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ADR1-Mediated Transcriptional Activation Requires the Presence of an Intact TFIID Complex†

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The yeast transcriptional activator ADR1, which is required for ADH2 and other genes’ expression, contains four transactivation domains (TADs). While previous studies have shown that these TADs act through GCN5 and ADA2, and presumably TFIIB, other factors are likely to be involved in ADR1 function. In this study, we addressed the question of whether TFIID is also required for ADR1 action. In vitro binding studies indicated that TADI of ADR1 was able to retain TAFI90 from yeast extracts and TADII could retain TBP and TAFI130/145. TADIV, however, was capable of retaining multiple TAFIIs, suggesting that TADIV was binding to the TFIID from yeast whole-cell extracts. The ability of TADIV truncation derivatives to interact with TFIID correlated with their transcription activation potential in vivo. In addition, the ability of LexA-ADR1-TADIV to activate transcription in vivo was compromised by a mutation in TAFI130/145. ADR1 was found to associate in vivo with TFIID in that immunoprecipitation of either TAFI90 or TBP from yeast whole-cell extracts specifically coimmunoprecipitated ADR1. Most importantly, depletion of TAFI90 from yeast cells dramatically reduced ADH2 derepression. These results indicate that ADR1 physically associates with TFIID and that its ability to activate transcription requires an intact TFIID complex.

The derepression of the glucose-repressible ADH2 gene from Saccharomyces cerevisiae requires the transcriptional activator ADR1 (16). ADR1 binds to a 22-bp palindromic sequence—UAS1—located 215 bp upstream of the transcription start site of the ADH2 gene (47). ADR1 also regulates the transcription of genes involved in glycerol metabolism (5, 29) and peroxisome biogenesis, and sequences similar to UAS1 of the ADH2 gene are found in the promoters of these genes (7, 36). Four regions of ADR1 have been identified that are required for its efficient activation of ADH2 transcription: transcription activation domain I (TADI) (residues 76 to 172), TADII (residues 263 to 357), TADIII (residues 420 to 462), and TADIV (residues 642 to 704) (5, 9, 12, 14, 39). TADII and TADIII are functionally redundant in the context of full-length ADR1 (12), suggesting that they may affect the same step in the process of transcriptional activation of the ADH2 gene. TADIV seems most important to the protein in that deletion of it reduces ADR1 function dramatically (9). In our earlier report, we had shown that individual activation domains of ADR1 can contact TFIIB, ADA2, and the histone acetyltransferase GCN5 in vitro (9). However, the deletion of ADA2 or GCN5 had only a moderate effect on the derepression of the ADH2 gene (9), suggesting the existence of additional activation mechanisms.

There are a number of potential targets for ADR1 activation domains among the core transcription factors. TFIID, TFIIF, TFIIB, RNA polymerase II (polII), TFIHH, and TFIIIIE have been implicated in mammalian and drosophila systems as being direct contacts for various transcription activators (48). For example, the glutamine-rich activation domain of Sp1 contacts TFIID component dTAFI110 (23); VP16 interacts with TFIIB, TBP, and histone-like dTAFI42/hTAFI31; and yeast GAL4 binds TBP and TFIIB (22, 26, 46). The ability of activators to contact multiple targets may be a reflection of activators displaying relatively weak binding to proteins and the requirement to recruit more than one component of the core transcriptional factors to obtain maximal activation potential (31).

TFIID is a multimeric complex consisting of TATA box binding subunit TBP and TBP-associated factors (TAFIIs) (6, 30). Both TBP and TAFIIs show significant degrees of evolutionary conservation in the eucaryotic kingdom (28, 37), suggesting that TFIID quaternary structure may also be conserved. Thirteen yeast TAFIIs have been cloned to date, with sizes ranging from 17 to 150 kDa (3, 25, 37). Most of these proteins are encoded by essential genes. The precise role of TAFIIs in transcription is largely unknown. In vitro studies have shown that activators have both qualitative and quantitative effects on TFIID binding to the TATA box (35). It has also been demonstrated that the TFIID binding step is first and rate limiting in the assembly of the initiation complex at many promoters (11, 24), making it a likely target for transcriptional activators. In vitro data suggest that these proteins are required for activated transcription and that they can serve as direct targets in vivo for activation domains of DNA-binding transcriptional activators of higher eucaryotes (23, 33, 46). In vivo data, however, indicate that yeast TAFIIs are not universally required for polII transcription and are dispensable for activated transcription of a number of yeast genes (27, 43). Although encoded by essential genes, temperature-sensitive alleles of TAFI130/145 and TAFI90 affect the expression of only a small fraction of yeast genes (1, 43). Similar results were observed when different TAFIIs were eliminated from the yeast cell by using a double-shutoff method (27). More recently, it has been shown that TAFI130/145 is required for expression of G1/S cyclins, several small ribosomal subunit
protein genes, and the inorganic phosphatase gene PPA1 (34, 44).

