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Artificial Recruitment of Mediator by the DNA-Binding Domain of Adr1 Overcomes Glucose Repression of *ADH2* Expression▽

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The transcription factor Adr1 activates numerous genes in nonfermentable carbon source metabolism. An unknown mechanism prevents Adr1 from stably binding to the promoters of these genes in glucose-grown cells. Glucose depletion leads to Snf1-dependent binding. Chromatin immunoprecipitation showed that the Adr1 DNA-binding domain could not be detected at the *ADH2* promoter under conditions in which the binding of the full-length protein occurred. This suggested that an activation domain is required for stable binding, and coactivators may stabilize the interaction with the promoter. Artificial recruitment of Mediator tail subunits by fusion to the Adr1 DNA-binding domain overcame both the inhibition of promoter binding and glucose repression of *ADH2* expression. In contrast, an Adr1 DNA-binding domain–Tbp fusion did not overcome glucose repression, although it was an efficient activator of *ADH2* expression under derepressing conditions. When Mediator was artificially recruited, *ADH2* expression was independent of *SNF1*, SAGA, and Swi/Snf, whereas *ADH2* expression was dependent on these factors with wild-type Adr1. These results suggest that in the presence of glucose, the *ADH2* promoter is accessible to Adr1 but that other interactions that occur when glucose is depleted do not take place. Artificial recruitment of Mediator appears to overcome this requirement and to allow stable binding and transcription under normally inhibitory conditions.

The glucose-repressed genes of *Saccharomyces cerevisiae* are excellent models for studying regulated promoters (8, 39). Glucose depletion can increase transcription of these genes several hundredfold, and much is known about the activators and binding sites involved. For example, the *ADH2* (alcohol dehydrogenase) gene is regulated by the zinc finger activator Adr1, which binds to the 22-bp, palindromic UAS1 (2, 5, 22, 41, 50), and Cat8, a zinc knuckle transcription factor that binds to UAS2, a carbon source response element adjacent to UAS1 (20, 24, 25, 39, 47, 59). Adr1 and Cat8 directly activate numerous other genes in nonfermentative metabolism (24, 47). Unlike Cat8, whose levels are low in glucose-repressed cells, Adr1 is present in the nucleus under these conditions (5, 42). Although the UAS1 sequence is in a nucleosome-free region (51), Adr1 appears to be regulated at the level of promoter binding, since chromatin immunoprecipitation (ChIP) assays fail to detect binding under repressing conditions (47, 58). When glucose is depleted, Adr1 binds to its cognate promoters in a Snf1-dependent fashion (58). If Snf1 is activated in the presence of glucose by inactivating Reg1, the regulatory subunit of the PP1-type protein phosphatase, Adr1 binding and transactivation can be detected at a low level (18, 58).

In the presence of glucose, Adr1 appears to be competent to bind DNA. Adr1 purified from repressed cells binds the UAS1 sequence in vitro (49). A recombinant "mini-Adr1" with the DNA-binding domain (DBD) and one of its four activation domains forms preinitiation complexes on an immobilized DNA template and activates transcription using nuclear extracts from either repressed or derepressed cultures (58). Altering chromatin structure in vivo by deleting histone H3 N-terminal tails or histone deacetylase genes leads to promoter binding in the presence of glucose by Adr1 (46, 52). Overexpression of *ADR1* from a strong promoter, high-copy-number plasmid, or multiple integrated copies of the gene leads to a weak constitutive expression of *ADH2*, which suggests that mass action can force DNA binding of Adr1 at sufficiently high concentrations (6, 15, 19, 27).

The mechanism that permits Adr1 promoter binding is unknown. Three possibilities are low-glucose-induced changes in chromatin structure, posttranslational modification of Adr1, or stabilizing interaction with coactivators. The chromatin structure of Adr1-dependent promoters undergoes a dramatic change in derepression (1, 51), but since these large changes require *ADR1*, they are presumed to occur after Adr1 interacts with the promoter. The region around the phosphorylated Ser230 of Adr1 appears to have an inhibitory influence on Adr1 activity (14, 16), but this is not part of the DBD, and an S230A mutation that enhances *ADH2* expression does not affect DNA binding assayed in vitro (49). The third possibility, not mutually exclusive of the others, is stabilization by coactivators that are recruited to the promoter upon derepression. Tanaka (48) found that activation domains influence transcription factor DNA binding, with increased numbers of activation domains corresponding to an increase in stable binding, suggesting the possibility that interactions with the recruited initiation complex contribute to binding.

To study the requirements for stable binding by Adr1, we analyzed Adr1 binding by ChIP and assessed *ADH2* expression by quantitative real-time PCR (qRT-PCR). To test the hypothesis that Adr1 binding is stabilized through coactivator inter-

