NGG1p/ADA3p forms a coactivator/repressor complex (ADA complex) in association with at least two other yeast proteins, ADA2p and GCN5p, that is involved in regulating transcriptional activator proteins including GAL4p and GCN4p. Using a two-hybrid analysis, we found that the carboxyl-terminal transcriptional activation domain of PDR1p, the primary regulatory protein involved in yeast pleiotropic drug resistance, interacts with the amino-terminal 373 amino acids of NGG1p (NGG1p1–373). This interaction was confirmed by coimmunoprecipitation of epitope-tagged derivatives of NGG1p and PDR1p from crude extracts. An overlapping region of the related transcriptional activator PDR3p was also found to interact with NGG1p. Amino acids 274–307 of NGG1p were required for interaction with PDR1p. This same region is required for inhibition of transcriptional activation by GAL4p. The association between NGG1p1–373 and PDR1p may be indirect, possibly mediated by the ADA complex since the two-hybrid interaction required the presence of full-length NGG1. A partial requirement for ADA2 was also found. This suggests that an additional component of the ADA complex, regulated by ADA2p, may mediate the interaction. Transcriptional activation by a GAL4p DNA binding domain fusion of PDR1p was enhanced in ngg1 and ada2 disruption strains. Similar to its action on GAL4p, the ADA complex acts to inhibit the activation domain of PDR1p.

Transcriptional activator proteins are regulated both positively and negatively by associated factors. As a group, factors that positively influence activator function are called coactivators and include components of the RNA polymerase II holoenzyme (1) and the TATA-binding protein (TBP)-associated factors and include component of the basal transcriptional machinery (8). Recently an interaction between components of the ADA complex and TBP has been demonstrated by affinity chromatography and immunoprecipitation (14).2 In this ability to associate with TBP, the ADA complex resembles other coactivators involved in transcriptional activation and repression (13).

As coactivators the ADA proteins were predicted to interact with a component of the basal transcriptional machinery (8). Recently an interaction between components of the ADA complex and TBP has been demonstrated by affinity chromatography and immunoprecipitation (14).2 In this ability to associate with TBP, the ADA complex resembles other coactivators including the TBP-associated factor complex (15, 16), SU1p (17, 18), and SPT3p (19), as well as repressor proteins such as MOT1p (20, 21), which are biochemically and/or genetically linked to TBP. In its role as a coactivator/pressor, the ADA complex may provide a regulatory link between the activator protein and TBP. In addition to the genetic evidence that NGG1p interacts with GAL4p (6), biochemical evidence for the bridging function of the ADA complex exists as components of the ADA complex associate with the activation domains of VP16, GCN4p, and GAL4p (14, 22, 23). The ability of recombinant ADA2p to interact with VP16 in vitro suggests that ADA2p may have a principal role in these interactions (14).

We have used a two-hybrid screen (24) to identify molecules

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank/EMBL Data Bank with accession number(s) U52042.

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The abbreviations used are: TBP, TATA box-binding protein; PCR, polymerase chain reaction; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis.

NGG1p/ADA3p forms a coactivator/repressor complex (ADA complex) in association with at least two other yeast proteins, ADA2p and GCN5p, that is involved in regulating transcriptional activator proteins including GAL4p and GCN4p. Using a two-hybrid analysis, we found that the carboxyl-terminal transcriptional activation domain of PDR1p, the primary regulatory protein involved in yeast pleiotropic drug resistance, interacts with the amino-terminal 373 amino acids of NGG1p (NGG1p1–373). This interaction was confirmed by coimmunoprecipitation of epitope-tagged derivatives of NGG1p and PDR1p from crude extracts. An overlapping region of the related transcriptional activator PDR3p was also found to interact with NGG1p. Amino acids 274–307 of NGG1p were required for interaction with PDR1p. This same region is required for inhibition of transcriptional activation by GAL4p. The association between NGG1p1–373 and PDR1p may be indirect, possibly mediated by the ADA complex since the two-hybrid interaction required the presence of full-length NGG1. A partial requirement for ADA2 was also found. This suggests that an additional component of the ADA complex, regulated by ADA2p, may mediate the interaction. Transcriptional activation by a GAL4p DNA binding domain fusion of PDR1p was enhanced in ngg1 and ada2 disruption strains. Similar to its action on GAL4p, the ADA complex acts to inhibit the activation domain of PDR1p.