We have found that ADR1 TADIV can specifically retain TFII D from yeast whole-cell extracts and that ADR1 communoprecipitates with TAF90 and with TBP. Moreover, TADIV activation potential was specifically reduced by a temperature-sensitive allele of TAF1130/145, and transcriptional activation of the ADH2 gene did not occur in vivo if the yeast cell was depleted of TAF90. These results suggest that transcriptional activation of the ADH2 gene by ADR1 is mediated through its contacts with the TFII D complex.

MATERIALS AND METHODS

Strains and cultures. The yeast strains used in this study are listed in Table 1. Strains 938-9b and yBY40-8 were used for transformation with plasmids expressing ADR1 from the G3PDH promoter. Strains 1427-1 and 1415-9 were derived following tetrad analysis from a cross between both YSW87 (TAF1130/145) and YSW90 (tafII145-ts-1) (43) and 612-4a. Strains containing the TAF1130/145-ts allele fail to grow at 37°C but appear to grow normally at 30°C. Strains ZMY60 and ZMY68 were a gift from Z. Moqtaderi and K. Struhl, and the ade4-411 allele fail to grow at 30°C. Strains containing the ade4-411 allele fail to grow at 30°C but appear to grow normally at 37°C. Strains 500-16 containing different defined ADR1 dosages and yeast whole-cell extracts were used for transformation with plasmids expressing ADR1 from the G3PDH promoter. Strains 1427-1 and 1415-9 were derived following tetrad analysis from a cross between both YSW87 (TAF1130/145) and YSW90 (tajII145-ts-1) (43) and 612-4a. Strains containing the tajII145-ts allele fail to grow at 37°C but appear to grow normally at 30°C. Strains ZMY60 and ZMY68 were a gift from Z. Moqtaderi and K. Struhl, and the ade4-411 allele fail to grow at 30°C. Strains containing the ade4-411 allele fail to grow at 30°C but appear to grow normally at 37°C.

Strains used in this study

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<th>Strain</th>
<th>Relevant genotype</th>
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<td>EGY188</td>
<td>MATa ura3-52 his3 leu2 trpl1 LexA&lt;sup&gt;4&lt;/sup&gt;-LEU2</td>
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<td>MATa ura3-52 his4 LexA&lt;sup&gt;-&lt;/sup&gt;-LEU2 adh1-d1 ad r1</td>
</tr>
<tr>
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</tr>
<tr>
<td>yBY40-8</td>
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</tr>
<tr>
<td>yKG178</td>
<td>MATa ura3 leu2 his3 trp1::LEU2 pRS415-HA1-BF1</td>
</tr>
<tr>
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<td>MATa ura3 trpl1 his3 leu2 tajf145 LEU2 pRS313-tajf145-ta1</td>
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</tr>
<tr>
<td>500-6</td>
<td>MATa adh1-1 adh3 ad r1 ura1 his4 trpl</td>
</tr>
<tr>
<td>411-40</td>
<td>Isogenic to 500-16 but with adh1-1::ADR1-TRP1</td>
</tr>
<tr>
<td>411-26</td>
<td>Isogenic to 500-16 but with trpl1::S-ADR1-TRP1</td>
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</tr>
<tr>
<td>612-4a</td>
<td>MATa adh1-1 ura1 his4 trpl</td>
</tr>
</tbody>
</table>

ADH1 and β-galactosidase activity assays. ADH1 and β-galactosidase activity assays were conducted as previously described (9), except that the medium used for β-galactosidase assays lacked uracil and threoninate.

