ADR1 Activation Domains Contact the Histone Acetyltransferase GCN5 and the Core Transcriptional Factor TFIIB

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ADR1 Activation Domains Contact the Histone Acetyltransferase GCN5 and the Core Transcriptional Factor TFIIB*

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The yeast transcriptional activator ADR1, which is required for ADH2 and peroxisomal gene expression, contains four separable and partially redundant activation domains (TADs). Mutations in ADA2 or GCN5, encoding components of the ADA coactivator complex involved in histone acetylation, severely reduced LexA-ADR1-TAD activation of a LexA-lacZ reporter gene. Similarly, the ability of the wild-type ADR1 gene to activate an ADH2-driven promoter was compromised in strains deleted for ADA2 or GCN5. In contrast, defects in other general transcription cofactors such as CCR4, CAF1/POP2, and SNF/SWI displayed much less or no effect on LexA-ADR1-TAD activation. Using an in vitro protein binding assay, ADA2 and GCN5 were found to specifically contact individual ADR1 TADs. ADA2 could bind TAD II, and GCN5 physically interacted with all four TADs. Both TADs I and IV were also shown to make specific contacts to the C-terminal segment of TFIIB. In contrast, no significant binding to TBP was observed. TAD IV deletion analysis indicated that its ability to bind GCN5 and TFIIB was directly correlated with its ability to activate transcription in vivo. ADR1 TADs appear to make several contacts, which may help explain both their partial redundancy and their varying requirements at different promoters. The contact to and dependence on GCN5, a histone acetyltransferase, suggests that rearrangement of nucleosomes may be one important means by which ADR1 activates transcription.

In Saccharomyces cerevisiae, the transcriptional activator ADR1 is required for expression of the glucose-repressible alcohol dehydrogenase gene (ADH2) under nonfermentative conditions (1). It also regulates genes required in glycerol metabolism (2, 3) and peroxisome function and biogenesis (4, 5). ADR1 is a zinc finger, DNA-binding protein that is 153 kDa in size (6, 7). Its regulation of ADH2 under nonfermentative growth conditions occurs by binding to UAS1, a palindromic sequence (bp) located 110 upstream of the ADH2 TATAA sequence (7). Similar UAS1 elements are located upstream of other genes that ADR1 controls (4, 8). Three regions of ADR1 have been identified that are required for its efficient activation of ADH2 transcription: transcription activation domain (TAD) I (76–172), TAD II (263–357), and TAD III (359–509) (2, 9, 10, 11). A fourth region (642–1323) has been implicated for efficient peroxisomal gene expression (5). The presence of four transactivation regions suggests that ADR1 may make multiple protein contacts to transcriptional cofactors and/or core transcriptional components. The observation that TADs II and III are functionally redundant (9) suggests that some of these contacts may be made to the same protein.

There are a number of potential targets for ADR1 activation domains. Core transcriptional components including TBP, TFIIB, TFIIF, TFIIE, and TAFs have been implicated in mammalian systems as being direct contacts for transcriptional activators (12). In yeast, the GAL4 activation domain has been shown to bind TBP but not TFIIB in vitro (13). In addition to these core transcriptional factors, other cofactors or coactivators may mediate the action of activators. The ADA2 complex is one such coactivator complex. These proteins have been shown to bind activators like VP16 and GCN4 (14, 15) and to be required for maximal transcriptional activity of several yeast activators (16). However, some yeast activators like HAP4 and GAL4 (16, 17) are slightly affected or not affected by defects in the ADA2 complex. Because the ADA2 complex has been also shown to bind TBP (14), it has been suggested that the ADA2 complex acts as a direct mediator between activators and core factors. Recent evidence indicates that GCN5 is a histone acetyltransferase (18). Activator recruitment of the ADA2 complex may result, therefore, in histone acetylation that would help relieve a repressive chromatin structure.