Transcriptional activator proteins are regulated both positively and negatively by associated factors. As a group, factors that positively influence activator function are called coactivators and include components of the RNA polymerase II holoenzyme (1) and the TATA-binding protein (TBP)–associated factor complex (2). Several examples of activator proteins being regulated by negative factors also exist (3). From a mechanistic standpoint dual function regulators, with the ability to activate or repress transcription depending upon different promoter contexts or environment signals, represent particularly interesting models (4). The yeast ADA complex (5) is such a dual function regulator.

We initially isolated NGG1 based on its requirement for the full inhibition of transcriptional activation by GAL4p in glucose media (6). Expression of a GAL10-lacZ reporter is increased 300-fold in glucose media in a gal80 ngg1 background. Approximately 10–15-fold of this effect is attributable to ngg1. We believe that GAL4p is the direct target for NGG1p action since inhibition is seen for independent GAL4p binding sites, requires GAL4, but does not require the GAL4 promoter (6). In addition, the synergistic effect with gal80 is consistent with NGG1p regulating the DNA binding or activity of GAL4p.

Independently, NGG1/ADA3 was isolated by the Guarente laboratory based on the ability of mutations to suppress the toxic effects of overexpression of the viral activator VP16 in yeast (7). Four additional ADA genes were isolated in these screens (ADA1 to ADA5; Refs. 8 and 9). As the toxicity of VP16 was thought to arise from squelching of essential transcription factors, the ADA genes were predicted to encode coactivators required for transcriptional activation by VP16 (8). ADA2, NGG1/ADA3, GCN5/ADA4, and ADA5 are in fact required for transactivation by a set of transcription factors that includes the chimeric molecules GAL4p-VP16 and LexA-GCN4p (7–10). GCN5p had also been identified because it is required for maximal activation by GCN4p (11). Using genetic and in vitro biochemical techniques, the Guarente and Theires laboratories have shown that the ADA proteins probably act in a complex that contains at least ADA2p, NGG1p, and GCN5p (7, 9, 10, 12). Direct interaction, in vitro, has been observed between ADA2p and both GCN5p and the carboxyl-terminal 250 amino acids of NGG1p (12).

Based on the finding that single and double disruptions of ngg1 and ada2 have similar effects on inhibition of GAL4p, we suggested that the same or related ADA complexes are involved in transcriptional activation and repression (13).

As coactivators the ADA proteins were predicted to interact with a component of the basal transcriptional machinery (8). Recently an interaction between components of the ADA complex and TBP has been demonstrated by affinity chromatography and immunoprecipitation (14).2 In this ability to associate with TBP, the ADA complex resembles other coactivators including the TBP-associated factor complex (15, 16), SU1p (17, 18), and SPT3p (19), as well as repressor proteins such as MOT1p (20, 21), which are biochemically and/or genetically linked to TBP. In its role as a coactivator/pressor, the ADA complex may provide a regulatory link between the activator protein and TBP. In addition to the genetic evidence that NGG1p interacts with GAL4p (6), biochemical evidence for the bridging function of the ADA complex exists as components of the ADA complex associate with the activation domains of VP16, GCN4p, and GAL4p (14, 22, 23). The ability of recombinant ADA2p to interact with VP16 in vitro suggests that ADA2p may have a principal role in these interactions (14).

We have used a two-hybrid screen (24) to identify molecules

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1 The abbreviations used are: TBP, TATA box-binding protein; PCR, polymerase chain reaction; HA, hemagglutinin; PAGE, polyacrylamide gel electrophoresis.

2 A. Saleh and C. J. Brandl, manuscript in preparation.
NGG1p/ADA3p Interacts with PDR1p

TABLE I

| Strain | Parent | GAL4 | GAL80 | NGG1 | ADA2 | GAL reporters | Reference |
|--------|--------|------|-------|------|------|---------------|-----------|
| Y187   | wt     | wt   | lacZ  | 26   |      |               |           |
| Y190   | wt     | wt   | lacZ  | 26   |      |               |           |
| CY922  | wt     | wt   | HIS3  | 26   |      |               |           |
| CY958  | wt     | wt   | HIS3  | This work |      |               |           |
| CY959  | wt     | wt   | HIS3  | This work |      |               |           |
| JY335  | estr+  | estr+ | HIS3  | This work |      |               |           |
| CY960  | wt     | estr+ | HIS3  | This work |      |               |           |
| CY957  | estr+  | estr+ | HIS3  | This work |      |               |           |
| SY6–2  | estr+  | estr+ | HIS3  | This work |      |               |           |

* wt, wild type.

that interact with NGG1p in vivo. In this report we show the interaction of the amino-terminal 373 amino acids of NGG1p with the carboxyl-terminal transcriptional activation domains of PDR1p and PDR3p. PDR1p and PDR3p belong to the C6 zinc cluster family of DNA-binding proteins, which includes GAL4p (reviewed in Ref. 25). By regulating expression of membrane transporter proteins, such as PDR5p, PDR1p, and PDR3p are key regulatory proteins required for multidrug resistance in Saccharomyces cerevisiae (25). The interaction between NGG1p and PDR1p was confirmed by their coimmunoprecipitation from crude yeast extracts. Furthermore, we show that similar to its action on GAL4p, NGG1p, and ADA2p inhibit transcriptional activation by the PDR1p transcriptional activation domain.