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TABLE 1. Yeast strains

Strain	Relevant characteristics	Reference or source
TYY201 (W303-1a)	*MAT***a** *ade2 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	57
TYY202	TYY201 *adr1*Δ1::*LEU2*	57
TYY203	TYY201 *ADH2*::YIp*ADH2*/*lacZ*	57
TYY204	TYY202 *ADH2*::YIp*ADH2*/*lacZ*	57
TYY309	PJ69-4a (*MAT***a** *trp1-901 leu2-3,112 ura3-52 his3-200 gal4*Δ *gal80*Δ *GAL2-ADE2 LYS2*::*GAL1-HIS3 met2*::*GAL7-lacZ*) Δ*adr1*Δ*1*::*LEU2*	This study
TYY317	TYY309 *gcn5*Δ	This study
TYY497	TYY204 (W303-1a) *adr1*Δ1::*LEU2 ADH2*::YIp*ADH2*/*lacZ*(*trp1*::*HIS3*)	This study
TYY498	TYY497 *snf1*Δ::*URA3*	This study
TYY540	TYY497 *med15*Δ::*NAT1*	This study
TYY541	TYY497 *med2*Δ::*kanMX*	This study
TYY542	TYY497 *med3*Δ::*kanMX*	This study
TYY543	TYY497 *med16*(*sin4*)Δ::*kanMX*	This study
TYY804	TYY497 *med17*(*srb4*)Δ::*NAT1* plus YCp*srb4-138*(*leu2*::*URA3*)	This study
ECY1	*MAT*α *his3-*Δ*200 leu2-3,112 trp1 ura3-52* YIp-*ADH2* promoter-*lacZ*::*URA3* Δ*adr1*::*NAT1*	This study
ECY4	ECY1 with *SNF1-ADR1*$_{(1–172\ DBD)}$-myc::*HIS3*	This study
ECY5	ECY1 with *MED15-ADR1*$_{(1–172\ DBD)}$-myc::*HIS3*	This study
ECY6	ECY1 with *GCN5-ADR1*$_{(1–172\ DBD)}$-myc::*HIS3*	This study
ECY10	ECY1 with *MED3-ADR1*$_{(1–172\ DBD)}$-myc::*HIS3*	This study
ECY11	ECY1 with *MED4-ADR1*$_{(1–172\ DBD)}$-myc::*HIS3*	This study
ECY12	ECY1 with *MED18-ADR1*$_{(1–172\ DBD)}$-myc::*HIS3*	This study
ECY13	ECY10 with *MED14*-HA::*kanMX*	This study
ECY14	ECY11 with *MED15*-HA::*kanMX*	This study
ECY15	ECY12 with *MED14*-HA::*kanMX*	This study
ECY16	ECY12 with *MED14*-HA::*kanMX*	This study
CTYTY61	*MAT*α *his3-*Δ*200 leu2-3,112 trp1 ura3-52* YIp-*ADH2* promoter-*lacZ*::*URA3*	This study
CTYTY66	*MAT*α *his3-*Δ*200 leu2-3,112 trp1 ura3-52* YIp-*ADH2* promoter-*lacZ*::*TRP1* Δ*snf1*::*URA3*	This study
CTYTY75	ECY10 with Δ*snf1*::*kanMX*	This study

action, we fused the Adr1 DBD to coactivator subunits and tested for the binding and activation of *ADH2*. This approach, known as activator bypass or artificial recruitment, has been used to study coactivator functions at the *CUP1* (38) and *GAL* (34) promoters and, more generally, to test the recruitment model of preinitiation complex formation (36). In some cases, recruiting Tbp, Mediator, Tafs, SAGA, or even Snf1 to a promoter is sufficient to transcribe a reporter in the absence of an activation domain (9–11, 21, 26, 33, 37, 56). Several features of Adr1 make it attractive for this analysis. Its domains and recognized promoters are extensively characterized, so instead of an engineered reporter system, we can use the chromosomal loci of activated genes to assay binding and gene expression. The DBD alone is transcriptionally inactive even when expressed from the strong *ADH1* promoter on a multicopy plasmid (31). When fused to the VP16 herpesvirus transcription activation domain, the DBD confers regulated expression upon a UAS1-containing reporter gene (42). UAS1 is nucleosome free in several Adr1-dependent promoters, including *ADH2* (1, 51), providing access to the chromatin. In addition, *ADH2* expression is Snf1 dependent like many glucose-repressed genes but is not repressed by Mig1 or other DNA-bound repressors (18, 31, 39), which makes its activation by Adr1 easier to study.

We found that the Adr1 DBD alone did not stably bind to the *ADH2* promoter, but when fused to a tail subunit of Mediator, it bound to the *ADH2* promoter even under repressing conditions. Moreover, the Adr1 DBD-Mediator fusion protein was able to activate the transcription of *ADH2* in the absence of Snf1 or subunits of SAGA or Swi/Snf. In contrast, *ADH2* expression activated by the artificial recruitment of Tbp was strongly glucose repressed. Thus, Mediator recruitment to the *ADH2* promoter may play an important role in overcoming glucose repression.

MATERIALS AND METHODS

Strains and primers. The *Saccharomyces cerevisiae* strains used are shown in Table 1. TYY309 and TYY317 are based on PJ69-4a (28). Epitope tags were introduced by the method of Knop et al. (32). Sequences of the oligonucleotides are available upon request. Yeast strains were grown as described previously (40). Repressing medium contained 5% glucose; derepressing medium contained 0.05% glucose.

ChIP and real-time qPCR. ChIP and gene-specific PCR with gel electrophoresis were performed as described previously (47). Real-time qPCR data from the ChIP experiments were generated with an MJResearch Chromo4 system, using ABI SYBRMaster mix. Data were analyzed using the method of Steger et al. (44) or of Bryant and Ptashne (7). Briefly, the amounts of DNA in the ChIP and total DNA samples were quantified relative to a standard curve for an Adr1-bound promoter and for a nonbound telomeric control region. The ratio of the DNAs was determined using the formula (ChIP $DNA_{bound\ promoter}$/total $DNA_{bound\ promoter}$)/(ChIP $DNA_{telomeric\ control}$/total $DNA_{telomeric\ control}$). The data are presented as the ratios of specific to nonspecific binding, expressed as percentages or increases over background measured with primers to the telomeric control.

