Identification of Native Complexes Containing the Yeast Coactivator/Repressor Proteins NGG1/ADA3 and ADA2*

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NGG1p/ADA3p and ADA2p are dual function regulators that stimulate or inhibit a set of yeast transcriptional activator proteins. In vitro, NGG1p and ADA2p associate in a complex that also contains GCN5p (Horiiuchi, J., Silverman, N., Marcus, G. A., and Guarente, L. (1995) Mol. Cell. Biol. 15, 1203–1209). We have found that NGG1p and ADA2p are coimmunoprecipitated from yeast whole cell extracts. In fact, <2% of cellular ADA2p was not associated with NGG1p. Also in agreement with their association in vivo, the stability of ADA2p and NGG1p depended on the presence of each other. In addition, three NGG1p- and ADA2p-containing peak fractions were resolved by Q-Sepharose Fast Flow ion-exchange chromatography of whole cell extract. The presence of another high molecular mass complex was supported by the separation of one of the NGG1p- and ADA2p-containing peak fractions by gel-filtration chromatography. Together, the combination of ion-exchange and gel-filtration chromatography suggests a total of four complexes, two with sizes of 2 MDa and single complexes of ~500 and 200 kDa. At least one of these complexes is found to associate with the TATA-binding protein (TBP) since TBP was present in immunoprecipitates with NGG1p. The association of TBP with the ADA proteins required amino acids 274–307 of NGG1p, a region of NGG1p required for activity. This supports a role for NGG1p in the interaction with TBP and suggests that the interaction with TBP is functionally relevant.

Activated transcription by RNA polymerase II requires the action of the basal transcriptional machinery, sequence-specific activator proteins, and coactivator or mediator proteins (1). Coactivators positively influence activator function either by providing a regulatory interface between the basal machinery and the activator protein or by enabling these components to contend with a DNA template in the form of chromatin. Coactivators have generally been found as components of regulatory complexes. The TBP–TBP-associated factor complex, the RNA polymerase II holoenzyme complex, and the Swi/Snf complex represent principal examples (reviewed in Refs. 2–4). The mechanisms and component structure of these complexes may overlap as suggested by the finding of Swi/Snf components (5) within the RNA polymerase II holoenzyme (6), the TBP-associated factor complex, and transcription factor IIF (7).

Probably through related mechanisms, coactivator complexes can also be involved in repression. We initially discovered NGG1 based on its requirement for full inhibition of the GAL4 activator protein in glucose media (8). NGG1p likely acts as a more general repressor because transcriptional activation by the carboxyl-terminal activation domain of PDR1p is enhanced in a nsg1 background (9). Guarente and co-workers (10) independently isolated NGG1/ADA3 based on suppression of the toxicity of overexpression of VP16 in yeast. Mutations within four ADA genes, ADA2, NGG1/ADA3, GCN5/ADA4, and ADA5, suppress the toxicity of VP16 by inhibiting its ability to activate transcription (11, 12). These genes are also required for transactivation by GCN4p (11–13); in fact, GCN5p had been identified because it is required for maximal activation by GCN4p (14). Genetic and in vitro biochemical evidence suggests that the ADA proteins likely act in a complex that contains at least ADA2p, NGG1p, GCN5p, and ADA5p (10–13, 15–18). Direct interaction in vitro has been observed between ADA2p and both GCN5p and the carboxyl-terminal 250 amino acids of NGG1p (13, 16). Based on the finding that single and double disruptions of nsg1 and ada2 have similar effects on inhibition of GAL4p and PDR1p, we suggested that the same or related ADA complexes are involved in transcriptional activation and repression (9, 18). Other coactivator complexes also appear to be involved in the positive and negative regulation of transcription. The RNA polymerase II holoenzyme contains components (SIN4p, RGR1p, and ROX3p) that are required for transcriptional repression (19, 20).