Genetic studies have implicated several general transcriptional factors as possibly mediating ADR1 activator function. The CCR4 and CAF1/POP2 proteins, components of a multisubunit transcriptional regulatory complex, are required for proper expression of a number of yeast genes including ADH2 (19–22). In previous studies we have analyzed the dependence of ADR1 TADs on the CCR4 and CAF1 cofactors, but ccr4 and caf1 defects generally had only 2–3-fold effects on ADR1 TAD function (22, 23). Moreover, ccr4 and caf1 defects can affect ADH2 expression under conditions when ADR1 is inactive (19, 22). The SNF/SWI factors involved in nucleosomal remodeling (24) are also known to be important to ADH2 derepression (25–27). Consistent with the role of the SNF/SWI factors in ADH2 expression are the presence of two repressing nucleosomes at the ADH2 promoter that are removed in an ADR1-dependent manner during ADH2 derepression (28). Neither of the repressing nucleosomes in the ADH2 promoter occupies the

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UA51 site to which ADR1 binds (28), which may explain the limited effects of snf4Δ mutations on ADH2 derepression (25, 27).

In this paper we continue our investigation into the factors required for ADR1 function. We report that mutations in ADA2 and GCN5 severely compromise the ability of ADR1 TADs to activate gene expression in vitro. In addition, we demonstrate that there is physical interaction between ADA2 and GCN5 and the ADR1 TADs in vitro. TAD IV binding to GCN5 was shown to directly correlate with TAD IV activation function. TADs I and IV were also shown to make specific contacts to TFIIB but not to TBP. These results suggest that ADR1 TADs activate gene expression in yeast through direct physical contacts with multiple proteins.

EXPERIMENTAL PROCEDURES

Yeast Strains

Yeast strains are listed in Table I. Strain EGY188 was used for transformation with plasmids expressing LexA-ADR1 fusion proteins. The ada2::URA3 and gen5::URA3 disruptions in strain PSY316 were a gift from L. Guarente, and strains CY26 and CY57 were provided by C. Peterson.

Plasmid Constructions

LexA-ADR1 Fusions—All LexA-containing plasmids encoded full-length LexA (1-292) except for LexA-ADR1-(1-220), which contained only the DNA binding domain of LexA (residues 1-87). The LexA fusion proteins were expressed from an ADH1 promoter and were encoded in yeast on a 2 µ plasmid containing the selectable marker URA3 (9). Plasmids containing LexA-ADR1-(1-1323) (full-length), LexA-ADR1-(1-220)-TAD I, and LexA-ADR1-(262-559)-TAD II have been previously described (9). LexA-ADR1-(420-462)-TAD III was constructed following polymerase chain reaction using oligonucleotides designed to generate Nco I and Xho I sites at the ends of the 420-462 fragment of ADR1 (oligonucleotides: 5'-GTCATGCCATGGATCCTGATTTCGTCGAT-3' and 5'-GCGTACTCGAGAAGCTTGACGTGGCGGTAG-3'). The resultant ADR1 polymerase chain reaction product after cleavage with Nco I and Xho I was ligated into the Nco I-Xho I sites of plasmid LexA-ADR1-(262-559) (9) to generate LexA-ADR1-(420-462), LexA-ADR1-(642-704)-TAD IV and its deletion derivatives were derived from plasmid LexA-ADR1-(420-1323) (9) by selecting for LexA-ADR1-(642-1323) derivatives that could activate the LexAop-LEU2 reporter in strain EGY188. All LexA proterotrofes analyzed were the result of alterations in the amino acid region 672-704 of ADR1 that created truncated LexA-ADR1-(642-704)-TAD IV derivatives. Each of these proteins was expressed to a comparable extent as analyzed by Western analysis. Sequencing of each of these resultant plasmids revealed stop codons in the DNA sequence that were in agreement with the relative size of each of the LexA-ADR1-(420-704) protein derivatives.