MATERIALS AND METHODS

Yeast Strains, Media, and Growth Conditions—Yeast strain Y190 (Ref. 26; MATa gal4 gal80 his3 trp1–901 ade2–101 ura3–52 leu2–3,112 ura3–52 lacZ LYS2::GAL–lacZ HIS3::GAL–lacZ) is isogenic to CY922 but contains a Tn10LUK (28) disruption of ADA2 (Ref. 7; kindly provided by J. Horiuchi and L. Guarente). JY335 is a derivative of CY922 in which NGG1 was replaced by NGG1+, a NGG1 allele with a nonsense mutation at Leu-62 (6). GAL4p, expressed from its own promoter (kindly provided by I. Sadowski) in the URA3 integrating vector Yplac211 (29) was integrated into yeast strains CY922, CY933, and JY335 at ura3–52 after digestion with EcoRV to make CY958, CY959, and CY960, respectively. SY6–2 is a derivative of CY756 (Ref. 30) containing Tn10LUK disruptions of ada2–101, his3–200, and ura3–52. CY756 was replaced by a hemagglutinin epitope-tagged derivative of NGG1 (HA-NGG1) (29,31). CY947 is also a derivative of CY756 containing Tn10LUK disruptions of GAL80 and ADA2 and a Myc-tagged NGG1 allele expressed from the DED1 promoter integrated at his3 (13). See Table I for the relevant genotypes of yeast strains used in this study.

In general, yeast strains were grown at 30 °C in liquid suspension or on 2% Bacto agar plates in YPD broth (1% yeast extract, 2% peptone, 2% dextrose) at 4°C with 200 μl of whole cell extract was transferred to the 2 g plasmid YEplac181 (29) for a Saff–SstI fragment.

A DNA fragment encoding the carboxyl-terminal 212 amino acids of PDR3 (31) was generated by PCR from genomic DNA and ligated into the BamHI–SalI sites of pACTII (26; kindly provided by S. Elledge) (32) by transferring a BamHI–SalI fragment from YCP87–his–G25 lacZ (6).

Two hybrid Analysis—Yeast strain Y190 containing the plasmid GAL4DBD–NGG1,373 was transformed with a yeast cDNA library fused to the GAL4p activation domain in pACT (Ref. 26; received from S. Elledge). Approximately 1.25 × 106 transformants were plated on selective media containing 50 mM 3-aminotriazole. After 3–5 days at 30 °C, colonies were replica plated onto Trp–Leu–ura3–52 media containing 500 mM 3-aminotriazole (27). Yeast strains containing only the pACT–DNA plasmids were obtained by growing strains to saturation then plating on media lacking leucine, followed by screening for tryptophan auxotrophy. The growth rates of diploids generated by mating these strains with Y187 derivatives expressing either GAL4DBD–SNF4p, GAL4DBD–p53, or GAL4DBD–lamin, were assayed to determine the specificity of the interaction. DNA sequence was determined using the T7 polymerase kit from Pharmacia Biotech Inc.

β-Galactosidase Assays—Yeast strains were grown in liquid culture in minimal media to an A600 of 1.0–1.5. Cells were pelleted, washed and concentrated 2–5-fold in LACZ buffer (33). β-Galactosidase activity was determined after disruption of the cells with glass beads as described by Himmel et al. (34). Activity was standardized to protein concentrations determined by the Bio-Rad protein assay. For yeast strains expressing potent activator proteins, β-galactosidase activity was assayed according to Ausubel et al. (35) and standardized to cell density.