The ADA proteins were predicted to interact with the basal transcriptional machinery and perhaps act as a regulatory bridge between activators and this machinery (11). An interaction between the ADA complex and TBP has been demonstrated by affinity chromatography (21). Recent genetic experiments showing that mutations in the gene encoding TBP (SPT15) have partial resistance to overexpression of GAL4p–VP16 (the ADA phenotype) also support a link between the ADA proteins and TBP (17). However, the component(s) of the complex required for the interaction with TBP is unknown. Components of the ADA complex have also been found to interact with activator proteins. PDR1p interacts with the amino-terminal 373 amino acids of NGG1p in a two-hybrid analysis and by coimmunoprecipitation (9). ADA2p in yeast extracts associates with the activation domains of VP16, GCN4p, and GAL4p on affinity columns (21–23). The ability of recombinant ADA2p to interact with VP16 in vitro suggests that ADA2p may have a principal role in these interactions (21), although disruption of ADA2 did not totally abolish the two-hybrid interaction between NGG1p and PDR1p (9).

To determine the functional associations of NGG1p and ADA2p that occur in vivo, we have begun a biochemical analysis of these proteins using epitope-tagged derivatives. We have found that virtually all of cellular ADA2p is associated with another protein liquid chromatography.

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The abbreviations used are: TBP, TATA-binding protein; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis; FPLC, fast protein liquid chromatography.
with NGG1p in multiple high molecular mass complexes. In addition, we have found that TBP coimmunoprecipitates with NGG1p. The significance of the interaction with TBP is suggested by the fact that deletion of amino acids 274–307 of NGG1p results in the slow growth phenotype and loss in repression of GAL4p seen in a nlg1 disruption (18), also results in loss of association with TBP.

**MATERIALS AND METHODS**

**DNA Constructs**

To epitope tag NGG1p, a Nol restriction site was introduced into YCplac33-NGG1 directly upstream of the TAA stop codon (8). An oligonucleotide encoding a triple hemagglutinin (HA) epitope (kindly provided by M. Manolson) was cloned into this site. Yplac211-HA-NGG1 was constructed by ligating a 2.7-kilobase pair Ssal-PstI fragment from this allele (including the NGG1p coding region) with an EcoRI-SalI fragment that contains 2.5 kilobase pairs of sequence upstream of NGG1 into the EcoRI and PstI sites of Yplac211 (24). Yplac211-HA-NGG1

** Yeast Strains, Media, and Growth Conditions**

Yeast strain CY756 (8) is a derivative of KY320 (26) that contains a URA3 disruption. CY946 is isogenic to CY756, but contains a Tn10 LUK disruption of the TRP1 gene. CY946 containing YCp88-HA-ADA2, prepared in 40 mg/ml Tris-HCl (pH 7.4), 150 mM NaCl, 0.1% Tween 20, 10% glycerol, 1 mM EDTA, and 1 mM dithiothreitol in the presence of the protease inhibitors and fractionated on a 100-ml open column of Sephacryl S-500 HR (Pharmacia Biotech Inc.) at a flow rate of 0.2 ml/min. 200-μl aliquots of alternate 1.6-ml fractions were precipitated in the presence of 0.5% trichloroacetic acid, separated by SDS-PAGE, and assayed for the presence of HA-ADA2p and HA-NGG1p by immunoblotting with anti-HA antibody.

**Immunoprecipitation of NGG1p and ADA2p Complexes**

For immunoprecipitation of Myc-NGG1p with HA-ADA2p, whole cell extracts were prepared from yeast strains CY947 containing YCp88-HA-ADA2, CY946 containing YCp88-HA-ADA2, and CY707 in 50 mM HEPES (pH 7.4), 150 mM NaCl, 0.1% Nonidet P-40, and 10% glycerol. Fractions 20–24 (250 mM NaCl eluting peak) were concentrated by 10% trichloroacetic acid, precipitated in the presence of 10% trichloroacetic acid, separated by SDS-PAGE, and assayed for the presence of HA-ADA2p and HA-NGG1p by immunoblotting with anti-HA antibody.