GST-ADR1 Fusions—The construction of GST-ADR1-(1-292)-TAD I was conducted by removing residues 1-262 from pJc100 (29) with Nco I and Xho I and ligating into pGEM-5f (+) cut with Nco I and EcoRV. The resultant plasmid was then digested with Nco I and Sal I and ligated into Nco I and Xho I sites of pGEX-KG (30) to create GST-ADR1-(1-262), GST-ADR1-(263-359)-TAD II was constructed by inserting a BamHI-SalI fragment of LexA-ADR1-TAD II into BamHI-SalI sites of pGEX-KG. GST-ADR1-(420-462)-TAD III was constructed by inserting a Nco I fragment of LexA-ADR1-TAD III into the Nco I-HindIII sites of pGEX-KG. In the construction of GST-ADR1-TAD IV-(462-704), a DNA fragment from LexA-ADR1-TAD IV-(462-704) was synthesized by polymerase chain reaction with oligonucleotides 5'-GCTTCAACATTTGCTCGGTATT-3' and 5'-CCAGATTGCACAGTTGATGTCGTT-3' designed to create Smal-EcoRI restriction sites at the ends. This fragment was ligated in Smal-EcoRI sites of pGEX-KG that had been pre-cut with Xba I and BamHI, blunt ended with the large subunit of E. coli DNA polymerase (Klenow), and religated. For all other GST-ADR1-TAD IV truncations, a Smal-EcoRI fragment from the LexA-ADR1-TAD IV truncations was inserted into Smal-EcoRI sites of pGEX-KG whose BamHI site had been filled in with Klenow to create the proper reading frame. GST-Vpu contains the hydrophilic segment of the HIV protein Vpu (residues 33-81) and was a gift of B. Kemp (St. Vincent's Institute of Medical Research, Melbourne, Australia). GST-CAF1 and GST-CC4 were provided by J. Meegan and S. Fontaine (University of New Hampshire, Durham, NH), respectively.

Transformations, Enzyme Assays, and Growth Conditions

All yeast transformations were conducted by using the lithium acetate method (31). ADH II and ß-galactosidase enzyme assays were conducted as described (9). Conditions for growth of cultures on minimal medium lacking uracil and histidine or YEP medium containing either 8% glucose, 3% ethanol or 2% ethanol, 2% glycerol have been described elsewhere (9).

In Vitro Binding Assay

GST fusion proteins were expressed and bound to glutathione-agarose beads (Sigma) in binding buffer (1 × phosphate-buffered saline, 1% Triton X-100, 4 times with binding buffer and then incubated for 60 min at 4°C in A300 buffer (20 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol, 300 mM potassium acetate (KAc), 1% Triton) containing 1 mg/ml E. coli extract and 40–200 ng of [35S]methionine-labeled in vitro translated proteins. In vitro translation of T7 fusion proteins was carried out by using the TNT-coupled transcription translation system (Promega). Unbound proteins were removed by four washes with A300 buffer, and specifically bound proteins were analyzed by SDS-PAGE after elution with 50 mM reduced glutathione in 50 mM Tris, pH 8.0, or by boiling beads directly in sample buffer. High salt washes of radiolabeled proteins bound to GST fusions were conducted as described above except that A300 buffer was changed to the appropriate KAc concentration (600, 900, or 1200 mM). The T7-GCN5, -ADA2, and -ADA3 plasmids were gifts of L. Guarente (MIT). T7-TFIIB was provided by M. Hampsey (University of New Hampshire, Durham, NH), respectively.

Deletion of Residues 642–704 of ADR1 at Its Chromosomal Locus

The ADR1 moiety in pBR322–411B (32) was cut with Bgl II and Xho I and ligated into the 3.2-kb Xho I-Bgl II fragment of pBR322-411B. This fragment was then ligated into the Sma I and Xho I sites of pGEX-KG (30) to create GST-ADR1-(1-262), GST-ADR1-(263-359)-TAD II was constructed by inserting a BamHI-SalI fragment of LexA-ADR1-TAD II into BamHI-SalI sites of pGEX-KG. GST-ADR1-(420-462)-TAD III was constructed by inserting a Nco I fragment of LexA-ADR1-TAD III into the Nco I-HindIII sites of pGEX-KG. In the construction of GST-ADR1-TAD IV-(462-704), a DNA fragment from LexA-ADR1-TAD IV-(462-704) was synthesized by polymerase chain reaction with oligonucleotides 5'-GCTTCAACATTTGCTCGGTATT-3' and 5'-CCAGATTGCACAGTTGATGTCGTT-3' designed to create Smal-EcoRI restriction sites at the ends. This fragment was ligated in Smal-EcoRI sites of pGEX-KG that had been pre-cut with Xba I and BamHI, blunt ended with the large subunit of E. coli DNA polymerase (Klenow), and religated. For all other GST-ADR1-TAD IV truncations, a Smal-EcoRI fragment from the LexA-ADR1-TAD IV truncations was inserted into Smal-EcoRI sites of pGEX-KG whose BamHI site had been filled in with Klenow to create the proper reading frame. GST-Vpu contains the hydrophilic segment of the HIV protein Vpu (residues 33-81) and was a gift of B. Kemp (St. Vincent’s Institute of Medical Research, Melbourne, Australia). GST-CAF1 and GST-CC4 were provided by J. Meegan and S. Fontaine (University of New Hampshire, Durham, NH), respectively.