RNA isolation from 10 to 20 ml of cells was performed by acid phenol extraction at 65°C for 1 h (13). Residual DNA in the RNA preparation was reduced by treatment with DNase (Ambion) by following the manufacturer's recommendations. cDNA synthesis was performed with SuperscriptIII (Invitrogen) by following the manufacturer's protocol. qRT-PCR for measuring mRNA levels was performed as described above, in duplicate, using a 1:300 dilution of the cDNA. A standard curve was generated from *ACT1* primers and used to quantitate all of the RNA levels.

Immunoprecipitations and Western blots. All antibodies were obtained from Santa Cruz Biochemicals (Santa Cruz, CA). Immunoprecipitations to concentrate samples for Western blots and coimmunoprecipitations were carried out as described by Strahl-Bolsinger et al. (45), without DNase I treatment and using 2 μg monoclonal antihemagglutinin (anti-HA) (F-7) or 6 μg monoclonal anti-myc (9E10). Western blot analyses were performed according to the manufacturer's

instructions for the Odyssey infrared imaging system (Licor Biosciences, Lincoln, NE), using a dilution of 1:500 to 1:1,000 of polyclonal anti-HA (Y-11) or monoclonal anti-myc (9E10) as the primary antibody.

Artificial recruitment strains. Plasmids encoding the 172 N-terminal amino acids of Adr1 fused to Med15 (Gal11) or Tbp were constructed by generating PCR fragments of the 284 C-terminal amino acids of Med 15 or the entire open reading frame (ORF) product of TBP (minus the first three amino acids). The primers generated PstI restriction sites for cloning into a pRS314-based plasmid containing a portion of the *ADR1* gene with a His_6 tag. Digestion with PstI and ligation of the PCR fragment allowed the insertion of the C terminus of Med15 or the Tbp ORF product at Adr1 amino acid 172.

Mediator-Adr1 protein fusion strains were created by integrating the portion of *ADR1* that encodes the DBD (amino acids 1 to 172), in frame, to the 3′ end of candidate genes, using a PCR-based epitope-tagging method (32). The integrating fragments also added a 3-myc tag and the *HIS3* marker. They were generated by PCR using the plasmid pEC2 as a template. To generate pEC2, a PCR fragment was made using the Roche Expand PCR kit with primers CTO ADR1 1–172 S2 and CTO ADR1 1–172 S3 and plasmid pYM4 (32) as a template. Yeast strain BY4741 was cotransformed with the resulting PCR fragment and the *ADR1*-containing plasmid pKD16 (19). In vivo recombination between pKD16 and the ADR1 1–172 PCR fragment truncated the *ADR1* ORF in pKD16 with a myc tag and *kanMX6* marker. The *kanMX6* marker was switched to *HIS3* (53) to generate pEC2. When used as the template in a PCR with primers that had 40 to 60 homologous nucleotides on either side of the stop codon of a target gene, a fragment was generated that would add *ADR1* encoding amino acids 1 to 172 and a 3-myc tag, all marked with *HIS3*, to the 3′ end of a target gene. Mediator-*ADR1* fusions were confirmed by colony PCR and Western blot analyses.

β-Galactosidase assays. β-Galactosidase assays (23) were performed using three cultures or transformants.

RESULTS

Adr1-DBD is not stably bound to the *ADH2* promoter. By several criteria, Adr1 has access to its binding site in the *ADH2* promoter even under repressing conditions. We hypothesized that one reason Adr1-promoter binding cannot be detected under these conditions is because stable binding requires the recruitment of coactivators. To test this hypothesis, we assayed for the binding of the Adr1 DBD to the *ADH2* promoter. The Adr1 DBD alone cannot activate transcription but can effect a slight remodeling of chromatin (17). To measure promoter binding directly, ChIP analysis was performed with the epitope-tagged Adr1 DBD. No occupation of the *ADH2* promoter could be detected (Fig. 1A). The same low levels of promoter DNA were detected in the ChIP samples by qPCR when samples from either repressing or derepressing conditions were assayed, levels that were comparable to the level of nonspecific binding to a telomeric control sequence. Since full-length Adr1 exhibits regulated binding and activation (58), and since fusion to an activation domain, either VP16 or TA-DIII of Adr1, allows the regulated transcription of *ADH2* (42), adding an activation domain to the Adr1 DBD is sufficient for stable binding and transcriptional activation.

Artificial recruitment of Mediator by Adr1 relieves glucose repression of *ADH2*. Since the primary known role of an activation domain is to bring coactivators to the promoter (36), we tested the effects of artificial recruitment of coactivators by fusing them directly to the Adr1 DBD. Fusion of the Adr1 DBD to Med15 (Gal11) (see the review by Biddick and Young [3] for nomenclature and the arrangement of subunits) and Med3 (Pgd1), two subunits of the tail module of Mediator, created fusion proteins that activated *ADH2* transcription in the presence and absence of glucose. Table 2 shows the activation of an *ADH2-lacZ* reporter gene by various fusion proteins and by wild-type Adr1. The first Adr1 DBD fusion protein tested, Adr1-Med15, contained the 280 C-terminal amino acids of Med15 fused to the C terminus of the Adr1 DBD. This fusion protein was 50 times more active under repressing conditions than wild-type Adr1, and its activation increased a further fivefold under derepressing conditions, reaching the same high level of activity as that promoted by wild-type Adr1 (Table 2). When the entire Med15 ORF product was fused in frame to the N terminus of the Adr1 DBD by an integrative targeting method, the fusion protein (designated Med15-Adr1 to distinguish it from the other Med15 fusion protein) was also active under repressing conditions (Table 2).