**Western Blotting**

Western blotting with a primary antibody from ascites fluid derived from the Myc1-9E10 cell line (32) was coupled to cyanogen bromide-activated Sepharose (Pharmacia Biotech Inc.). For analysis of TBP in the complexes, whole cell extracts were prepared in SY6-2, SY7-3, and KY320. Immunoprecipitations were performed with 200 μg of extract using monoclonal anti-HA antibody covalently bound to N-hydroxysuccinimide-activated Sepharose (Beckeley Antibody). Protein was eluted from the beads by incubation at 40°C in 4.0 mM urea.
Coimmunoprecipitation of NGG1p and ADA2p from yeast whole cell extract. A, 50 mg of whole cell extract prepared from yeast strains CY947 containing YCp88-HA-ADA2 (myc-NGG1 HA-ADA2, lane 1), CY946 containing YCp88-HA-ADA2 (nga1 HA-ADA2, lane 2), and CY1077 (myc-NGG1 HA-ADA2, lane 3) was incubated with anti-HA antibody and protein A-Sepharose. After washing, protein was eluted by heating at 95 °C in SDS gel loading buffer, separated by SDS-PAGE, and analyzed by Western blotting with anti-Myc antibody to detect native ADA complexes. The bands at ~67 kDa is shown on the right. The band at ~67 kDa is the heavy chain of anti-Myc antibody that serves as a loading control. The migration of molecular mass protein standards (kDa) is shown on the right. The band at ~67 kDa is the heavy chain of anti-Myc antibody that serves as a loading control.

RESULTS

Coimmunoprecipitation of NGG1p and ADA2p—When expressed in vitro, NGG1p interacts with ADA2p (13). Genetic experiments including the similar slow growth phenotype and relief of inhibition on GAL4p shown by single and double mutants also suggest that NGG1p and ADA2p act in a complex (13, 18). To address whether these proteins associate in vivo, we determined whether they can be coimmunoprecipitated from a yeast whole cell extract.

Epitope-tagged derivatives were constructed to immunoprecipitate and identify NGG1p and ADA2p. NGG1p tagged with a Myc epitope at its amino terminus is functional (18). ADA2p was tagged at its carboxyl terminal with a HA epitope (35, 36). This derivative was also functional as determined by its ability to complement ada2 disruptions in restoring wild-type growth rates and repression of GAL4p (data not shown). HA-ADA2p expressed from the DED1 promoter was introduced into yeast strains CY947 (myc-NGG1 ada2) and CY946 (nga1::TRP1 ada2) on a URA3 to centromeric plasmid (YCp88-HA-ADA2). Whole cell extracts were prepared from these strains and from CY1077 (myc-NGG1 ADA2). The extracts containing HA-ADA2p were treated with anti-HA antibody, separated by SDS-PAGE, and Western-blotted with anti-Myc antibody to detect Myc-NGG1p (Fig. 1A). The presence of Myc-NGG1p in immunoprecipitates with HA-ADA2p (lane 1) shows that NGG1p and ADA2p expressed in vivo are associated. The specificity of the association was shown by the absence of a band of ~116 kDa that reacts with anti-Myc antibody in immunoprecipitates from strains lacking HA-ADA2p (lane 3) or Myc-NGG1p (lane 2).