In Vivo Binding Assay

GST fusion proteins were expressed and bound to glutathione-agarose beads (Sigma) in binding buffer (1 × phosphate-buffered saline, 1% Triton X-100, 4 times with binding buffer and then incubated for 60 min at 4°C in A300 buffer (20 mM HEPES, pH 7.6, 1 mM EDTA, 1 mM dithiothreitol, 20% glycerol, 300 mM potassium acetate (KAc), 1% Triton) containing 1 mg/ml E. coli extract and 40–200 ng of [35S]methionine-labeled in vitro translated proteins. In vitro translation of T7 fusion proteins was carried out by using the TNT-coupled transcription translation system (Promega). Unbound proteins were removed by four washes with A300 buffer, and specifically bound proteins were analyzed by SDS-PAGE after elution with 50 mM reduced glutathione in 50 mM Tris, pH 8.0, or by boiling beads directly in sample buffer. High salt washes of radiolabeled proteins bound to GST fusions were conducted as described above except that A300 buffer was changed to the appropriate KAc concentration (600, 900, or 1200 mM). The T7-GCN5, -ADA2, and -ADA3 plasmids were gifts of L. Guarente (MIT). T7-TFIIB was provided by M. Hampsey (University of New Hampshire, Durham, NH), respectively.
β-Galactosidase activities (units/mg) represent averages of at least three separate transformants measured in isogenic strains PSY316 (wild type), PSY316-ada2 (ada2), and GMY26 (gen5) and the isogenic strains CY26 (SNF2), and CY57 (snf2) using the LexA-lacZ 1840 reporter gene as previously described (9). The 1840 reporter plasmid contains a single LexA operator site upstream of the GAL1-lacZ promoter. LexA-ADR1 fusions are described under “Experimental Procedures.” TADs I and II were defined as activation domains previously (9, 10); TAD III previously localized to residues 359–506 (9) was more precisely located to 420–462 (J. Ssario and T. Young, personal communication); and TAD IV was defined as shown in Fig. 3 and Table V. Strains were grown on minimal medium lacking uracil and histidine and supplemented with 8% glucose as previously described (9). LexA fusions were expressed to comparable levels as determined by Western analysis. The LexA-ADR1 fusion proteins were approximately 50-fold more abundant than single copy ADR1. S.E. values were less than 20% in each case.

The resulting construct was cut with SmaI and transformed into strain 500-16 to site-specifically integrate at the adr1-1 locus. Identification of single integrants and their subsequent analysis were conducted as described previously (33).

RESULTS

The ADA2 Complex Is Required for ADR1 Activation of Transcription—ADR1 contains three separate transcription activation domains, and a fourth activation region has been implicated in residues 642-1323 (5, 9). TAD IV was subsequently more precisely localized to residues 642–704 (see below). In order to identify the factors through which the four individual ADR1 TADs act, the effect of deleting different general transcription cofactors or adaptors on LexA-ADR1-TAD transactivation has been analyzed.

Deletion of the ADA2 or GCN5 gene dramatically reduced the ability of LexA-ADR1 (full-length) to activate the LexA-lacZ reporter gene (Table II). Moreover, the activation function of each LexA-ADR1-TAD was shown to be highly dependent on functional ADA2 complex components (Table II). The expression of the LexA-protein fusions was unaffected by deletions in ADA2 or GCN5 as analyzed by Western analysis (data not shown). An ada2 or gen5 deletion does not uniformly affect all LexA transactivators (16, 17), and its reduction of ADR1 activation ability (20–50-fold effects) appeared to be one of the most severe of the several activators previously tested (17, 34). The observation that both an ada2 and a gen5 deletion affected LexA-ADR1-TAD function is in agreement with previous results demonstrating that inactivation of any component of the ADA2 complex reduces the function of the complex (17, 35). In contrast to these results, the deletion of the SNF2/SWI2 gene, which is known to severely reduce the activity of GAL4 and other LexA transactivators (36), displayed little or no effect on ADR1 TAD function (Table II). Similarly, deletion of the SNF5 and SWI1 genes, two other components of the SNF/SWI complex, resulted in little or no diminution of ADR1 TAD function (data not shown). In addition, we have previously shown that mutation of either CCR4 or CAP1, two components of a multi-subunit transcription complex required for maximal ADH2 expression, had generally only 2–3-fold effects on LexA-ADR1-TAD activation of a LexA-lacZ reporter (22, 23).