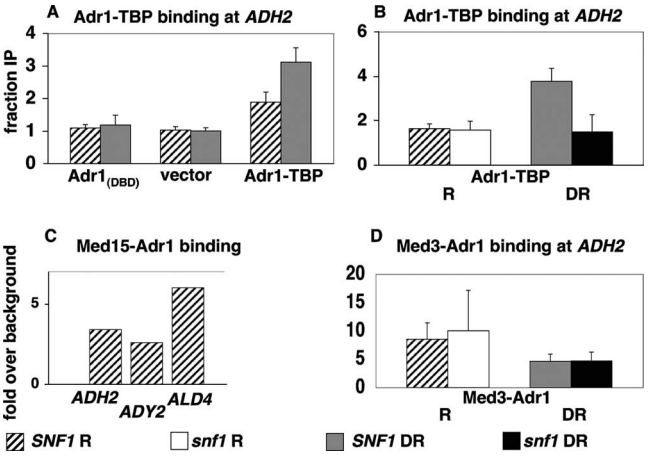

FIG. 1. Binding of Adr1 DBD fusions to Adr1-dependent promoters. ChIP was performed and the results were quantitated as described in Materials and Methods with strains with the indicated Adr1 DBD fusions or controls. (A) ChIP for the Adr1 DBD only (plasmid $pAdr1_{\Delta 172}$ [17]), negative control vector (pKD8 [17]), or Adr1-TBP (Materials and Methods), all in ECY1 and grown under repressing conditions (hatched bars) or after 4 h of derepression (gray bars). Data are expressed as the ratios described in Materials and Methods. (B) ChIP of Adr1-TBP in a *SNF1* strain (TYY497), repressed (R) (hatched bar) or 4-h derepressed (DR) (gray bar), and a *snf1*Δ strain (TYY498), repressed (white bar) or 4-h derepressed (black bar). Data are expressed as described for panel A. (C) ChIP of Med15-Adr1 under repressing conditions using strain ECY5. Data are expressed as increases over background from an untagged, repressed *adr1*Δ strain (ECY1). (D) ChIP of Med3-Adr1 in repressed *SNF1* (ECY10) (hatched bar) and *snf1*Δ (CTYTY75) (white bar) strains and in the same strains after 4 h of derepression (gray and black bars, respectively). Data are expressed as described for panel C. Error bars in panels A, B, and D indicate standard errors of the means for two or three biological replicates.

Fusion of the entire ORF product of another Mediator tail component, Med3, to the N terminus of the Adr1 DBD created a fusion protein that was even more active under repressing conditions than that created by the fusion of Med15 to the Adr 1 DBD (Table 2), and its activity was comparable to that of wild-type Adr1 under derepressing conditions. The high constitutive activation is not the result of overexpression of the fusion protein genes. Expression of the *MED3-ADR1* gene was 3.5-fold lower than that of the *ADR1* gene, as determined by qPCR of mRNA. Expression of *ADR1-MED15* was 2.3-fold higher than that of *ADR1*, yet this fusion is a weaker activator than Med3-Adr1. Also, *ADR1-MED15* was expressed from the same promoter on the same plasmid as an *ADR1*-TBP ORF

TABLE 2. Activation of an integrated *ADH2* promoter-*lacZ* reporter by coactivator-Adr1 DBD fusions

Strain[a]	Activator (on a plasmid or integrated)[b]	β-Gal activity[c]	
		Repressed	Derepressed
TYY204	None	5.4 (2)	55 (9)
	Wild-type Adr1	5 (2)	810 (100)
	Adr1-Med15 (Gal11)	260 (90)	1,400 (100)
ECY10	Med3 (Pgd1)-Adr1	1,000 (87)	980 (120)
ECY11	Med4-Adr1	18 (1.8)	56 (6.1)
ECY12	Med18 (Srb5)-Adr1	90 (1.8)	150 (8.8)
ECY5	Med15-Adr1	160 (31)	1,600 (540)
ECY4	Snf1-Adr1	14 (6.2)	68 (13)
ECY6	Gcn5-Adr1	44 (5.1)	110 (19)
TYY497	None	5 (1)	25 (5)
	ADR1-TBP	5 (2)	250 (4)
	Wild type	2 (1)	230 (10)

[a] Strains are described in Table 1. All strains are *adri*Δ.
[b] Activators for strains TYY204 and TYY497 were on plasmids, and the activators for ECY strains were integrated at the coactivator locus as described in Materials and Methods. Adr1-Med15 (Gal11) is the Adr1 DBD fused to the 280 C-terminal amino acids of Gal11 as described in Materials and Methods. It is carried on a *TRP1-CEN3-ARS1* plasmid. Adr1 DBD-Tbp fusion contains amino acids 1 to 172 of Adr1 fused at the C terminus to the entire *SPT15* ORF, as described in Materials and Methods, and is carried on a *TRP1-CEN3-ARS1* plasmid.
[c] β-Galactosidase activity is expressed in Miller units with standard deviations in parentheses. Derepression, 4 h in 0.05% glucose.