To determine the relative amount of ADA2p associated with NGG1p, we performed an immunodepletion experiment. 600 μg of whole cell extract containing Myc-NGG1p and HA-ADA2p (CY947 containing YCp88-HA-ADA2) was incubated with anti-Myc antibody coupled to cyanogen bromide-activated Sepharose 4B or to Sepharose 4B alone (lanes 1 and 2). The supernatant (Sup; lanes 2 and 3) was removed, and after washing, protein was eluted from the beads by boiling in SDS gel loading buffer (Bound; lanes 1 and 4). Protein in each of the fractions was separated by SDS-PAGE on an 8% gel, and HA-ADA2p was detected by Western blotting with anti-HA antibody. The first two lanes contain 150 μg of whole cell extract from strains lacking or containing HA-ADA2p and HA-NGG1p and serve as negative and positive controls, respectively. These are followed by the column fractions numbered as they were eluted. The positions of HA-ADA2p and HA-NGG1p are labeled. Arrowheads indicate the peak fractions for elution of ADA2p and NGG1p. B, shown are the densitometric scanning profiles of ADA2p (solid line) and NGG1p (dashed line) obtained from the immunoblot shown in A. The relative amounts of ADA2p and NGG1p represent the intensity of each band expressed in integration units. WT, wild type.
of ADA2p and NGG1p was detected by Western blotting with anti-HA antibody (Fig. 2A). Densitometric scanning revealed that NGG1p eluted in four peaks centered at fractions 4 (~50 mM NaCl), 10 (100 mM NaCl), 22 (250 mM NaCl), and 36 (450 mM NaCl) (Fig. 2B). ADA2p cofractionated with NGG1p, with the exception of the 450 mM NaCl eluting fraction. Individually, the 50, 100, and 250 mM NaCl complexes were reapplied to the Fast Q column to ensure that they were chromatographing true. Each NGG1p-containing complex eluted in a single peak at the same position as it had first chromatographed (data not shown). The occurrence of three separable fractions containing both NGG1p and ADA2p suggests that they are associated and found within multiple complexes.

To provide additional support for the association of ADA2p and NGG1p in multiple complexes and to obtain approximate sizes of these complexes, the elution profiles of the tagged derivatives were examined after separation of whole cell extract on gel-filtration columns. Extract containing HA-ADA2p and HA-NGG1p was fractionated on a Superose 6 column in 300 mM NaCl (Fig. 3A). Equal volumes of alternate fractions were examined by SDS-PAGE, and the presence of ADA2p and NGG1p was detected by Western blotting with anti-HA antibody. NGG1p eluted in three peaks centered at fractions 14, 34, and 40. By comparison with protein standards, these fractions correspond to apparent molecular sizes of ~1.5 MDa (this value was obtained by linear extrapolation from the calibration proteins and is an underestimate of its size; see below), 200 kDa, and 90 kDa, respectively. Approximately 5% of NGG1p fractionated at a size indicative of a monomer (90-kDa peak); the amount of this form varied from <3 to 12% in four independent experiments. HA-ADA2p had a virtually identical elution profile to HA-NGG1p, lacking only the peak at fraction 40 and having a slightly more prominent shoulder at fraction 20. A peak corresponding to monomeric ADA2p was not apparent.

To determine the relationship between the putative complexes identified by ion-exchange and gel-filtration chromatography, the Fast Q peak fractions were fractionated on Superose 6 (Fig. 3B). The 50 and 100 mM NaCl eluting fractions from the Fast Q column had apparent sizes of ~1.5 MDa. The 250 mM NaCl fraction was resolved into two complexes containing NGG1p with approximate sizes of 900 and 200 kDa. The 900-kDa peak may represent the shoulder at fraction 20 seen when whole cell extract was fractionated on Superose 6 (see below). The 450 mM NaCl fraction from the Fast Q column contained ADA2p and NGG1p obtained after fractionation of yeast whole cell extract on a Superose 6 column. 16 mg of whole cell extract from strains expressing HA-NGG1p and HA-ADA2p was fractionated on Superose 6. Equal volumes of alternate fractions were separated by SDS-PAGE and assayed for the presence of HA-ADA2p and HA-NGG1p by immunoblotting with anti-HA antibody. The dashed line represents NGG1p; the solid line represents ADA2p. The void volume was determined with high molecular mass DNA and occurs at fraction 10. Arrowheads depict the peak fractions containing the following calibration proteins: thyroglobulin (669 kDa), apoferritin (443 kDa), β-amylase (200 kDa), and bovine serum albumin (66 kDa). Note that the scale for ADA2p has been magnified 2-fold in this graph as compared with Fig. 2. B, densitometric scanning profiles of immunoblots of NGG1p obtained after fractionation of the NGG1p- and ADA2p-containing Fast Q peak fractions on a Superose 6 column. Fast Q fractions 2–5 (50 mM NaCl eluting peak), fractions 9–12 (100 mM NaCl eluting peak), fractions 20–24 (250 mM NaCl eluting peak), and fractions 35–39 (450 mM NaCl eluting peak) were concentrated using a Centricon 30 concentrator and then diluted in extraction buffer to a final concentration of ~300 mM NaCl and a volume of 500 μl. Individually, the concentrated samples were fractionated on Superose 6 as described above. Equal volumes of alternate fractions were precipitated, separated by SDS-PAGE, and assayed for the presence of HA-NGG1p by immunoblotting with anti-HA antibody. The void volume was determined with high molecular mass DNA and occurs at fraction 10. Arrowheads depict the peak fractions containing the calibration proteins with the sizes (kDa) indicated.
the 90-kDa form of NGG1p. The combination of gel-filtration and ion-exchange chromatography allowed us to identify four complexes containing both ADA2p and NGG1p, two with apparent molecular masses of ~1.5 MDa and individual complexes of ~900 and 200 kDa.