Binding assays. GST fusion proteins were expressed and bound to glutathione-agarose beads as previously described (9). Yeast whole-cell extracts were prepared from cells grown to a density of 5 x 10<sup>6</sup>, collected by centrifugation, and disrupted by bead beating in 2 volumes of A300 buffer (20 mM HEPES (pH 7.6), 1 mM DTT, 1 mM dithiothreitol, 300 mM potassium acetate, 1% Triton X-100, protease inhibitors). Washed glutathione-agarose beads were incubated with 1 μg of yeast whole-cell extract protein in 250 μl of A300 buffer for 2 h at 4°C on a rocking platform. Unbound proteins were removed by four washes with 1 ml of A300 buffer, and specifically bound proteins were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after boiling beads directly in sample buffer. Proteins resolved by SDS-PAGE were transferred to a polyvinylidene difluoride (PVDF) membrane and analyzed by Western blotting as previously described (20). Antibody to YTA1 was kindly provided by M. Green, and antibody to the RPBI subunit of RNA polII was a gift from J. Jaehning.

IP. Preparation of yeast extracts for immunoprecipitation (IP) was done as described in reference 45, except that dithiothreitol was omitted from the final dialysis step. IP of triple HA1-tagged TAF90 from yeast whole-cell extracts was conducted in IP incubation and washing buffer (25 mM KPO<sub>4</sub>, pH 7.6, 150 mM KCl, 1 mM Na<sub>2</sub>EDTA, 1 mM Na<sub>2</sub>SO<sub>4</sub>, 5 mM Mg<sub>2</sub>EDTA, 10% glycerol, 1% Nonidet P-40, protease inhibitor cocktail). A 20-μl volume of packed protein A-agarose beads was incubated with 2 μl of monoclonal antibody 12CA5 for 1 h at 4°C and in 250 μl of IP incubation and washing buffer, washed once with the same buffer to remove excess antibodies, and once with 2 μl of yeast cell extract protein in 500 μl of IP incubation and washing buffer. Incubation was allowed to proceed for 2 h at 4°C with constant rocking. The beads were washed four times with 1 ml of the same buffer to remove unbound proteins from the yeast extract, mixed with SDS loading buffer, boiled for 5 min, and separated on a 10% polyacrylamide gel prior to Western analysis (20). TBP IPs were conducted in a similar manner, except that strains were used that were isogenic to 500-16 containing different defined ADR1 dosages and yeast whole-cell extracts were used (12).

Northern analysis. Total yeast RNA was isolated by the hot acidic phenol method (2, 13) and quantified by spectrophotometry (A<sub>260</sub>). Approximately 40 μg of total RNA per sample was denatured with glyoxal and dimethyl sulfoxide and mixed with gel loading buffer as described in reference 32. Denatured RNA samples were loaded in duplicate and resolved on a 1.2% agarose gel, half of which was stained with ethidium bromide in 0.1 M ammonium acetate to verify the equality of loading, and the other half was transferred to a GeneScreen nylon membrane and hybridized to the β<sup>32</sup>P-labeled ADH1 oligonucleotide probe GTGGGTAACCTTATAGGCTTAC (18) in accordance with the membrane manufacturer’s instructions, except that all posthybridization washes were for 1 min each. To determine the levels of CCR4 and ADR1 mRNAs, CCR4 and ADR1 coding sequence fragments were first PCR amplified from plasmids pP1C and pP4K7, respectively. PCR products were subsequently purified with double phenol extraction-ethanol precipitation. β<sup>32</sup>P-labeled with the Amersham Corp. Random Priming Labeling Kit, and used as probes in a hybridization reaction with the same membrane used to measure ADH2 mRNA levels.

RESULTS

TADIV specifically contacts TFII D. We used affinity chromatography to determine if components of TFII D interact with TADs of ADR1. GST fusions with TAD1, TADII,
TADII, TADIV, and Vpu, a human immunodeficiency virus type 1-encoded control protein, bound to glutathione-coupled beads were incubated with whole-cell extracts obtained from yeast strains expressing triple HA1-tagged TAF$_{90}$, TAF$_{25}$, or BRF1, a TAF subunit of TFIIIB but not of TFIIID (25, 30). As shown in Fig. 1, TADIV and, to a lesser extent, TADI were capable of specifically retaining both TAF$_{90}$-HA1$_{1}$ and TAF$_{25}$-HA1$_{1}$ but not BRF1-HA1$_{1}$. The GST-Vpu, GST-TADII, and GST-TADIII fusions and GST alone bound none of the tagged proteins examined. We subsequently examined if other components of TFIIID were being pulled down by TADIV and TADI. Yeast extracts were incubated with the same GST fusions described above, and the proteins which were retained were probed with antibodies to different components of the TFIIID complex: TAF$_{130/145}$, TAF$_{90}$, TAF$_{60}$, and TBP (Fig. 2). The GST-TADIV fusion retained all of these (Fig. 2, lane 4). The RPB1 subunit of RNA polII, however, was not retained by GST-TADIV. The most likely interpretation of this result is that TADIV retains the entire TFIIID complex. TADI displayed weak binding to TAF$_{90}$ and did not retain any of the other components of TFIIID (Fig. 2, lane 2). Weak binding of TBP to TADI was also observed, although our previous analysis indicated that in vitro-expressed TBP does not bind TADI (9). TADI-7c, containing a mutation that enhances ADR1 transcriptional activity, was not retained by GST-TADIV (Fig. 2, lane 5). GST-TADII displayed weak binding to TBP, as shown previously (9), and to TAF$_{130/145}$ (Fig. 2, lane 6), while GST-TADIII did not bind any components of the TFIIID complex (Fig. 2, lane 7).