TABLE 3. Adr1-coactivator activation of *ADH2* expression measured by real-time qRT-PCR

Strain	Activator	*ADH2* mRNA level/*ACT1* mRNA level[a]	
		Repressed[b]	Derepressed[c]
TYY497	None	0.025 (0.0015)	NA[d]
	Wild-type Adr1	0.012 (0.01)	1.45 (0.08)
	Adr1-Med15	0.92 (0.09)	NA
CTYTY61	Wild-type Adr1	NA	1.3
ECY10	Med3-Adr1	1.9	15.5
ECY1	None	0.002	0.012
	Adr1-TBP	0.030	2.0

[a] Standard deviations, when measured, are given in parentheses.
[b] 5% glucose.
[c] For 4 to 6 h (0.05% glucose).
[d] NA, not assayed.

fusion, whose phenotype was very different (see below), even though its expression level should have been comparable.

Fusion of the Adr1 DBD to the C terminus of two other Mediator components, Med4 and Med18, subunits of the middle and head modules of Mediator, respectively, produced fusion proteins that were less active under both repressing and derepressing growth conditions than fusions to the tail subunits of Mediator (Table 2). Fusion of the entire ORF product of *GCN5*, a component of the coactivator SAGA, and the entire ORF product of *SNF1* to the Adr1 DBD produced fusion proteins that were weakly active, as assayed by reporter gene expression (Table 2). Wishing to concentrate on the strongest phenotypes, we used the Med3 and Med15 fusions for further expression analyses.

Fusion of the entire ORF product of TBP to the Adr1 DBD produced a fusion protein that was comparable in its regulation and activity to wild-type Adr1 (Table 2), activating *ADH2-lacZ* expression only in the absence of glucose. Thus, Tbp behaved like a typical activation domain when fused to the Adr1 DBD: it activated expression, and the activation of *ADH2-lacZ* expression was strongly glucose repressed.

To analyze the expression of Adr1-dependent genes from endogenous chromosomal loci, the activity of the Mediator and Tbp fusions to the Adr1 DBD was confirmed by qPCR analysis of *ADH2* mRNA. Consistent with the reporter assay data in Table 2, Adr1-Med15 activated *ADH2* under repressing conditions to nearly the wild-type derepressed level (Table 3). Fusion to the entire ORF product of the Mediator tail component *MED3* produced the most active fusion protein under both repressing and derepressing conditions (Table 3). Adr1-TBP stimulated a small amount of expression of the endogenous *ADH2* locus under repressing conditions, and activation under derepressing conditions was comparable to that of wild-type Adr1 (Table 3). In summary, while fusion of the Adr1 DBD to Gcn5, Tbp, or Snf1 produced weak constitutive activators, fusion to Mediator tail subunits could completely overcome the glucose repression of *ADH2* expression.

Artificial recruitment of Mediator by Adr1 causes constitutive DNA binding. ChIP assays were performed to see if the fusion proteins were affecting Adr1-dependent genes indirectly or directly. Med15-Adr1, Med3-Adr1, and Adr1-Med15 were detected at the *ADH2*, *ADY2*, and *ALD4* promoters under repressing conditions, suggesting that they activate *ADH2* expression directly (Fig. 1 and data not shown). In contrast, the Adr1 DBD-TBP fusion, which activated a low level of *ADH2* expression under repressing conditions (Table 2), showed only slightly higher than background levels of repressed *ADH2* binding, and binding increased approximately twofold under derepressing conditions (Fig. 1A and B). The low level of Adr1-TBP binding and the lack of binding seen for the Adr1 DBD only (Fig. 1A) indicate that constitutive binding and expression are neither general phenomena of all Adr1 DBD fusions nor properties of the Adr1 DBD itself, when liberated from its transactivation domains.

Adr1-Mediator fusions incorporate into Mediator complexes. Mutations in some Mediator components allow activator-independent gene expression (29, 54). Thus, an aberrant form of Mediator might act at *ADH2* outside the context of the normal Mediator complex. We used two assays to test for the possibility of anomalous Mediator activation. First, an *ADH2-lacZ* reporter gene was assayed in strains carrying an Adr1-Med15 plasmid and deleted for each one of the Mediator tail subunit genes. Expression was reduced under both repressing and derepressing conditions when *MED2*, *MED3*, and *MED16* were deleted but not when *MED15* was deleted, presumably because the fusion protein could functionally replace wild-type Med15 (Table 4). The requirement for other tail subunits suggests that Adr1-Med15 functions within the context of an intact Mediator tail.