To ensure that the high molecular mass ADA complex did not result from the formation of nonspecific aggregates that fractionate near the void volume of the Superose 6 column, we analyzed the elution profile of ADA2p and NGG1p after separation on Sephacryl S-500 (Fig. 4). The void volume was determined with high molecular mass DNA, and the column was calibrated with blue dextran 2000 (Pharmacia Biotech Inc.), thyroglobulin (669 kDa), and b-amylase (200 kDa). Three peak fractions containing NGG1p and ADA2p were observed, corresponding to the 1.5-MDa, 900-kDa, and 200-kDa complexes seen on the Superose 6 column. The 900-kDa complex was resolved from the larger complexes after chromatography on Sephacryl S-500. The high molecular mass complexes were not excluded from the Sephacryl S-500 column and eluted one fraction before blue dextran 2000. Considering the general differences in fractionation of dextrans and globular proteins by gel filtration, this suggests that these complexes have predicted sizes of >2.0 MDa.

Stability of NGG1p and ADA2p Is Co-dependent—The integrity of a protein complex is often critical for the stability of its component members. To determine if the stability of ADA2p was dependent on NGG1p, we expressed HA-ADA2p from the constitutive DED1 promoter (17) in yeast strains containing (CY947) or not containing (CY946) NGG1. The DED1 promoter is not influenced by NGG1, allowing an analysis of protein stability (18). The presence of HA-ADA2p was detected by Western blotting with anti-HA antibody (Fig. 5A). Almost no full-length ADA2p was found in CY946. To further support that NGG1p regulates the stability of ADA2p rather than its expression, we have observed a band of ~10 kDa reacting with anti-HA antibody in the CY946 extract, indicative of a proteolytic fragment of HA-ADA2p (data not shown). We conclude that the stability of ADA2p is highly dependent on NGG1p.

The reciprocal experiment was performed in which Myc-NGG1p was expressed in strains containing or not containing ADA2p. Myc-NGG1p was detected by Western blotting with anti-Myc antibody. As shown in Fig. 5B, little or no NGG1p was detectable in the strain lacking ADA2p, even when 300 µg of total protein was analyzed. The dependence of the stability of ADA2p and NGG1p on the presence of each other again supports their association in vivo.

NGG1p Is a Nuclear Protein—The action of NGG1p as a transcriptional coactivator/repressor predicts that it should be found in the nucleus. ADA2p has been found in nuclear extracts (21); however, cytoplasmic extracts were not examined. Furthermore, ADA2p-independent NGG1p might potentially be involved in a cytoplasmic process. To determine the cellular localization of NGG1p, nuclear and cytoplasmic fractions were prepared from a yeast strain expressing HA-NGG1p and analyzed by Western blotting with anti-HA antibody (Fig. 6A). The integrity of the fractions was examined by reprobing the filter with antibody to the carboxyl-terminal domain of the large subunit of RNA polymerase II (Fig. 6B). Like RNA polymerase II, HA-NGG1p was almost exclusively found in the nuclear fraction. On extensive overloading, trace amounts of HA-NGG1p could be detected in the cytoplasmic fraction.