The ability of TADIV to bind TFIIID correlates with the ability of LexA-TADIV fusions to activate transcription. A set of GST-ADR1 truncation derivatives of TADIV that are modeled on LexA-TADIV derivatives which differ in the ability to activate transcription of a LexA$_{op}$-lacZ reporter construct was tested for the ability to retain TAF$_{90}$-HA1$_{1}$ from yeast extracts. LexA-TADIV C-terminal deletions activated a LexA$_{op}$-lacZ reporter to different extents, depending on the length of the ADR1 moiety present in the fusion. LexA-ADR1-(642-704), LexA-ADR1-(642-701), and LexA-ADR1-(642-698) retained significant transcriptional activity, whereas shorter derivatives were less active (9; see the bottom of Fig. 3). As evident from Fig. 3, the most active TADIV truncation derivatives bound TAF$_{90}$-HA1$_{1}$ to the greatest extent, suggesting a functional relevance of the observed binding. Similarly, TAF$_{130/145}$, TAF$_{90}$, TAF$_{60}$, and TBP were not retained by the transcriptionally inactive TADIV moiety represented in GST-TADIV-642-679 (Fig. 2, lane 3).

We had shown previously that deletion of TADIV from the full-length ADR1 protein drastically reduces its ability to activate transcription of ADH2 and of a LexA$_{op}$-lacZ reporter gene under the control of a single LexA operator binding site (9). Adding back a part of TADIV (residues 642 to 675) which does not bind any of the TFIIID components was found not to restore the ability of LexA-ADR1 to activate transcription from the LexA$_{op}$-lacZ reporter gene: the $\beta$-galactosidase activities measured in strains expressing LexA-ADR1, LexA-ADR1-642-704, and LexA-ADR1-675-704 were 900, 50, and 105 mU/mg of protein, respectively, under glucose growth conditions. All of these LexA-ADR1 proteins were expressed to equivalent extents in yeast cells as determined by Western analysis (data not shown). This result indicates that it is the region capable of binding TFIIID that is required for ADR1 function.

LexA-ADR1-TADIV activation function is dependent on TAF$_{130/145}$. The above results suggest that it is the ADR1-
TADIV interaction with TFIID that would mediate part of the ability of ADR1 to activate transcription. To examine ADR1-TADIV functional dependency on TFIID, the activation ability of LexA-ADR1-TADIV was quantitated in a strain containing the temperature-sensitive taf130/145-1 allele that was defective in TAF130/145 function at the restrictive temperature of 37°C (43). LexA-ADR1-TADIV displayed an over fourfold reduction in the ability to activate a LexA-lacZ reporter in a strain containing a taf130/145 allele compared to a wild-type strain (Table 2). In contrast, LexA-B42, a non-ADR1 activator, was unaffected by the taf130/145 allele. LexA-ADR1-TADII, which did not bind TFIID in vitro, did not display a significant reduction in activation ability as caused by the taf130/145 allele. LexA-ADR1-TADIII, which bound weakly to TBP, and TAF130/145 were reduced in function by 1.7-fold by a taf130/145 allele. Equivalent levels of these LexA fusion proteins were observed in the wild-type and taf130/145 strains (data not shown). These results establish a correlation between TADIV retention of TFIID in vitro as determined by Western analysis. A polyclonal antibody raised against ADR1 residues 208 to 231 was used for ADR1 detection; antibody 12CA5 was used for TAF90-HA1 detection. A 100-μg protein sample was loaded in each crude extract lane. The level of ADR1 protein present in the cell was about 30- to 40-fold higher than that observed for ADR1 expressed at its normal dosage. IgG, immunoglobulin G.