In order to verify the physiological relevance of the requirement of the ADA2 complex for ADR1 activation, we examined whether the activation ability of the wild-type full-length ADR1 protein at its normal cellular concentration was also compromised. We found that deletion of the ADA2 or GCN5 gene dramatically reduced the ability of ADR1 to activate an ADH2-lacZ reporter under derepressed growth conditions (Table III). The derepression of the ADH2-lacZ reporter gene has been shown previously to be strictly dependent on ADR1 (7), and ADH2-lacZ contains all of the promoter sequences necessary for complete derepression of ADH2 (37). In contrast, an ada2 or gen5 deletion had at most 2-fold effects on the similar CYC1-lacZ reporter gene, which is under the control of the HAP1 and HAP2/3 activators (Table III). Relatively, the LexA-HAP4 transactivation ability is not affected by ada2 or gen5 deletions (16, 17).

We subsequently analyzed the effect of ada2 and gen5 defects at the wild-type ADH2 promoter. ADH2 expression was reduced 2.5-fold by an ada2 deletion under ethanol growth conditions (Table IV). Similarly, the ability of an ADR1 allele to bypass glucose repression and allow increased ADH2 expression was reduced about 3-fold by an ada2 defect (Table IV). The ada2 allele, in contrast, had no effect on spt10-enhanced ADH2 expression under glucose-repressed conditions, which occurs in an ADR1-independent manner (Table IV) (19). A gen5 allele also reduced the ability of the ADH2 gene to derepress (Table IV). These results indicate that the ADA2 complex is required for ADR1-dependent activation of transcription in different promoter contexts.

Components of the ADA2 Complex Physically Interact with Each of the TADs of ADR1—Since the activation ability of the ADR1 TADs was strongly ADA2 complex-dependent, we tested if this dependence was the result of ADA2, GCN5, and ADA3 proteins making direct contacts with ADR1 TADs. GST fusions to individual ADR1 TADs were constructed (Fig. 1A), and their ability to bind to [35S]methionine-labeled in vitro translated ADA2, ADA3, and GCN5 proteins (Fig. 1B) was examined (Fig. 1C). As shown in Fig. 1C, second row, GST-ADR1-TAD II could bind the ADA2 protein. ADA2 did not bind control proteins GST and GST-Vpu, nor did it display significantly increased binding to GST-ADR1-TAD I, III, or IV (Fig. 1C). In control experiments, in vitro translated luciferase was incubated with each of the GST-ADR1-TADs and GST, and no binding to any of these fusion proteins was observed (Fig. 1C). In vitro translated ADA3 did not bind to any of the four GST-ADR1-TADs (Fig. 1C, third row). In contrast, in vitro translated GCN5, while incapable of binding to GST alone, did bind to all four GST-ADR1-TADs (bottom row). GCN5, however, did not display binding to other GST-fusions such as GST-CAP1 or GST-Vpu (bottom row). For ADA2 binding to TAD II and GCN5 binding to each of the TADs, generally about 1–5% of the input radioactivity was retained by the GST-TADs (data not shown).

The stability of binding of ADA2 and GCN5 to ADR1 TADs was further tested by determining the effect of increasing salt concentrations on each of these interactions. As shown in Fig. 2, the binding between GST-ADR1-TAD II and ADA2 was

<table>
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<th>β-Galactosidase activity (units/mg)</th>
<th>wt</th>
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<th>gen5</th>
<th>SNF2</th>
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<td>ADR1-(1–1323) (full-length)</td>
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<td>100</td>
<td>58</td>
<td>1100</td>
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<tr>
<td>ADR1-(220–262) TAD I</td>
<td>23</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>ADR1-(262–359) TAD II</td>
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<td>7.5</td>
<td>280</td>
<td>220</td>
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<tr>
<td>ADR1-(420–462) TAD III</td>
<td>46</td>
<td>1.3</td>
<td>1.8</td>
<td>41</td>
<td>22</td>
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<tr>
<td>ADR1-(642–704) TAD IV</td>
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<td>4.3</td>
<td>5.0</td>
<td>160</td>
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<tr>
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<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>&lt;2</td>
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</table>
stable to salt concentrations up to 1.2 M KAc. Similarly, the binding between GST-ADR1-TAD IV and GCN5 was relatively insensitive to increasing salt concentrations (Fig. 2). GCN5 binding to ADR1 TADs I, II, and III was also stable at high salt concentrations (Fig. 2). These results suggest that the dependence of ADR1 TAD activation on the ADA2 complex was the result of specific ADR1 TAD interactions with GCN5 and ADA2.