Coimmunoprecipitation of PDR1p and NGG1p—Yeast whole cell extract was prepared as described by Schultz et al. (36) with minor modifications. For immunoprecipitation of Myc-tagged proteins, 50 mg of whole cell extract was rotated for 1 h at 4 °C with 200 μl of a slurry of Sepharose CL–4B (Pharmacia) equilibrated in IP buffer (12; 50 mM Hepes, pH 7.3, 100 mM sodium glutamate, 1 mM magnesium acetate, 1 mM EDTA, 0.3% Nonidet P-40, 0.5% bovine serum albumin, 10% glycerol). Unbound protein was mixed with 7 μl of an anti-Myc ascites fluid derived from the Myc–9E10 cell line (37) and rotated at 0°C for 1 h. 100 μl of Protein A-Sepharose beads (Pharmacia), preincubated with 20 μg of rabbit anti-mouse IgG and IgM (Jackson ImmunoResearch Labs, Inc.) and equilibrated in IP buffer, were added to the mixtures and rotated 3 h at 4°C. Protein-bound beads were pelleted by centrifugation and washed four times in IP buffer without bovine serum albumin. Immunoprecipitated complexes were separated from the beads by boiling for 5 min in SDS gel loading buffer. Protein was separated by SDS-PAGE and analyzed by Western blotting. For immunoprecipitation of hemagglutinin (HA)-tagged proteins, a similar protocol was followed substituting anti-HA monoclonal antibody, clone 12B12, covalently bound to N-hydroxysuccinimide-activated Sepharose (Berkley Antibody Co.), for the Myc ascites fluid and Protein A-Sepharose.

Western Blot Analysis—Western blotting with anti-Myc primary an-
FIG. 2. Deletion mapping of NGG1p to define a region required for PDR1p interaction. Deletion derivatives of NGG1p with the indicated amino acids were fused to the GAL4p DNA binding domain sequence in pAS1 and coexpressed in Y190 with either GAL4AD-SNF4 or GAL4AD-NGG1p derivatives. Transformants grown in minimal media containing 2% glucose were assayed for expression of the GAL-lacZ reporter and the values standardized to total protein. Each value represents the average of three independent transformants.

NGG1p/ADA3p Interacts with PDR1p

RESULTS

Isolation of NGG1p Interacting Proteins—We have used the yeast two-hybrid system (24) to identify proteins that interact with the amino-terminal 373 amino acids of NGG1p (NGG1p1–373). This segment of NGG1p contains a region essential for inhibition of GAL4p (amino acids 274–307; Ref. 13). Based on the role of NGG1p and the ADA complex in regulating transcriptional activators (6–9, 12, 13), we chose to further explore the interaction of genes required for pleiotropic drug resistance (39–41). Based on the role of NGG1p and the ADA complex in regulating transcriptional activators (6–9, 12, 13), we chose to further explore the interaction of genes required for pleiotropic drug resistance (39–41).

The four cDNAs were sequenced and compared with sequences in the GenBank data base. Interestingly, one of the cDNAs encoded the carboxyl-terminal 251 amino acids of the transcriptional activator protein PDR1p (39). PDR1p contains a DNA binding domain related to GAL4p and activates expression of genes required for pleiotropic drug resistance (39–41). Based on the role of NGG1p and the ADA complex in regulating transcriptional activators (6–9, 12, 13), we chose to further characterize the interaction between NGG1p and PDR1p and its functional significance as described below.

In addition to PDR1p, we identified three cDNAs encoding proteins that interact with the amino-terminal 373 amino acids of NGG1p. The NIF3 (NGG1p interacting factor) cDNA contained sequence not found in the data base. The protein fragment responsible for the interaction with NGG1p1–373 was probably a 54-amino acid GAL4p fusion since a frameshift mutation at the GAL4 DNA activation domain junction resulted in a loss of interaction. A second cDNA was in frame with the coding sequence of SYGP-ORF43 (accession no. L11119), an open reading frame coding for 357 amino acids with no described function. The third cDNA coded for the carboxyl-terminal 180 amino acids of a yeast ubiquitin-specific protease, UBP3p (42). These gene products are being further analyzed to determine the functional significance of their interaction with NGG1p.

Mapping the Region of NGG1p Required for Interaction with PDR1p—To localize the region within the amino-terminal 373 amino acids of NGG1p that is able to recruit PDR1p, we analyzed internal deletions of GAL4AD-NGG1p1–373 in a two-hybrid analysis with GAL4AD-PDR1p1–1063 (Fig. 2). Because GAL4AD-NGG1p fusions are transcriptional activators (12, 13), the GAL4AD-NGG1p deletions were also coexpressed with GAL4SNF4, which does not interact with NGG1p1–373 (Fig. 1), to control for changes in GAL10-lacZ induction related to its function as an independent activator. GAL4AD-NGG1p1–373 coexpressed with GAL4AD-PDR1p1–1063 resulted in an 8-fold increase in GAL10-lacZ expression as compared to its coexpression with GAL4AD-SNF4, indicating that amino acids 276–376 are sufficient for interaction with PDR1p1–1063. By comparison, an internal deletion of amino acids 274–307 did not show the increased expression of the GAL10-lacZ reporter in the presence of GAL4AD-PDR1p1–1063. This suggests that amino acids 274–307 of NGG1p, a region required for inhibi-
NGG1p/ADA3p Interacts with PDR1p