**TABLE 2. Effect of taf130/145 allele on ADR1-TAD activation**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>LexA fusion</th>
<th>Mean β-Galactosidase activity (U/mg) ± SEM</th>
<th>Fold decrease</th>
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<tr>
<td>Wild type</td>
<td>LexA-TADIV</td>
<td>171 ± 32</td>
<td>4.3</td>
</tr>
<tr>
<td>taf130/145</td>
<td>LexA-TADIV</td>
<td>40 ± 5.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Wild type</td>
<td>LexA-B42</td>
<td>160 ± 18</td>
<td>1.0</td>
</tr>
<tr>
<td>taf130/145</td>
<td>LexA-B42</td>
<td>160 ± 27</td>
<td>1.0</td>
</tr>
<tr>
<td>Wild type</td>
<td>LexA-TADII</td>
<td>42 ± 4.9</td>
<td>1.4</td>
</tr>
<tr>
<td>taf130/145</td>
<td>LexA-TADII</td>
<td>30 ± 5.4</td>
<td>1.4</td>
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<tr>
<td>Wild type</td>
<td>LexA-TADIII</td>
<td>110 ± 7.1</td>
<td>1.7</td>
</tr>
<tr>
<td>taf130/145</td>
<td>LexA-TADIII</td>
<td>66 ± 12</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*β-Galactosidase activities were determined as described previously (12) on at least five separate transformants following growth in glucose-containing medium at the permissive temperature of 30°C. The pBl840 reporter plasmid used contains one LexA operator upstream of the lacZ gene (12). LexA-TADIV contains residues 642 to 704 of ADR1 fused to LexA-202, LexA-B42 consists of the E. coli-derived B42 activator, LexA-TADIII contains residues 420 to 462 of ADR1, and LexA-TADII contains residues 263 to 359 of ADR1 (12).
ADR1 gene integrated into the genome: 16, 8, 4, and 1 copy, respectively (12, 15). Immunoprecipitating TBP with anti-TBP antibody from cells grown on ethanol-containing medium resulted in the coimmunoprecipitation of ADR1 from each of these strains containing a low dosage of ADR1 (Fig. 5, lanes 5 and 6; data not shown). The amount of ADR1 immunoprecipitated with anti-TBP antibody was proportional to the abundance of ADR1 present in the cell (Fig. 5, lanes 2 and 3; data not shown). In contrast, IPs using CCR4 antibody (Fig. 5, lanes 9 to 12) or preimmune serum (data not shown) did not immunoprecipitate ADR1. These data confirm that ADR1, when expressed at its normal physiological concentration, does associate with TFIID in vivo.

TAFII90 is required for ADH2 derepression. TAFII90 has been implicated in coactivator function in various in vitro systems by a number of studies (23, 33, 46). Recent results (27, 34, 43, 44) indicate that yeast TAFII90 are not universally required for transcription, although they are required for transcription of some yeast genes. By using a double shutoff system designed to eliminate TAFII90 (27), it was shown, for instance, that the expression of the GAL7 gene occurred in an unimpeded manner when cells were shifted from a repressing carbon source to a nonrepressing carbon source. We therefore used the same strain and protocol for depletion of TAFII90 to test if TAFs are required for derepression of ADH2 transcription. Yeast cells were depleted of TAFII90 (Fig. 6B) and then tested for the ability to activate the ADH2 gene upon depletion of glucose. As shown in Fig. 6A, in the control strain, ADH2 mRNA appeared 1 h after glucose removal. In contrast, in the strain depleted of TAFII90, ADH2 mRNA synthesis did not occur as normalized to the level of either 25S and 18S rRNA or the CCR4 mRNA standard. The observed failure to derepress ADH2 as a result of depletion of TAFII90 was not due to a decrease in ADR1 transcription, since ADR1 mRNA levels were found to be unaffected by TAFII90 depletion (data not shown). Also, use of this TAFII90 shutoff procedure has shown previously that HIS3, DED1, GAL7, CUP1, and SSA4 expression was unaffected by depletion of TAFII90 (27). TRP3 gene expression, however, was found to be sensitive to TAFII90 depletion (27). Other studies have indicated that inactivation...
of any one of the TAFs can have a general effect on the inactivation of other TAFs and, hence, TFIID in general (43). Our results suggest a requirement for intact TFIID in ADR1-dependent activated transcription.