**Binding of GCN5 to ADR1 TAD IV Deletion Derivatives Correlates with Their Ability to Activate Transcription**—Deletion analysis of ADR1 suggested a fourth possible activation domain in the C-terminal 642-1323 region (9). We have subsequently shown that LexA-ADR1-(642–704) is capable of activating its regions important for activation and binding to GCN5. To further examine the specificity of this interaction, the C-terminal half of TFIIB (residues 135–345) was found to retain the ability to bind to both TADs I and IV, whereas the N-terminal segment of TFIIB (residues 1–134) did not bind GST-TADs I or IV (Fig. 5). In contrast to TFIIB, TBP displayed little or no increased binding to GST-ADR1-TADs I or IV as compared with the control GST-Vpu (Fig. 4). As shown with GCN5 binding to these TAD IV deletion variants, the correlation between TAD IV activation ability and binding to TFIIB was nearly exact (Fig. 3A).

The region of TFIIB that interacted with TAD I and TAD IV was localized using the in vitro binding assay in order to further examine the specificity of this interaction. The C-terminal half of TFIIB (residues 135–345) was found to retain the ability to bind to both TADs I and IV, whereas the N-terminal segment of TFIIB (residues 1–134) did not bind GST-TADs I or IV (Fig. 5). In contrast to TFIIB, TBP displayed little or no increased binding to GST-ADR1-TADs I or IV as compared with the control GST-Vpu (Fig. 4). TBP also did not bind TAD III and interacted with TAD II only about 2-fold better than to GST-Vpu, suggesting that this interaction between TAD II and TFIIB may not be significant.

**TADs I and IV of ADR1 Bind Specifically to TFIIB**—The observation that a gen5 or ada2 disruption resulted in only partial blockage of ADR1 activation of ADH2 (Table IV) suggested that ADR1 was interacting with factors in addition to the ADA2 complex. Because it is known that transcriptional activators can make contacts to several different cofactors and core transcriptional components, we analyzed further whether ADR1 TADs could bind the transcriptional factor TFIIB. Incubation of in vitro translated yeast TFIIB with GST-ADR1-TAD fusions demonstrated that TAD I and to a lesser extent TAD IV could retain TFIIB (Fig. 4). In vitro translated TFIIB, in contrast, did not display significant binding to GST alone, GST-Vpu, GST-TAD II, or GST-TAD III (Fig. 4). TFIIB was able to bind about 5-fold better to GST-TAD II than to GST alone but only about 2-fold better than to GST-Vpu, suggesting that this interaction between TAD II and TFIIB may not be significant. The binding of TFIIB to TADs I and IV was also stable to high salt washes (1.2 M KAc) (data not shown). The region of TAD IV that was capable of binding TFIIB was analyzed using the series of GST-TAD IV variants described above. Deletions of TAD IV that reduced its activation function (Fig. 3A) similarly reduced its binding to TFIIB (Fig. 3B). As shown with GCN5 binding to these TAD IV deletion variants, the correlation between TAD IV activation ability and binding to TFIIB was nearly exact (Fig. 3A).

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### Table III

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>ADH II</th>
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<tr>
<td>Reporter gene</td>
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<td></td>
<td></td>
</tr>
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<tr>
<td>CYC1-lacZ</td>
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### Table IV