Second, strains were constructed in which both a Mediator component and a Mediator-Adr1 DBD fusion were epitope tagged. Coimmunoprecipitations were performed to assay for in vivo interactions. Figure 2 shows that Med14 (Rgr1) coimmunoprecipitated with Med18-Adr1 and Med3-Adr1 and that

TABLE 4. Adr1-Med15 activation depends on the Mediator tail

Strain	Activator	Mediator mutation	β-Gal activity[a]		*ADH2* mRNA level/*ACT1* mRNA level[b]	
			Repressed conditions	Derepressed conditions	Repressed conditions	Derepressed conditions
TYY204	Adr1-Med15	None (WT)	260 (100)	1,400 (100)		
TYY540	Adr1-Med15	*med15* (*gal11*)Δ	400 (150)	1,200 (86)		
TYY541	Adr1-Med15	*med2*Δ	36 (14)	250 (18)		
TYY542	Adr1-Med15	*med3* (*pgd1*)Δ	18 (6.9)	410 (29)		
TYY543	Adr1-Med15	*med16* (*sin4*)Δ	120 (46)	210 (15)		
TYY309	Adr1	None (WT)			0.002	7.1
TYY309	Adr1-Med15	None (WT)			0.92	13
TYY317	Adr1-Med15	*gcn5*Δ			0.90	1.5

[a] Values are expressed as Miller units, with percentages of β-galactosidase activity of the wild-type Mediator shown in parentheses. The standard deviation of triplicate assays was about 20%. Derepression, overnight in 0.05% glucose.
[b] Derepression, 14 h in 0.05% glucose.

Med18 and Med15 coimmunoprecipitated with Med4-Adr1. Interaction between the fusions and subunits from different Mediator modules (30) suggested that the Mediator-Adr1 DBD fusions could be incorporated into Mediator complexes.

Artificial recruitment of Mediator overcomes the requirement for SAGA and Swi/Snf at the *ADH2* promoter. Most promoters require several coactivators for efficient transcription. *ADH2* expression, for example, requires Mediator, SAGA, NuA4, and Swi/Snf for the efficient recruitment of polymerase II, chromatin remodeling, and transcription (4, 12, 52). To assess the role of SAGA in *ADH2* expression when it is activated by artificial recruitment of Mediator, qRT-PCR analysis was used to measure transcript levels in the absence of the histone deacetylase component, Gcn5, of SAGA. As shown in Table 4, Adr1-Med15 activated *ADH2* expression to a high level in the absence of Gcn5, whereas wild-type Adr1 has a strong dependence on this coactivator subunit (12). *ADH2* activation by Adr1-Med15 was also uncompromised by the deletion of another component of SAGA (*ADA1*) or of an essential subunit of Swi/Snf (*SNF2*; data not shown). This suggested that direct recruitment of Mediator could overcome the requirement for additional coactivators and that coactivators may be redundant with regard to Adr1 stabilization.

Adr1 can bind in the absence of individual subunits of Mediator. To test the hypothesis that Adr1 binding can be stabilized by any of several coactivators, we tested for the binding of wild-type Adr1 and the activation of *ADH2* in Med15 and other Mediator mutants. When *MED15*, *MED3* (*PGD1*), or *MED2* was deleted from a strain with wild-type Adr1, *ADH2* derepression was significantly slowed, although not abolished. Deletion of *MED16* (*SIN4*) did not reduce *ADH2* expression. In each of the mutants, there was a low level of constitutive *ADH2* expression detected that was Adr1 dependent (Fig. 3A and data not shown). Since the deletion of *MED15* had the strongest effect on *ADH2* expression, the binding of Adr1 in the strain with this deletion was measured by ChIP analysis. There was no effect on Adr1 binding in the absence of *MED15*, and significant binding was detected under repressing conditions (Fig. 3B). Thus, the absence of Med15 did not significantly reduce Adr1 binding, although the early kinetics of expression could be significantly affected.

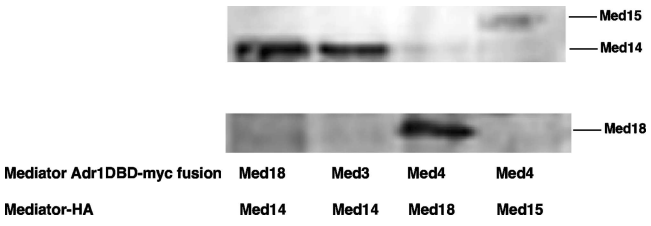

FIG. 2. Mediator subunits coimmunoprecipitate with Mediator-Adr1 DBD fusion proteins. Coimmunoprecipitation was performed with strains with Myc-tagged Mediator-Adr1 DBD fusions and HA-tagged Mediator components (ECY13, ECY14, ECY15, and ECY16). Immunoprecipitations with anti-Myc monoclonal antibodies were Western blotted with anti-HA polyclonal antibodies as described in Materials and Methods.

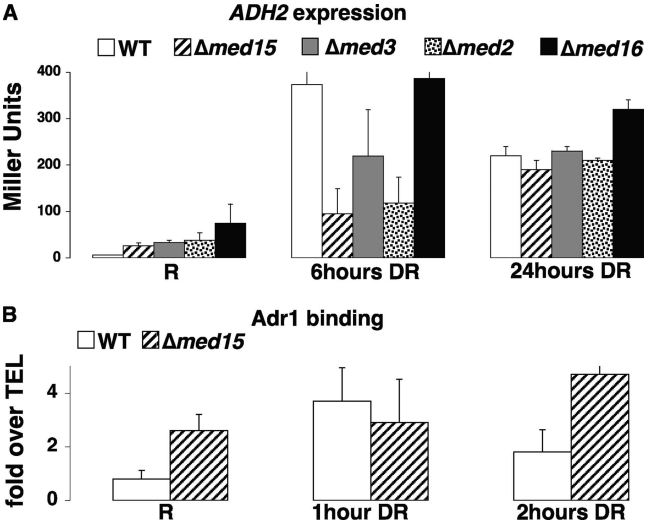

FIG. 3. Adr1 binding and activation in Mediator mutants. (A) Beta-galactosidase assays of strains TYY497 (wild type [WT]), TYY540 (*med15*Δ), TYY541 (*med2*Δ), TYY542 (*med3*Δ), and TYY543 (*med16*Δ), all with *CEN-TRP1* plasmid pKD16, which contains the *ADR1* gene with a C-terminal HA tag. Cultures were assayed under repressing conditions (R) or after 6 or 24 h of derepression (DR). (B) ChIP for Adr1 in the wild-type and *med15*Δ strains used for panel A under repressing conditions (R) or after 1 or 2 h of derepression (DR). Data are expressed as increases over background from an unbound telomeric (TEL) region. Error bars show standard deviations from triplicate measurements of each sample.