Amino Acids 274–307 of NGG1p Are Required for Interaction with TBP—Barlev et al. (21) have found that ADA2p contained in nuclear extracts will associate with TBP. Their inability to find a direct interaction for recombinant proteins suggests that the interaction between TBP and ADA2p is indirect. A possible role for NGG1p in the interaction is supported by the finding that amino acids 1–308 of NGG1p, when fused to a GAL4p DNA-binding domain, will activate transcription independently from ADA2p (18). This activation requires amino acids 274–307 of NGG1p. To test for the involvement of NGG1p in

**Fig. 4. Fractionation of ADA complexes on Sephacryl S-500 HR.** Shown are densitometric scanning profiles of immunoblots of ADA2p and NGG1p after fractionation of whole cell extract on a 100-ml open Sephacryl S-500 HR column. 120 mg of whole cell extract containing HA-NGG1p and HA-ADA2p was fractionated at a flow rate of 0.2 ml/min. 200 µl of 1.6-ml alternate fractions was precipitated, separated by SDS-PAGE, and analyzed for the presence of HA-ADA2p and HA-NGG1p by immunoblotting with anti-HA antibody. The solid line represents HA-ADA2p; the dashed line represents HA-ADA2p. The void volume was determined with high molecular mass DNA and occurs at fraction 18. Arrowheads depict the peak fractions containing the calibration markers as indicated.

**Fig. 5. Stability of ADA2p and NGG1p depends on the presence of each other.** A, whole cell extract from yeast strains CY946 containing YCp88-HA-ADA2 (ngg1 HA-ADA2; lanes 1 and 2), CY947 containing YCp88-HA-ADA2 (myc-NGG1 HA-ADA2; lanes 3 and 4), and CY1077 (myc-NGG1 ADA2; lane 5) was separated by SDS-PAGE and analyzed by Western blotting with monoclonal anti-HA antibody to detect HA-ADA2p. B, whole cell extract from yeast strains CY1077 (myc-NGG1 ADA2; lane 1), KY320 (NGG1 ADA2; lane 2), CY947 containing YCp88-HA-ADA2 (myc-NGG1 HA-ADA2; lanes 3 and 4), and CY947 (myc-NGG1 ada2; lanes 5 and 6) was separated by SDS-PAGE and analyzed by Western blotting with anti-Myc antibody to detect Myc-NGG1p. The NG1G1 and ADA2 alleles found in each strain and amount of protein analyzed are indicated at the top of each lane. WT, wild type.
the association of an ADA complex with TBP, we immunoprecipitated HA-NGG1p and HA-NGG1p<sub>p274–307</sub> from whole cell extracts with anti-HA antibody and probed for TBP by Western blotting (Fig. 7A). TBP coimmunoprecipitated with HA-NGG1p (compare lanes 1 and 3). TBP was not found in the immunoprecipitate from the HA-NGG1p<sub>p274–307</sub> extract (lane 2); therefore, amino acids 274–307 of NGG1p are essential for interaction of an ADA complex with TBP.

Although the expression of NGG1p<sub>p274–307</sub> is comparable to the full-length protein (18), the inability of TBP to coimmunoprecipitate with HA-NGG1p<sub>p274–307</sub> could be indirect, resulting from the lack of association of NGG1p<sub>p274–307</sub> with an ADA complex. Therefore, we analyzed for complex formation by HA-NGG1p<sub>p274–307</sub> by determining its elution profile from a Superose 6 column. HA-NGG1p<sub>p274–307</sub> was found in complexes of ∼900 and 200 kDa (data not shown). The largest complex containing the deletion derivative eluted approximately four fractions later than that for the wild-type protein, suggesting that amino acids 274–307 are required for protein-protein contacts necessary in the formation of the largest ADA complexes.