PDR1p

FIG. 4. Homology between carboxyl-terminal regions of PDR1p and PDR3p. Amino acids 806-1063 of PDR1p (upper lines) were aligned with PDR3p (lower lines) using the BESTFIT program (Genetics Computer Group, Madison, WI). This region of PDR1p shares 27% identity with amino acids 765–969 of PDR3p including a transcriptional activation domain defined by Delaveau et al. (31). The boxes outline two regions that share 62% and 71% amino acid identity. The dots on the lower line indicate 3 gaps introduced in PDR3p to maintain the alignment. Amino acid identity and conservative differences between PDR1p and PDR3p are indicated by the vertical lines and the single or double dots, respectively.

PDR1p was not found in immunoprecipitates with HA-tagged NGG1p1–373 when the proteins were transcribed and translated in vitro (data not shown).

NGG1p Also Interacts with the Carboxyl-terminal Activation Domain of PDR3p—Mutations in a Second Genetic Locus. PDR3p, result in similar multidrug resistance phenotypes as for PDR1p (31, 45, 46). PDR3p and PDR1p share 36% amino acid identity (31) and are thought to recognize the same promoter elements (45, 47). Two regions of PDR3p, amino acids 1–109 and 765–976, activate transcription when fused to the LexA DNA binding domain (31). The latter activation domain contains two stretches of 21 and 24 amino acids that are 62% and 71% identical, respectively, with the carboxyl-terminal domain of PDR1p (Fig. 4). Based on the sequence similarity and the apparent functional overlap of PDR1p and PDR3p, we tested for interaction between NGG1p1–373 and the carboxyl-terminal region of PDR3p by two-hybrid analysis. Y190 containing either GAL4AD-NGG1p1–373 or the nonspecific bait protein, GAL4AD-SNF1p, were transformed with a plasmid expressing the GAL4p activation domain fusion, GAL4AD-PDR3p1–976. This 212-amino acid region of PDR3p corresponds to the carboxyl-terminal 258 amino acids of PDR1p. Resulting transformants were assayed for two-hybrid interaction in direct comparison to strains expressing GAL4AD-PDR1p1–1063 (Table II). Coexpression of GAL4AD-PDR3p1–976 with GAL4BD-NGG1p1–373 resulted in increased expression of GAL1-lacZ approximately 5-fold above that with GAL4BD-SNF1p; results equivalent to those for GAL4AD-PDR1p1–1063. This indicates that NGG1p interacts with both PDR1p and PDR3p and suggests that their carboxyl-terminal activation domains share a related protein motif responsible for the interaction.

Diversity of GAL4p (6), is essential for the interaction with PDR1p.

Communoprecipitation of NGG1p and PDR1p—Communoprecipitation was used to verify the two-hybrid interaction between NGG1p and PDR1p. Whole cell extracts were prepared from yeast strains expressing NGG1p tagged with a HA hemagglutinin epitope (43, 44) with or without coexpression of Myc-PDR1p from an episomal plasmid, were incubated with anti-Myc antibody. After washing, proteins were eluted in SDS loading buffer, separated by SDS-PAGE, and analyzed by Western blotting with anti-HA antibody. Lanes 1 and 2 contain 150 μg of whole cell extract from SY6–2 strains expressing HA-NGG1p or HA-NGG1p and Myc-PDR1p, respectively. Lane 3 contains 150 μg of whole cell extract from yeast strain CY947 (an isogenic strain that does not express HA-NGG1p). The position of HA-NGG1p and the light chain of the anti-Myc antibody (Lc), which serves as a loading control, are labeled. Relevant molecular mass protein standards (in kDa) are indicated. B, the whole cell extracts described above were incubated with anti-HA antibody coupled to Sepharose. Immunoprecipitates from SY6–2 strains expressing HA-NGG1p with (lane4) or without (lane 3) coexpression of Myc-PDR1p and 50 μg of the corresponding whole cell extracts (lanes 1 and 2) were Western-blotted with anti-Myc antibody. The positions of HA-NGG1p and the light chain of the anti-HA antibody (Lc) are labeled.

Amino acids 806-1063 of PDR1p (Genetics Computer Group, Madison, WI). This region of PDR1p shares 27% identity with amino acids 765–969 of PDR3p including a transcriptional activation domain defined by Delaveau et al. (31). The boxes outline two regions that share 62% and 71% amino acid identity. The dots on the lower line indicate 3 gaps introduced in PDR3p to maintain the alignment. Amino acid identity and conservative differences between PDR1p and PDR3p are indicated by the vertical lines and the single or double dots, respectively.