Transcription by artificial recruitment of Mediator does not require Snf1. Since Snf1 is normally required for promoter binding by Adr1 (58), we determined whether DNA binding and transcriptional activation by Mediator-Adr1 DBD fusions also require Snf1. Snf1 is inactive in in vitro kinase assays when isolated from glucose-grown cells (55), so it seemed likely that the expression caused by Adr1-Mediator fusions under repressed conditions would be Snf1 independent, even though the expression of most Adr1-dependent genes is Snf1 dependent (57). qPCR of RNA isolated from strains containing either wild-type Adr1 or Med3-Adr1 DBD fusions grown under repressing and derepressing conditions showed that the activation of several Adr1-dependent genes by Med3-Adr1 in the presence of glucose was independent of Snf1 (Table 5). As observed previously for *ADH2* (Table 3), the derepression of several other genes was elevated relative to their expression in the presence of wild-type Adr1 when Med3-Adr1 was the activator (compare the values in Table 5 for Med3-Adr1 and wild-type Adr1 for *SNF1*). The levels of *ADH2* and *ATO3* expression were comparable in the presence and absence of Snf1, and repressed expression was similar to activation in derepressed *SNF1* with wild-type Adr1 as the activator. The constitutive activation of *ALD4* and *ACS1* by Med3-Adr1 was lower than the activation of the derepressed wild type but still had a high degree of Snf1 independence. Many of the genes (*ADH2*, *ATO3*, *ALD4*, *ACS1*, the *FDH* genes, and *ADY2*) that were strongly Snf1 dependent when wild-type Adr1 was the activator showed enhanced derepression in the presence of Adr1-Med3 in the *snf1* mutant *ADH2* expression when Adr1-Med15 was the activator was also Snf1 independent (data not shown), indicating that Snf1 independence is not a unique property of the Med3-Adr1 fusion.

The Snf1-independent activation of Adr1-dependent genes suggested that the Mediator-Adr1 fusion might be causing a transcription enhancement of all glucose-repressed genes. To test this possibility, the transcript levels of several Snf1- and Cat8-dependent genes (*FBP1*, *MLS1*, *ICL1*, and *MDH2*) were measured. These genes were expected to be relatively unaffected by Med3-Adr1, since Adr1 makes a minor contribution to their derepression (57). The data in Table 5 show that under repressing conditions, Med3-Adr1 had no effect on the expression of *FBP1*, *ICL1*, and *MLS1* and activated *MDH2* about twofold. As expected, the derepression of these genes was strongly Snf1 dependent. Only *MDH2* derepression had a significant *SNF1*-independent component when Med3-Adr1 was present, suggesting that Med3-Adr1 can activate *MDH2* expression in a Snf1-independent manner. Med3-Adr1 reduced derepression of *FBP1*, *ICL1*, and *MLS1* about eightfold (Table 5), and an array analysis of gene expression in cells with Adr1-Med15 revealed both activating and repressing effects on some Cat8-dependent genes (unpublished data). These results are consistent with previous reports that Med15 can function in both repression and activation (35). With regard to the overall effects of the Adr1-Mediator fusions on glucose-repressed genes, the data indicated that the fusions were not acting as a nonspecific activator. They could, however, bypass the *SNF1* requirement for the activation of some Adr1-dependent genes.

If Adr1-Mediator fusions are able to overcome glucose repression in a Snf1-independent manner, promoter occupancy should be independent of Snf1. In agreement with this interpretation, quantitative ChIP showed that while the Adr1-TBP fusion was *SNF1* dependent like wild-type Adr1, Med3-Adr1 fusions occupied the *ADH2* promoter in *SNF1* wild-type and *snf1* deletion strains under repressing and derepressing conditions (Fig. 1B and D).

TABLE 5. Snf1-independent activation by artificial recruitment of Mediator

Gene	Growth condition[a]	Relative expression level with activator[b]:			
		Med3-Adr1 DBD[c]		Adr1 WT	
		SNF1	*snf1*	*SNF1*	*snf1*
ADH2	R	120	100	0.5	0.5
	DR	1,700	300	126	0.4
ATO3	R	41	67	6.3	3.4
	DR	266	150	50	4.5
ALD4	R	4.0	3.5	0.1	0.0
	DR	110	33	9.7	0.2
ACS1	R	20	6.0	1.1	0.9
	DR	950	120	190	3.9
FDH	R	0.2	0.2	0.2	0.2
	DR	56	4.6	2.3	0.4
ADY2	R	1.5	1.6	0.5	0.4
	DR	2,900	100	240	0.7
ICL2	R	0.9	0.8	0.9	0.7
	DR	18	0.9	7.3	1.2
FBP1	R	0.2	0.2	0.2	0.1
	DR	17	0.3	130	0.6
ICL1	R	1.5	1.7	1.1	0.8
	DR	4.9	1.5	87	1.4
MLS1	R	0.3	0.2	0.2	0.2
	DR	12	0.4	87	0.4
MDH2	R	4.3	3.0	1.9	1.5
	DR	58	12	43	3.9

[a] R, repression (5% glucose); DR, derepression for 6 h (0.05% glucose).
[b] mRNA levels were calculated by qRT-PCR as described in Materials and Methods. The quantities determined for each primer pair were divided by the quantity determined for the control *ACT1*. WT, wild type.
[c] Strains were ECY10, CTYTY75, CTYTY61, and CTYTY66.

