Post-translational Regulation of Adr1 Activity Is Mediated by Its DNA Binding Domain

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**Materials and Methods**

ADR1 encodes a transcriptional activator that regulates genes involved in carbon source utilization in *Saccharomyces cerevisiae*. ADR1 is itself repressed by glucose, but the significance of this repression for regulating target genes is not known. To test if the reduction in Adr1 levels contributes to glucose repression of ADH2 expression, we generated yeast strains in which the level of Adr1 produced during growth in glucose-containing medium is similar to that present in wild-type cells grown in the absence of glucose. In these Adr1-overproducing strains, ADH2 expression remained tightly repressed, and UAS1, the element in the ADH2 promoter that binds Adr1, was sufficient to maintain glucose repression. Post-translational modification of Adr1 activity is implicated in repression, since ADH2 derepression occurred in the absence of de novo protein synthesis. The N-terminal 172 amino acids of Adr1, containing the DNA binding and nuclear localization domains, fused to the Herpesvirus VP16-encoded transcription activation domain, conferred regulated expression at UAS1. Nuclear localization of an Adr1-GFP fusion protein was not glucose-regulated, suggesting that the DNA binding domain of Adr1 is sufficient to confer regulated expression on target genes. A Gal4-Adr1 fusion protein was unable to confer glucose repression at GAL4-dependent promoters, suggesting that regulation mediated by *ADR1* is specific to UAS1.

Proteins that regulate transcription are themselves regulated in a variety of ways. Post-translational mechanisms, including covalent alterations such as phosphorylation/dephosphorylation, are common ways of controlling the activity of transcription factors that mediate environmental influences where the response is rapid or transient (1).

In the yeast *Saccharomyces cerevisiae*, Adr1 is the principal transcriptional activator of the glucose-repressible alcohol dehydrogenase (ADH2) gene (2–4). Adr1 also regulates the expression of genes involved in glycerol metabolism (5) and in peroxisome function and biogenesis (6, 7). These *ADR1*-regulated genes are not expressed when yeasts are growing in the presence of glucose, but are turned on when glucose is exhausted.

At the ADH2 promoter, activation of gene expression requires the binding of two monomers of Adr1 to a 22-base pair dyad-symmetric sequence designated UAS1. Two Cys2-His2-type zinc fingers and the region immediately preceding the fingers, make up its DNA binding domain, designated ABD (8–10). Adr1 appears to contain multiple transcription activation domains (9, 11, 12) that interact with components of a histone acetyltransferase complex, TFIIB, and components of TFIID (13, 14). A regulatory region of Adr1, responsible for glucose repression, has not been identified (11, 12).

Glucose repression in yeast involves multiple mechanisms. The best understood of these requires three genes: *MIG1*, encoding a DNA-binding protein, *TUP1*, and *SSN6*. A Tup1-Ssn6 complex is recruited by Mig1 to the promoter of glucose-repressed genes, where it blocks transcription (15–17). Regulated phosphorylation of Mig1, most likely by Snf1, modulates its nuclear entry and ability to repress transcription (18).

ADH2 is glucose-repressed by a mechanism that does not involve Mig1, Ssn6, or Tup1 (19). Instead, glucose repression of *ADH2* expression is mediated by modulation of the activity or the amount of the positive transcription factor Adr1 (20). Phosphorylation-dephosphorylation is involved in the activity of Adr1, since repression requires the protein phosphatase Gdc7 and its regulatory subunit, Reg1 (59), and derepression requires Snf1 and the regulatory subunit of the cAMP-dependent protein kinases (22, 23). Whether Adr1 itself is the subject of regulated phosphorylated has not been demonstrated (19, 24–27).

The other alternative, that regulation mediated by Adr1 is due to changes in its expression, is also a possibility. *ADR1* expression is repressed 3–20-fold when glucose is present in the medium (25, 28). Since ADH2 derepression requires occupation of both binding sites in UAS1, cooperative binding to UAS1 might be sensitive to small changes in Adr1 concentration. A 5-fold increase in the level of the activator Gal4 accounts for glucose repression of *GAL4*-dependent genes (29–32). However, the importance of the increase in *ADR1* expression for ADH2 derepression, has not been addressed.

While overexpression of *ADR1* leads to constitutive ADH2 expression, further increases in ADH2 expression occur upon derepression, indicating that glucose repression is still active in the presence of high level expression of *ADR1* (33).

We have investigated the relationship between Adr1 levels and ADH2 derepression and identified a glucose-regulated domain in Adr1. Three integrated copies of *ADR1* increase the level of Adr1 during glucose repression to a level equivalent to the derepressed level in wild-type cells. In the presence of derepressing levels of Adr1, ADH2 expression remains fully repressed during growth in glucose. Adr1 appears to be rapidly converted to an active form upon derepression because ADH2 expression is activated within minutes after glucose is removed from the medium, and the rate of accumulation of ADH2 mRNA or β-galactosidase activity from an ADH2/lacZ gene

phenylindole; GFP, green fluorescent protein; ADH1 and ADHII, alcohol dehydrogenase I and II, respectively.
fusional analysis is present on a CEN-HIS3 plasmid, pJS193.