PDR1p was not found in immunoprecipitates with HA-tagged NGG1p1–373 when the proteins were transcribed and translated in vitro (data not shown).
NGG1p/ADA3p Interacts with PDR1p

GAL4DBD-NGG1p and GAL4AD-SNF1p, two proteins known to observe the two-hybrid interaction between PDR1p813–1063 and PDR1p. Interestingly, disruption of NGG1p1–373. In contrast, reporter (6) in wild type and significant, disruption of NGG1p should alter transcriptional activation by PDR1p. We analyzed expression of the GAL10-lacZ reporter (6) in wild type and ngg1–1 strains containing

| GAL4p DNA Binding Domain Fusion | GAL4p Activation Domain Fusion | Yeast Strains |
|--------------------------------|-------------------------------|---------------|
| CY922                          | (ngg1, ada2)                  |               |
| CY933                          | (ngg1-1)                      |               |
| **NGG1**                       | **PDR1**                     | 17.42±1.340   | 5.91±1.0     |
| **NGG1**                       | **SNF1**                     | 2.29±1.060    | 2.69±0.4     |
| **SNF1**                       | **PDR1**                     | 9.13±3.731    | nd           |
| GAL4148–762                    | GAL4148–762                   |               |
| **NGG1**                       | **PDR1**                     | 65±15         | 45±2.7       |
| 63±15                          |                               |               |

**TABLE II**

NGG1p interacts with PDR3p by two-hybrid interaction. PDR3 sequence encoding amino acids 765–976, was fused to coding sequence for the GAL4p activation domain in pACTII (GAL4pDNA binding domain with amino acids 813–1063 and transformed into yeast strain Y190 containing GAL4DBD-NGG1p,373 or GAL4AD-SNF1p (control for NGG1p-independent expression of the reporter). Transforms were grown in minimal media containing 2% glucose and assayed for expression of the GAL-lacZ reporter in direct comparison to equivalent strains containing GAL4AD-PDR1p113–1063. Each value represents the average β-galactosidase activity of at least 3 transformants, standardized to total protein. The far right column indicates the ratio of β-galactosidase activity for strains containing GAL4DBD-NGG1p,373 versus GAL4DBD-SNF1p as a measure of the two-hybrid interaction.

NGG1p and ADA2p Inhibit Transcriptional Activation by PDR1p

NGG1p and ADA2p Inhibit Transcriptional Activation by PDR1p: NGG1p and ADA2p inhibit transcriptional activation by GAL4p (6, 13); in contrast, these proteins are required for full activity of LexA-GCN4p and LexA-VP16 (7-10). If the interaction between NGG1p and PDR1p is functionally significant, disruption of NGG1p should alter transcriptional activation by PDR1p. We analyzed expression of the GAL10-lacZ reporter (6) in wild type and ngg1–1 strains containing GAL4DBD-PDR1p113–1063 and a fusion of the GAL4p DNA binding domain with amino acids 813–1063 of PDR1p (Fig. 6A). The presence of a transcriptional activation domain at the carboxy terminus of PDR1p was confirmed by the 16-fold increase in expression of GAL10-lacZ by GAL4DBD-PDR1p113–1063 as compared with GAL4DBD-SNF1p in the wild type strain CY922. In the ngg1–1 strain (Y335) expression of GAL10-lacZ increased approximately 4.5-fold in the presence of GAL4DBD-PDR1p113–1063 as compared to CY922. This effect was not due to the influence of the GAL4p DNA binding domain because a similar increase in expression was not found in Y335 for GAL4DBD-SNF1p. The increase in activity of GAL4DBD-PDR1p113–1063 in the ngg1–1 background is comparable to that for full-length GAL4p integrated into CY922 and CY335. These results demonstrate that, similar to GAL4p, the activity of the transcriptional activation domain of PDR1p is inhibited by NGG1p.

To determine if other components of the ADA complex are involved in inhibiting PDR1p-mediated activation, a similar analysis was performed with the ada2 disruption strain, CY933. For GAL4DBD-PDR1p113–1063 and GAL4DBD-SNF1p, a GAL4p-regulated lacZ reporter with five tandemly repeated GAL4p DNA binding sites, his3-G4-lacZ (6) was used to increase the relative expression of β-galactosidase. Similar to ngg1–1 strains, disruption of ada2 resulted in a 4.5-fold increase in GAL4DBD-PDR1p113–1063-mediated expression of his3-G4, while expression mediated by GAL4DBD-SNF1p was relatively unaffected (compare CY933 with CY922; Fig. 6B). Therefore, both ADA2p and NGG1p, are involved in negatively regulating transcriptional activation by PDR1p. Based on the known interaction between NGG1p and ADA2p (12), and the involvement of ADA2p and NGG1p in the interaction between PDR1p113–1063 and NGG1p,1–373, this suggests that the ADA complex inhibits PDR1p.