The effect of ada2 and gen5 alleles on ADH2 expression

ADH2 enzyme activities were conducted as described under “Experimental Procedures.” Strains were grown either on 8% glucose (R) or 3% ethanol (DR). Wild type, strain 612–1d; ada2, isogenic to 612–1d except ada2::ura3; gen5, segregants from cross-EGY188-g1 (gen5) and 612–1d-a1; adr1, data taken from Ref. 9. ADR1–5c ada2, and ADR1–5c segrengants were from cross-612–1d-a1 (ada2) and 787–6b (ADR1–5c); spt10 ada2, and spt10 segrengants were from cross-612–1d-a1 (ada2) and 1005–2-3b (spt10). For the wild-type segrengants from cross-EFY188-g1 and 612–1d-a1, ADR1–5c ada2; trp1–5c ada2; gcn5, data taken from Ref. 9. ADR1–5c ada2; trp1–5c ada2; gcn5, strains were grown in minimal medium lacking uracil and containing 2% each of glycerol and ethanol (DR) or 8% glucose (R). S.E. values were less than 20%.
Fig. 1. Binding of ADR1 TADs to ADA2 and GCN5. A, Coomasie-stained GST-TAD fusions. GST fusions were induced as described (30), bound to glutathione-agarose beads, and eluted from glutathione-agarose beads by boiling and separation by SDS-PAGE. Fifty μg of GST-TAD fusion is represented in each lane. GST-TAD I contains residues 1–262 of ADR1; GST-TAD II contains residues 262–359; GST-TAD III contains residues 420–462; and GST-TAD IV contains residues 642–701. B, in vitro translated proteins. T7 fusion proteins were translated in vitro with [35S]methionine as described under “Experimental Procedures.” One μl of radioactive proteins was separated by SDS-PAGE and identified following fluorography. C, binding of in vitro translated proteins to GST-TAD fusions bound to glutathione-agarose beads. Forty μl of in vitro translated proteins were incubated with 50 μg of GST fusions and, after washing, eluted by boiling. Similar results were obtained when bound proteins were eluted with excess glutathione. In general, a protein was considered to bind specifically if at least 15–20-fold more radioactive protein was retained by the GST-TAD than by the GST-Vpu control and 20–40-fold more than for GST as observed in at least three separate in vitro binding analyses. Densitometric analysis using an EC-210 densitometer was used to quantitate relative intensities of bound proteins. Top row, binding of radioactive luciferase to GST and GST-TADs; second row, binding of ADA2 to GST, GST-Vpu, and GST-TADs; third row, binding of radioactive ADA3 to GST and GST-TADs; bottom row, binding of radioactive GCN5 to GST, GST-Vpu, GST-CAF1, and GST-TADs.

**DISCUSSION**

The ADA2 Complex Mediates ADR1 Activation—ADIR1 is the primary transcriptional activator of the ADH2 gene and has been shown previously (9) and herein to contain four separable domains capable of activating transcription. These individual activation domains were found to be highly dependent on a functional ADA2 complex for their ability as LexA fusions to activate a LexA reporter system. Individual LexA-TAD fusions were nearly completely blocked for activation when either the ADA2 or GCN5 gene was deleted. Other LexA-transactivators such as LexA-GCN4 have also been shown to function at reduced levels when components of the ADA2 complex are deleted (17, 34). In contrast, LexA fusions such as LexA-HAP4 are nearly fully transcriptionally active in the absence of components of the ADA2 complex (17). Full-length ADR1, when fused to LexA, also displayed a 20–30-fold reduction in activation function when components of the ADA2 complex were defective. The observation that LexA-ADR1 (full-length) retained a substantial, albeit reduced, function suggests that the activation domains in full-length LexA-ADR1 in this context must also be capable of binding targets in addition to the ADA2 complex.

Several other cofactors known to be required for ADH2 derepression were similarly investigated in their requirement for LexA-ADR1-TAD function. Defects in components of the SNF/SWI complex had essentially no effect on ADR1 activation of the LexA-lacZ reporter in contrast to their sizable reduction in LexA-GAL4 activation of the same reporter gene (36). The CCR4 and CAF1 transcriptional regulatory factors that form a multisubunit complex reduced LexA-ADR1-TAD function generally about 2–3-fold (22, 23). This dependence on CCR4 and CAF1 appears, however, to represent an indirect requirement, since in vitro binding assays have been unable to substantiate any specific interaction between ADR1 TADs and CCR4 or CAF1 (23) and since ccr4 and caf1 can affect ADH2 expression independent of ADR1 activity (19, 22). Core transcriptional factors may represent other potential targets for ADR1 (see below).