**Growth of Yeast Cultures**—Yeast strains were grown in YPD or synthetic medium prepared according to standard methods (37). For maintaining glucose repression, cultures were started in YP or synthetic medium containing 8% glucose. Cell densities were kept below about 5 × 10^5 during growth in glucose and in minimal media, because we observed ADH2 expression at higher cell densities even in the presence of glucose. For growing yeast under derepressing conditions, cultures were grown in YP or synthetic medium containing 3% ethanol and 0.1% glucose. Alternatively, yeast cultures were derepressed in 0.05% glucose.

**Construction of an ADH1-GFP Expression Plasmid**—A 0.9-kb fragment encoding the F64L,S65T-enhanced version of green fluorescent protein (GFP) (38) with an AarII site introduced immediately 5' to sequences coding for an amino-terminal alanine-glycine flexible linker was created by polymerase chain reaction using plasmid pL2000, which was generously supplied by G. Muller, as template with primers Xhol-GFP-3' (5'-CACTATCTGCAATTTTGAGCTTCGGTACCAG-3') and AarII-GFP-5'-N' (5'-TCTAGAGCTGCGAGCCGGCGTGGTGTGC-3'). The resulting polymerase chain reaction fragment was digested with AarII and Xhol, gel-purified, and then ligated to the 10.5-kb AarII–Xhol vector fragment of pKD84 to create pKD110. This plasmid was digested with AarII, treated with T4 polymerase to blunt the ends, and then digested with Xhol. A 0.9-kb blunt Xhol fragment from the diggingested plasmid was ligated to a 2.8-kb BglII–BamHI fragment of pKD84 (25), which contains a portion of the ADH1 open reading frame, and an 8.8-kb BglII–SalI fragment of pKD84, which contains the 5'-end of the ADH1 gene and pRS314 vector sequences. The resulting CEN plasmid, pKD113, has the ADH1 promoter and open-reading frame fused in frame at codons 1245 to sequences coding for the flexible linker amino-terminal to GFP coding sequences.

**Fluorescence Microscopy of Yeast Cells**—Cells were stained in culture with 2,6-diamidino-2-phenylindole (DAPI, Sigma) as described by Shero et al. (39). A 5-μl aliquot of each DAPI-stained culture was mounted on a coverslip under a 0.1-mm-thick slab of 0.8% agarose containing fresh growth medium and suspended over a concave well on a multwell slide. Cells were viewed as described by Moser et al. (40) using a Zeiss Axioskop microscope fitted with the appropriate filters for differentiating between DAPI and GFP fluorescence. Images were processed using Adobe Photoshop and prepared for publication using Microsoft PowerPoint software.

**Protein Extracts and Western Blotting**—Denatured whole-cell extracts and nitrocellulose protein blots were prepared as described previously (18, 25). Blots were probed with polyclonal rabbit antibodies raised against amino acids 225–410 of ADH3 (α-ADH3) (19) and polyclonal anti-GFP antibodies (α-GFP), which were kindly provided by T. Davis.

**Enzyme Assays**—β-Galactosidase assays were performed on protein extracts or on permeabilized cells as described by Guarente (41). ADH activity assays were performed as described previously (19).

**RNA Extraction and Northern Analysis**—Total yeast RNA was prepared by disrupting yeast cells with glass beads in the presence of guanidine isothiocyanate followed by phenol extraction (37). RNA samples (16 μg) were incubated in a solution of 50% formamide, 2.2 M formaldehyde, 10 mM sodium phosphate (pH 7.4), and 0.5 mM EDTA at 65° for 10 min and then separated on a 1.2% agarose gel containing 10 mM sodium phosphate at pH 7.4. The RNA was transferred to ZetaProbe membrane (Bio-Rad) and treated according to the Zeta-Probe instruction manual. ADH2 mRNA was specifically detected using the 32P-labeled oligonucleotide 3′-CAGCGACCGGACGCAATTGCAT-5′, which does not hybridize with ADH1 mRNA in the conditions used (26). ACT1 mRNA was detected using a 560-base pair ClaI fragment of the ACT1 gene as a probe.

**RESULTS**

**Kinetics of Accumulation of Adr1 and ADH2 mRNA**—We first examined the time course of appearance of Adr1 and ADH2 mRNA in a strain of A. thaliana (17) that contains only the endogenous copy of ADR1. If Adr1 had to accumulate to a critical level to activate the ADH2 promoter, the GAL4 DNA binding domain (amino acids 1–147), and ADH2-encoded amino acids 21–1323 was constructed in a series of steps (details will be provided upon request). The gene fusion was very rapid. Moreover, ADH2 derepression in these conditions can occur in the absence of protein synthesis, suggesting that inactive Adr1 is converted post-translationally to an active form and that de novo synthesis of proteins is not required for ADH2 derepression. Utilizing gene fusions, we show that nuclear targeting is not glucose-regulated and that the DNA binding domain of Adr1 is the likely target of glucose repression.