**DISCUSSION**

Using the two-hybrid system, we have found that the carboxy-terminal 251 amino acids of the transcriptional activator protein, PDR1p, associate with amino acids 1–373 of NGG1p. This was confirmed by the communoprecipitation of epitope-tagged derivatives of NGG1p and PDR1p from yeast extracts. Based on the 5-fold increase in activation by GAL4DBD-PDR1p113–1064 in both ngg1 and ada2 strains, we also conclude that, in common with GAL4p (6, 13), NGG1p and ADA2p inhibit transcriptional activation by the activation domain of PDR1p.
NGG1p/ADA3p Interacts with PDR1p

The interaction of NGG1p1–373 with PDR1p. The requirement for GAL4DBD-PDR1p813–1063 and GAL4DBD-SNF1p, while the units for NGG1p and PDR1p did not coimmunoprecipitate when trans-

interaction between NGG1p and PDR1p may be indirect. First, Ours experiments suggest that the component of the ADAComplex—

ponent of the ADA Complex—

by PDR1p. 813-1063) was fused to that for the DNA binding domain of GAL4p in plasmids containing

NGG1 for

was assayed in isogenic yeast strains CY922 (wild type; bars

FIG. 6. NGG1p and ADA2p inhibit transcriptional activation by PDR1p. A, transcriptional activation in ngg1-1 strains. Sequence encoding the carboxy-terminal 251 amino acids of PDR1p (amino acids 813-1063) was fused to that for the DNA binding domain of GAL4p in pAS1 (GAL4DBD-PDR1p813-1063). Transcription of a GAL10-lacZ reporter was assayed in isogenic yeast strains CY922 (wild type; stippled bars) and JY335 (ngg1-1; hatched bars) that had been transformed with plasmids containing GAL4DBD-PDR1p813-1063 or GAL4DBD-SNF1p, or had GAL4 integrated at the ura3-52 (CY958 and CY960; see Table I). Cells were grown in minimal media containing 2% glucose and 

NGG1p and ADA2p inhibit transcriptional activation by PDR1p. A, transcriptional activation in ngg1-1 strains. Sequence encoding the carboxy-terminal 251 amino acids of PDR1p (amino acids 813-1063) was fused to that for the DNA binding domain of GAL4p in pAS1 (GAL4DBD-PDR1p813-1063). Transcription of a GAL10-lacZ reporter was assayed in isogenic yeast strains CY922 (wild type; stippled bars) and JY335 (ngg1-1; hatched bars) that had been transformed with plasmids containing GAL4DBD-PDR1p813-1063 or GAL4DBD-SNF1p, or had GAL4 integrated at the ura3-52 (CY958 and CY960; see Table I). Cells were grown in minimal media containing 2% glucose and β-galactosidase activity quantitated for a minimum of three independent colonies from each transformation using glass bead lysis (34). The units of β-galactosidase labeled on the left axis correspond to values for activation by GAL4DBD-PDR1p813-1063 and GAL4DBD-SNF1p, while the units on the right axis correspond to activation by GAL4p. B, transcriptional activation in ada2 strains. Transcription of his3-gal1acZ, which contains a modified his3 promoter with five optimal GAL4p binding sites (6) was assayed in isogenic yeast strains CY922 (wild type; stippled bars) and CY933 (ada2; solid bars) that had been transformed with plasmids containing GAL4DBD-PDR1p813-1063 and GAL4DBD-SNF1p. For the isogenic strains integrated with GAL4 (CY958 and CY959) expression of GAL10-lacZ was assayed.

The NGG1p-PDR1p Association May Be Mediated by a Component of the ADA Complex.—Our experiments suggest that the interaction between NGG1p and PDR1p may be indirect. First, NGG1p and PDR1p did not coimmunoprecipitate when translated in vitro. Second, both ADA2 and NGG1 were required for the interaction of NGG1p1–373 with PDR1p. The requirement for NGG1 is particularly interesting. Full-length NGG1p may be required to form a dimer with NGG1p1–373, which then directly, or indirectly, associates with PDR1p813-1063. We think that this is unlikely based on our inability to demonstrate an interaction between NGG1p and NGG1p1–373 by two-hybrid

analysis (data not shown). Alternatively, full-length NGG1p may be required for the stability or expression of a component of the ADA complex mediating the interaction between NGG1p1–373 and PDR1p813-1063. Consistent with this, we have found that the stability of ADA2p and NGG1p are dependent on the presence of each other. 2 ADA2p, itself, is a possible candidate for mediating the interaction between NGG1p and PDR1p, since it has been found to interact with the VP16 activation domain in vitro (14). While we cannot exclude this possibility, the fact that disruption of ADA2 does not completely eliminate the two-hybrid interaction, and that NGG1p1–373 lacks the carboxy-terminal region of NGG1p that interacts with ADA2p (12), suggests that a third component is involved. The presence of this component must be less dependent on ADA2p than NGG1p.