We compared the elution profile of TBP with NGG1p from the Fast Q ion-exchange column as a first step in determining which of the NGG1p- and ADA2p-containing complexes associate with TBP (Fig. 7B). TBP was found in two peaks centered at fractions 4 and 22. The profile of TBP from the Fast Q column paralleled the elution of NGG1p found within one of the two 2-MDa complexes and that contained within the 900-200-kDa peak. Since TBP immunoprecipitates with ADA2p (Fig. 7A), this result indicates that TBP is associated with at least one of these complexes. Furthermore, the absence of TBP in the NGG1p peaks at fractions 12 and 36 indicates that TBP is not associated with one of the two largest ADA complexes or with free NGG1p.

**DISCUSSION**

*NGG1p and ADA2p Are Associated in Multicomponent Complexes*—Previous reports on components of the ADA complex demonstrated that ADA2p, NGG1p, GCN5p, and ADA5p associate in vitro and by two-hybrid analysis (12, 13, 16, 17). The similar effects of single and double disruptions of *ngg1*, *ada2*, and *gcn5* on transcriptional activation and repression have been used to support the functional association of the proteins in vivo (10, 12, 13, 18). The finding of Candau et al. (16) that the stability of ADA2p is dependent on GCN5 and our finding that the stability of ADA2p and NGG1p depends on the presence of each other provide additional evidence for their association in vivo. However, the instability of the proteins does limit the interpretation of previous gene disruption experiments. In this report, we provide biochemical evidence for the functional association of ADA2p and NGG1p. The association of NGG1p and ADA2p was revealed by their coimmunoprecipitation from yeast whole cell extracts. This was supported by the coelution of the proteins in multiple peaks after fractionation on both gel-filtration and ion-exchange columns. It should also be noted that Candau and Berger (38) have shown that the stability of LexA-ADA2p is dependent on the presence of NGG1p and that NGG1p and GCN5p coimmunoprecipitate with epitope-tagged ADA2p when the three proteins are overexpressed in vivo.

The combination of gel-filtration and ion-exchange chroma-
tography resolved four complexes containing both ADA2p and NGG1p (ADA complexes). Two of these had estimated sizes of ~2 MDa; the other two were ~200 and 900 kDa. The simplest explanation for the 200-kDa complex is that it represents a minimum complex containing ADA2p, NGG1p, and perhaps GCN5p. The presence of GCN5p would be consistent with the demonstrated interaction with ADA2p in vitro (13) and its stabilization of LexA-ADA2p (38).

The 2-MDa complexes and the 900-kDa ADA complex from the gel-filtration chromatography almost certainly contain additional NGG1p- and ADA2p-associated proteins. We do not believe that the high molecular mass ADA complexes arise as the result of the interaction of the 200-kDa complex with DNA. This possibility was suggested by the finding that the Swi5/Snf complex interacts with DNA (39). First, the yeast whole cell extract used for the chromatography experiments was treated with protamine sulfate to remove DNA. Second, ADA complexes fractionated on the Fast Q column were subsequently chromatographed on Superose 6 and shown to have approximate sizes of >1.5 MDa, 900 kDa, and 200 kDa. These complexes are almost certainly free of DNA since any residual DNA not removed by protamine sulfate treatment would be retained at low salt concentrations on the cation-exchange column.

Third, we have found the same elution profile on Superose 6 when the extract is chromatographed in the presence of ethidium bromide. 2

There are several potential reasons for the presence of four biochemically resolvable complexes containing ADA2p and NGG1p. Activation and repression may require complexes with different components. Stable subcomplexes (for example, the 200-kDa complex) may represent a core that is an intermediate in the formation of these forms rather than having an independent functional role. NGG1p and ADA2p may also function as components of non-ADA complexes. The finding of TBP in immunoprecipitates with NGG1p might suggest that one of the complexes is a TBP-TBP-associated factor complex; however, the sizes of proteins that coimmunoprecipitate with NGG1p do not resemble the sizes of the yeast TBP-associated factors (40, 41). The finding that ADA5p/SPT20p may associate with NGG1p/ADA3p (17) suggests that some of these complexes may contain the genetically related proteins SPT3, SPT7, and SPT8 (17, 42). While we cannot exclude the possibility that the smaller complexes dissociate from the larger complexes during the isolation procedure, independently, Cote et al. 3 have found similar chromatographically distinct, high molecular mass complexes containing GCN5p and ADA2p. In addition, the high molecular mass complexes did not dissociate after a second chromatography step on either Superose 6 or Fast Q columns.