**DISCUSSION**

The results reported herein demonstrate an association of ADR1 with the TFIID complex that appears to be important for ADR1 activation of ADH2. Six lines of evidence suggest that ADR1 acts through TFIID in vivo. (i) TADIV of ADR1 binds TFIID components from yeast crude extracts; (ii) the ability of derivatives of TADIV to bind TFIID components correlates with TADIV activation potential; (iii) deletion of TADIV from ADR1 severely compromises ADR1 function; (iv) the transcriptional potential of LexA-ADR1-TADIV was more severely compromised than that of other activation domains by a taf130/145 allele; (v) ADR1 coimmunoprecipitates with either TAF9 or TBP; and (vi) TFIID appears to be required for ADR1-dependent activation of ADH2 in vivo, as depletion of yeast cells of TAF9 prevents ADH2 derepression. These results correlate the in vitro binding of TFIID components by TADIV to the in vivo activation function by TADIV and to its dependency on TFIID. ADR1 physical association with TAF9 and TBP in vivo further correlates with the demonstrated TFIID requirement for ADH2 derepression. These data suggest a model whereby ADR1 either aids recruitment of TFIID to the promoter, induces structural reconfiguration of TFIID, or stimulates both processes, thereby allowing transcription to occur.

The relative proportion of TBP bound to GST-TADIV of the total present in the crude extract (Fig. 2) is likely to be about 1/10 of that of the TAF9p8 as determined by densitometric analysis (data not shown). Since there is approximately 10 times as much TBP in the cell as there are individual TAF9p8 in the cell (26a, 27, 43), it appears that TBP and TAF9p8 were binding stoichiometrically to GST-TADIV. Since TBP (9) and TAF9p25 (unpublished observation) do not bind GST-TADIV in the absence of other yeast proteins, these data suggest that TBP and the TAF9p8 were binding GST-TADIV as a complex. The ability of TADII to retain TBP and TAF9p130/145 from crude extracts further suggests that ADR1 activation domains other than TADIV may be important to ADR1-TFIID interactions. TADII may make a limited interaction with a subset of the TFIID complex. Although the physiological relevance of this interaction is unclear, a taf130/145 allele did result in a slight decrease in the ability of LexA-TADII to activate a LexA-lacZ reporter.

We had shown previously (9) that ADR1 TADs can also contact ADA2, GCN5, and TFIIB in vitro. Since ADR1 is required both for chromatin remodeling and for subsequent transcriptional activation (41, 42), it is likely that ADR1 makes multiple contacts to initiate transcription. The facts that two ADR1 molecules bind to its cognate DNA binding site (38) and that ADR1 contains at least four separate activation domains (9) further support the likelihood of distinct multiple interactions by ADR1. Because TADIV of ADR1 uses the same apparent sequence determinants for interactions with TFIID and GCN5 (695 to 704 of TADIV), it may be that the same ADR1 region binds both GCN5 and TFIID. ADR1 TADIV may first contact GCN5 to aid in nucleosome rearrangement and then contact TFIID. Other ADR1 activation domains may contribute to activation through their specific contacts with other components of the GCN5-ADA2 complex or with TFIIB (9). The GCN4 activator in yeast has also been shown to make multiple contacts by using the same apparent sequence determinants to do so. GCN4 has been shown to bind TAF9p8, the ADA2 and ADA3 proteins, and components of the RNA polII holoenzyme (21).

Notwithstanding the above considerations, it remains possible that the ADR1-TFIID interaction is dependent on other transcription factors. Since GCN5-ADA2 could make contact with TAF9p8 and TBP (4, 21), it may be that ADR1 interactions with GCN5 recruit TFIID. However, we have been unable to demonstrate an in vivo association of GCN5 with ADR1 as we have done with TFIID and ADR1, suggesting that GCN5 does not mediate TFIID interaction with ADR1.

We also observed that ADR1, when overexpressed under glucose growth conditions (at least eightfold), coimmunoprecipitated with TAF9p90 or TBP and did so in proportion to its abundance in the cell (unpublished observations). This level of overexpression of ADR1 allows partial derepression of the ADH2 gene on glucose, indicating that the ADR1 protein is functional (15). These results suggest that even in the presence of glucose, ADR1 can still make appropriate contacts with TFIID at the ADH2 promoter. Previous results have also indicated that ADR1 activation functions are not controlled by glucose (12). It is likely, therefore, that glucose repression affects not the ADR1-TFIID interaction specifically but other aspects of ADR1-dependent ADH2 promoter function. These other aspects include regulation of ADR1 protein (19, 40), control of ADR1-dependent chromatin remodeling that occurs only under derepressing conditions (41, 42), and binding of a repressor to ADR1 (12).

**ACKNOWLEDGMENTS**

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