DISCUSSION

The binding site for the Zn finger activator Adr1 is in a nucleosome-free region at several Adr1-regulated promoters, yet binding is not detected by ChIP under glucose-repressing conditions. Nonetheless, Adr1 appears to be competent to bind DNA, even when the glucose level is high. We tested binding by the DBD by ChIP analysis and were unable to detect promoter occupancy, suggesting that an activation domain is needed for stable promoter binding. We hypothesized that in the presence of glucose, Adr1 can bind its cognate promoters weakly and transiently but that its binding is not sufficiently stable to be detected by ChIP. Since Adr1 activation domains have been found to require SAGA components to be able to function and to interact with them in vitro (12), one possible stabilizing factor could be interaction with coactivator complexes that are recruited under derepressing conditions. We tested this possibility using an artificial recruitment assay. We fused the Adr1 DBD to coactivator subunits and found that Mediator tail fusions bound and activated several Adr1-dependent genes in the presence of the repressing carbon source glucose. Fusion to the Mediator head and middle components yielded weaker activators, possibly because of in-

correct orientation or steric hindrance when the Adr1 DBD was fused to this region of Mediator. Nonetheless, since the Mediator head and middle component fusions and the Gcn5 and Snf1 fusions were able to activate, albeit weakly, the stabilizing effect may extend to the rest of Mediator and possibly to other coactivators, any one of which can facilitate Adr1 binding when recruited to the promoter.

Fusion of the Adr1 DBD to Mediator creates a different kind of activator than the fusion of the Adr1 DBD to Tbp. Adr1-Mediator fusions are able to overcome the repressive mechanism at the *ADH2* promoter and bind to the promoter, subsequently activating transcription. Adr1-Tbp, on the other hand, is still subject to glucose repression. The ability of Adr1-Tbp to activate the *ADH2* promoter is surprising in light of a report that artificial recruitment of Tbp could not activate transcription at several promoters at which the TATA element is in a nucleosomal location (37). The *ADH2* promoter requires extensive chromatin remodeling that is Adr1 dependent, and one of the remodeled nucleosomes contains the TATA element (51). Nonetheless, Adr1-Tbp acts like a classical activator at the *ADH2* promoter. One possibility is that Adr1-Mediator binding is accompanied by or immediately recruits chromatin-modifying activities, whereas Adr1-Tbp may be unable to recruit the necessary activities to allow stable binding under repressing growth conditions. Alternatively, Adr1-Mediator could be part of a holoenzyme complex that brings RNA polymerase II to the promoter, regardless of chromatin structure.

Adr1 DBD-Mediator tail fusions were strong constitutive binders and activators. The fusions appeared to associate with the rest of the Mediator complex, supporting the hypothesis that activator binding can be stabilized at the promoter by the recruitment of a functional coactivator. Surprisingly, perturbing Mediator by *MED15* deletion did not affect the binding of wild-type Adr1. Since *ADH2* activation was noticeably delayed by coactivator deletions, a possible explanation is that Mediator, SAGA, and Swi/Snf all play important roles in *ADH2* expression but that individual coactivator subunits do not have important roles in Adr1 binding. Instead, the coactivators might be redundant with regard to Adr1 binding. In fact, we have found that individual subunits of SAGA and Swi/Snf can also be deleted without strongly affecting Adr1 binding (R. Biddick et al., unpublished data). Also consistent with the explanation of coactivator redundancy, we found that the Adr1-Med15 fusion could activate *ADH2* in the absence of SAGA or Swi/Snf subunits. Together, these results suggest that strong binding to Mediator can replace contacts with multiple coactivators.

Binding and activation by Adr1 DBD-Mediator tail fusions at several promoters were Snf1 independent in the presence of glucose and showed a reduced requirement for Snf1 in derepression. The mechanism by which Snf1 regulates Adr1 binding is unknown, but the ability of the fusions to bypass the Snf1 requirement suggests that Snf1 may aid coactivator recruitment, either directly or indirectly. In particular, Snf1 might be involved in Mediator recruitment, since there is both genetic and physical evidence for an interaction of Snf1 with Mediator (33, 43, 58). In summary, our model is that the binding of Adr1 to the *ADH2* promoter under normal repressing conditions is not detectable, because without the signals for derepression, Adr1 lacks stabilizing interactions with recruited coactivators. Our data with Adr1-Mediator fusions suggest that one factor in the stabilization of an activator and the subsequent initiation complex formation might be the interaction between the activator and Mediator at activated promoters. The fact that at least one Adr1-Mediator fusion can activate *ADH2* in mutants of Swi/Snf or SAGA subunits suggests that coactivators may be redundant with regard to the stabilization of factor binding.

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