Amino Acids 274–307 of NGG1p Are Required for Interaction with TBP—As coactivators, the ADA proteins were predicted to provide a regulatory link between the basal transcriptional machinery and activator proteins. Interactions between activators including GCN4p, VP16, GAL4p, and PDR1p with components of the ADA complexes have been observed by affinity chromatography, coimmunoprecipitation, and two-hybrid analysis (9, 21–23). A link between the ADA complex(es) and the basal machinery was established with the finding that ADA2p in nuclear extracts associated with a GST-TBP fusion protein on an affinity column (21) and is supported by the genetic findings that ADA5/SPT20 encodes a protein functionally related to TBP (17, 42). The inability of Barlev et al. (21) to detect an interaction between TBP and recombinant ADA2p suggested that the association might be mediated by another component of the ADA complex(es). Our finding of TBP in immunoprecipitates with NGG1p demonstrates in vivo the interaction between the ADA complex(es) and TBP that was found by affinity chromatography. Furthermore, we have shown that the association with TBP requires amino acids 274–307 of NGG1p. The role of NGG1p in the interaction with TBP is likely indirect since TBP was not found associated with monomeric NGG1p. This result, along with the finding that recombinant ADA2p does not interact with TBP (21) and the identification of GCN5p as a histone acetyltransferase (43), suggests that none of these three ADA components interacts directly with TBP.

The requirement for amino acids 274–307 of NGG1p for association with TBP agrees with the region being crucial for function of the molecule. Deletion of this region results in a loss of repression of GAL4p and in the slow growth phenotype typical of disruption of nng1 (18). Amino acids 1–308 of NGG1p activate transcription as a GAL4p fusion independent of ADA2p (18). This activation depends on amino acids 274–307 (18). Mutations can be isolated in this region that either stimulate or inhibit the action of NGG1p in repression of GAL4p and the activity of GAL4p-NGG1p fusions in transcriptional activation (18). Interestingly, this region contains a Phe-rich segment with homology to a group of proteins including human immunodeficiency virus group-specific antigen protein that strongly predicts to be an amphipathic α-helix (10, 18). An involvement of amino acids 274–307 in protein-protein interactions is also suggested by the finding that this region is required for a two-hybrid interaction with PDR1p (9).

Coactivators in Activation and Repression—NGG1p, ADA2p, and likely other components of the ADA complex(es) fit into a class of regulators that are able to stimulate or repress activator function. The link between activation and repression for NGG1p is particularly strong since a region essential for repression (amino acids 274–307) is also required for transcriptional activation as a GAL4p-NGG1p fusion (18) and for association with TBP. Mechanisms for activation and repression may be closely related. An activator protein may signal an ADA complex to either stimulate or repress the activities of TBP. Differences leading to activation or repression could arise because specific activators elicit different conformational changes in the complex, perhaps by targeting different components or possibly by acting on different ADA complexes. The finding by Brownell et al. (43) that GCN5p is a histone acetyltransferase also suggests that some of the activities of the complex may be mediated by chromatin modification, with others being dependent on interaction with the basal transcriptional machinery.

Other dual function regulators probably function to stimulate or inhibit the basal transcriptional machinery. PAP1p was isolated as a RNA polymerase II-associated protein that differentially activates or represses transcription (44). Recently, SIN4p, ROX3p, and RGR1p, which had previously been characterized with roles in activation and repression, were found as components of the RNA polymerase II holoenzyme (19, 20).

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