The ADR1 activation of transcription of the ADH2-lacZ reporter was also severely reduced by defects in the ADA2 complex. In addition, ada2 and gcn5 deletions reduced the ability of ADR1 to activate the ADH2 gene at its chromosomal location. These results confirm the importance of the ADA2 complex in ADR1 activation. Yet, the fact that an ada2 or gcn5 disruption does not give the same ADH2 phenotype as an ADR1 deletion highlights the fact that at the ADH2 locus ADR1 must also be capable of making additional contacts to activate transcription. It should be noted that the effect of ada2 or gcn5 deletions on ADR1 activation ability was more severe when the reporter gene (either LexA-lacZ or ADH2-lacZ) was on a high copy plasmid than when it was positioned in the chromosome (the ADH2 locus). The cause of this difference is unclear, although the plasmid-borne promoters and the chromosomal promoter may differ in terms of chromatin structure or in assembly of ADA2 complex-dependent transcription complexes.

Physical Interaction of ADR1 TADs with Components of the ADA2 Complex—Each of the ADR1 TADs could selectively bind components of the ADA2 complex. ADA2 interacted with TAD II and GCN5 with each of the TADs. These interactions appear specific for several reasons. First, for the interactions of GCN5 and ADA2 to the ADR1 TADs to be considered significant, at

![Fig. 2. Stability of TAD binding to ADA2 and GCN5 to high salt washes. After binding of radioactive proteins to GST-TADs as described in Fig. 1, different wash conditions were used. The numbers refer to the concentration (in mM) of KAc used in the wash buffer. Bound proteins were eluted with boiling and detected with fluorography following SDS-PAGE.](http://www.jbc.org/Downloaded from http://www.jbc.org/ at UNIV OF NEW HAMPSHIRE on February 17, 2015)
es of ADR1-TAD IV activation ability correlates with binding to GCN5 and TFIIB. A, effect on LexA-ADR1-(642–704) transcriptional activity by C-terminal deletions. LexA-ADR1-(642–704) and its deletion derivatives were derived as described under “Experimental Procedures.” Each derivative was expressed to a comparable extent in yeast strain EGY188 as analyzed by Western analysis (data not shown). β-Galactosidase activity represents averages of at least three separate transformants and were assayed as described in Table II. S.E. values were less than 20%. The LexA reporter was p1840 in all cases. Relative binding to GCN5 or TFIIB compares the amount of radiolabeled GCN5 or TFIIB bound to the GST-ADR1-TAD IV derivative with the amount bound to GST alone. Data for relative binding was obtained following densitometric analysis of the data displayed in B. B, the effect of C-terminal deletions of LexA-ADR1-(642–704) on its ability to bind GCN5 or TFIIB. GST-ADR1-TAD IV derivatives of length as indicated in the figure were expressed in E. coli and bound to glutathione-agarose beads as described under “Experimental Procedures.” The bottom row is a Coomassie Blue-stained SDS-PAGE separation of 50 μg of each of these proteins and illustrates the relative abundance of each of the GST-derivatives used for binding. The top row displays the binding of GCN5 to each of the GST-derivatives as analyzed by SDS-PAGE and fluorography, whereas the middle row displays the binding of TFIIB to each of the GST-derivatives.

least 20-fold more GCN5 or ADA2 had to be retained by the GST-ADR1-TAD constructs than by the GST and GST-Vpu controls (See legend to Fig. 1). In many cases, up to 30–40-fold more GCN5 was bound to the target than to the controls: e.g. GCN5 binding to GST-ADR1-TAD IV and GST-ADR1-TAD III. Second, GCN5 failed to bind to other GST fusions such as GST-Vpu and GST-CAP1. ADA2 also failed to bind well to TAD I, III, or IV. Each of the significant bindings of an ADR1 TAD to ADA2 or GCN5 was, moreover, shown to be stable even under high salt wash conditions. This result suggests that these contacts are not solely dependent on ionic interactions. Finally, the GST-ADR1-TADs, whereas they could bind ADA2 or GCN5, failed to bind a number of radioactively labeled proteins including luciferase, ADA3, CAF1, CCR4, TBP, and N-TFIIB.
acetylation would presumably relax the nucleosomal structure at the promoter and facilitate nucleosomal rearrangement. Since, upon derepression, ADR1 is required at the promoter and facilitate nucleosomal rearrangement. As acetylation would presumably relax the nucleosomal structure at the promoter and facilitate nucleosomal rearrangement.

References

Nucleic Acids, Protein Synthesis, and Molecular Genetics:
ADR1 Activation Domains Contact the Histone Acetyltransferase GCN5 and the Core Transcriptional Factor TFIIB

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