To date, GCN5p is the only other defined protein in the complex; however, we have recently found NGG1p and ADA2p as part of a complex that fractionates with an apparent size of greater than 1×10^6 kDa by gel filtration. 3 Immunoprecipitation studies with epitope-tagged NGG1p also reveal at least five interacting proteins in addition to ADA2p. 2 Similar studies using a derivative of NGG1p lacking amino acids 274–307, the region required for the PDR1p interaction, revealed a similar but slightly smaller complex containing NGG1p. 2 A protein that associates with NGG1p through interactions involving amino acids 274–307 may directly contact the activation domain of PDR1p. Clearly, this region of NGG1p is involved with the function of NGG1p, since its deletion results in loss of inhibition of GAL4p by NGG1p and imparts the slow growth phenotype typical of disruption of ngg1 (13).

Interaction of NGG1p with PDR1p and PDR3p.—The primary role of PDR1p in the multidrug resistance response is evident from the high frequency of PDR1 mutations that result in resistance to nearly 30 chemicals that affect cytoplasmic or mitochondrial functions (reviewed in Ref. 48). PDR1p regulates the expression of nine or more yeast genes, including membrane transporter proteins such as PDR5p (40, 41) and SNQ2p (25, 49). Mutations conferring similar multidrug resistant phenotypes map to PDR3 (45, 46, 50, 51). These data (along with the finding that PDR1 and PDR3 cross-complement null alleles, have related DNA binding motifs that bind the same consensus (45, 47), and overall share 36% amino acid identity (31, 39, 45, 52)) indicate that these two regulators functionally overlap (25).

Our finding that NGG1p also associates with the carboxy-terminal activation domain of PDR3p suggests that PDR3p and PDR1p share a motif that is involved in recruiting NGG1p. Two candidate regions are stretches of 21 and 24 amino acids having 62% and 71% identity starting at residues 858 and 1035, respectively, of PDR1p (Fig. 4). Interestingly, several mutations of PDR1p that result in increased drug resistance cluster around amino acids 800-1000 (25). These mutations may prevent the association between NGG1p and PDR1p allowing for increased activation.

Induction of the genes involved for multidrug resistance requires an interplay between PDR1p and PDR3p. A predominant role for PDR1p in this response is suggested by the finding that deletion of PDR1 has a more pronounced effect on sensitivity to cycloheximide than deletion of PDR3 (31, 45). However, PDR3p is clearly involved because the double disruption (pdr1 pdr3) has increased sensitivity as compared to either single disruption. Delahodde et al. (47) identified two sites in the PDR3 promoter, which bind either PDR1p or PDR3p. These sites are required for resistance to cycloheximide even in pdr1 strains, suggesting that autoregulation of PDR3 is involved in drug resistance. PDR1p may confer a rapid response to cellular
toxins by inducing expression of membrane transporters, such as PDR5p and enhance the response by activating expression of PDR3. Once induced PDR3p may potentiate drug resistance through further induction of membrane transporters and by activating its own autoregulatory loop (47). As inhibitors of PDR1p and probably PDR3p, NGG1p and ADA2p are involved in limiting the expression of genes involved in drug resistance, including PDR3, and may have a key role in terminating the response.

General Regulatory Role of NGG1p—Our first report on NGG1p focused on its regulation of GAL4p and in particular its action in glucose repression (6). Subsequently, we have shown that ADA2p is also required for inhibition of activation by GAL4p (13). As witnessed by their action on PDR1p, NGG1p and ADA2p can function as more general inhibitors of transcriptional activation, not limited to a role in glucose repression.

The proteins of the ADA complex are uniquely positioned to have a critical role in regulating transcription. Acting as a coactivator/repressor, this complex links components of the basal transcriptional machinery with activator proteins. The ADA proteins can provide gene-specific regulation through their association with different activators. Moreover, the complex can induce either gene-specific activation or repression as determined, presumably by the nature of the activator-complex interactions and how this influences the basal machinery.

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