). Smaller bands result from amino-terminal degradation, since the fragment is expressed with a carboxyl-terminal six-histidine fusion and purified from a nickel column. For the gel transferred to nitrocellulose and probed with radiolabeled ADA2, extract from 5 × 10⁷ cells expressing GST and 5 μg each of ADA3(580–702), ADA3(452–702), and DHFR were loaded. For the Coomassie blue-stained gel, extract from 10⁷ cells expressing GST, 1 μg of ADA3(580–702), 400 ng of ADA3(452–702), and 0.5 μg of DHFR were loaded. (B) *E. coli* extracts expressing ADA2, vector (pUH24.2ΔCAT), GST, and purified recombinant DHFR were subjected to SDS-PAGE. As above, the gels were either stained or transferred to nitrocellulose. The nitrocellulose blot was probed with [35S]-labeled ADA3(452–702). The 50-kDa band represents ADA2, and the smaller band is a degradation product of ADA2 (data not shown). Extracts from 2 × 10⁷ cells expressing ADA2, vector, or GST and 20 μg of purified DHFR were loaded on both gels.

**FIG. 3.** Coimmunoprecipitation experiments of ADA2 with the C-terminal domain of ADA3. Combinations of HA epitope-tagged or untagged ADA3(452–702), ADA2, and lucerase were cotranslated in an in vitro reticulocyte lysate system. Lanes 1 to 4 show SDS-PAGE analysis of the cotranslated products. The cotranslated products were immunoprecipitated (IP) with antibody (ab) directed against the HA epitope and analyzed by SDS-PAGE (lanes 5 to 8). The lower-molecular-weight bands in lanes 6 and 7 seem to be a degradation product of ADA3(452–702)HA.

**FIG. 4.** Coimmunoprecipitation experiments of ADA2, ADA3, and GCN5. ADA2, GCN5, and either HA epitope-tagged or untagged ADA3(214–702) were cotranslated (lanes 3 and 4). Lanes 1 and 2 show anti-HA antibody (ab)-immunoprecipitated (IP) products; lanes 5 and 7 show complexes immunoprecipitated with anti-ADA2 antibody, and lane 6 shows products immunoprecipitated by preimmune serum.
immunoprecipitated (IP) with anti-HA antibody (ab). Lanes 1 to 5 show cotranslation products; lanes 6 to 10 show products from the translation mix, the HA-tagged ADA3 carboxyl-terminal domain is unable to precipitate GCN5. This finding indicates that there is no direct contact between ADA3 and GCN5 and that GCN5 is recruited to the complex by ADA2. This model proposes that ADA2 is the linchpin in the complex, binding to both ADA3 and GCN5 (Fig. 6). An alternative explanation for our findings is that the conformation of ADA3 is altered when it binds to ADA2, allowing it to make direct contact with GCN5. In a separate analysis of GCN5, however, we found that it can bind directly to ADA2 (22). Therefore, we conclude that the simplest model from our data is that ADA2 is the linchpin in the trimeric complex.

Function of the amino-terminal domain—assembly of a heterotrimeric complex. Our in vitro experiments show that ADA2, ADA3, and GCN5 form a trimeric complex. This complex was first demonstrated by immunoprecipitation of cotranslated ADA2, GCN5, and an HA epitope-tagged version of ADA3 containing both the amino- and carboxyl-terminal domains. Precipitation of this translation mix with either a monoclonal antibody to HA or an antiserum to ADA2 brings down all three proteins. What region of ADA3 is required for formation of this complex? The carboxyl-terminal domain is clearly sufficient for assembly of the trimeric complex, as demonstrated by the following assays. First, this domain binds to ADA2 when the latter has been transferred to nitrocellulose in a far-Western experiment. Second, ADA2 binds to the carboxyl-terminal domain of ADA3 in the converse far-Western experiment. Third, HA antibody precipitates the three proteins in a cotranslation of ADA2, GCN5, and the HA-tagged carboxyl-terminal domain of ADA3. The region of ADA3 that is functional in these assays, residues 452 to 702, is partially active in the complementation experiments in vivo (Fig. 1B).

Architecture of the ADA complex. What are the binary protein-protein contacts that hold ADA2, ADA3, and GCN5 together? In an important experiment, when ADA2 is omitted from the translation mix, the HA-tagged ADA3 carboxyl-terminal domain is unable to precipitate GCN5. This finding indicates that there is no direct contact between ADA3 and GCN5, indicating that there is no direct contact between ADA3 and GCN5. Our in vitro experiments also show that ADA2, ADA3, and GCN5 form a trimeric complex. This complex was first demonstrated by immunoprecipitation of cotranslated ADA2, GCN5, and an HA epitope-tagged version of ADA3 containing both the amino- and carboxyl-terminal domains. Precipitation of this translation mix with either a monoclonal antibody to HA or an antiserum to ADA2 brings down all three proteins. What region of ADA3 is required for formation of this complex? The carboxyl-terminal domain is clearly sufficient for assembly of the trimeric complex, as demonstrated by the following assays. First, this domain binds to ADA2 when the latter has been transferred to nitrocellulose in a far-Western experiment. Second, ADA2 binds to the carboxyl-terminal domain of ADA3 in the converse far-Western experiment. Third, HA antibody precipitates the three proteins in a cotranslation of ADA2, GCN5, and the HA-tagged carboxyl-terminal domain of ADA3. The region of ADA3 that is functional in these assays, residues 452 to 702, is partially active in the complementation experiments in vivo (Fig. 1B).

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Model of the ADA complex. Figure 6 shows two parallel pathways, one direct and the other ADA dependent, that connect activators to basal factors. Because LexA-GCN4 still has a low level of activity in the absence of the ADA genes, we envision that an ADA-independent pathway may act in concert with an ADA-dependent pathway for activation. However, at this point, it is unclear whether the final target of the ADA complex is the basal factors or some other target such as nucleosomes. We favor the idea that the ADAs interact with basal factors since the transcriptional defect of a strain mutant for ADA2, ADA3, and GCN5 comprise a single complex will allow us to relate structural domains that reside in different subunits to a common function. ADA2 contains a Cys-rich domain that is conserved in the mammalian factor CBP(3). The latter protein has been proposed to be a coactivator because it binds to the transcription factor CREB and potentiates its activity (6). GCN5 contains a bromo domain, which is found in several factors in other important transcription complexes, such as TAF250, SW2, SPT7, and others (17, 32). The bromo domain has been shown to be important in GCN5 function (22) and may exemplify some common function that all of these complexes share.

The ADA genes were isolated from S. cerevisiae and are required for activator-dependent transcription for a subset of activators including GCN4 and GAL4-VP16. We show in this report that at least three of the ADA proteins form a heteromeric complex. Combined with the observation that the ADA complex binds to activators (29), we believe that we have identified a complex recruited by activators to help transcriptional activation.

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