**EXPERIMENTAL PROCEDURES**

**Strains and Plasmids**—The S. cerevisiae strains used are listed in Table I. Strains containing additional copies of ADR1 were derived from HY100 (MATα ura3 leu2 trp1 ade3) by a series of one-step gene disruptions or transformation with integrating plasmids. HY100 (MATα ade3 trp1 leu2 ade3) was derived from a cross of 521-6 (MATα ade3 trp1 leu2) and 3452-16-1 (MATα ura3 trp1 leu2). HY100 (MATα ade3 trp1 leu2 ade3) was obtained by transformation (34) of HY100 with a fragment containing ADH1 disrupted with LEU2 as described (28). A LEU2 derivative of HY100, JSY11, was generated by transformation of HY100 with an EcoRI digest of the LEU2 integrating plasmid pRS305 (35). This strain served as a wild-type control.

To generate a series of strains containing multiple copies of ADR1, a 6.8-kb fragment containing ADR1 from the pJS15 site at position −1097 from the start of translation) to the PstI site at position 5226 was assembled between the EcoRV and PstI sites in pBluescript II (SK++) (Strategene, La Jolla, CA) to create pJS10. The ADR1-containing fragment was excised from pJS10 using SalI and NotI and inserted between the SalI and NotI sites in pRS305 to create pJS15. This plasmid was digested with EcoRV and introduced into HY100. Leu+ colonies were isolated, and protein extracts from several of the transformants were examined by immunoblotting to determine the level of Adr1 under repressed and derepressed growth conditions. Three transformants (JSY12, JSY13, and JSY14) were selected that contained levels of Adr1 in glucose media equivalent to or greater than the level of Adr1 in derepressed JSY11. Southern analysis of ADR1 revealed that these strains contain about 2, 3, and 4 plasmid copies, respectively.

A TRP1-CAT plasmid containing the full-length bovine gene was constructed by excising ADR1 from pJS10 with SalI and NotI and inserting it into the SalI and NotI sites in pRS314 (35) to create pJS20. A truncated ADR1 gene encoding the N-terminal 172 amino acids of ADR1 was constructed by digesting pJS10 with EcoRI and BamHI and a 482-base pair EcoRI–BamHI fragment from the plasmid pJSL93 containing ADR1 between EcoRI and XhoI (5). The truncated ADR1 gene was excised from this plasmid using KpnI and NotI and inserted between the KpnI and NotI sites of pRS314 to create pJS21. A fusion of the DNA binding domain of Adr1 with the activation domain of the hepar simplex virus protein VP16 was created by cutting plasmid pJS21 with BamHI and inserting a VP16-encoding BglII–BamHI fragment from the plasmid pCFR2 (36). This plasmid was designated pJS22. A tripartite gene fusion containing the ADR1-CAT, the GAL4 DNA binding domain (amino acids 1–147), and ADR1-encoded amino acids 21–1323 was constructed in a series of steps (details will be provided upon request). The gene fusion is present on a CEN-HIS3 plasmid, pJS193.

**TABLE I**

| Strain | Genotype | Source |
|--------|----------|--------|
| HY10   | MATα ade3 leu2 trp1 uro3 | This laboratory |
| HY11   | MATα ade3 trp1 ade3 1::UER2 | This laboratory |
| JSY11  | MATα ade3 trp1 uro3 | This study |
| JSY12  | MATα ade3 trp1 ade3 UER2::PJS15 1 copy | This study |
| JSY13  | MATα ade3 trp1 ade3 UER2::PJS15 2 copies | This study |
| JSY14  | MATα ade3 trp1 ade3 UER2::PJS15 3 copies | This study |
| JSY20  | HY101 TRP1::YlpADH2 (lacZ/EcoRV) | This study |
| JSY21  | ADR1::YlpADH2 (lacZ/EcoRV) | This study |
| JSY22  | JSY12 TRP1::YlpADH2 (lacZ/EcoRV) | This study |
| JSY24  | JSY14 TRP1::YlpADH2 (lacZ/EcoRV) | This study |
| W303-1A | MATα ade2 cam1-100 his3-11,15 leu2-13,12 trp1-1 ura3-1 | This study |
| TYY303 | W303-1a ade3 1::UER2 | This study |

J. S. Sloan, unpublished data.
mRNA accumulation increases at a faster rate than that of Adr1, suggesting that a threshold level of Adr1 is not necessary to trigger ADH2 transcription. This observation agrees with previous results showing that a low level of ADR1 expression is capable of activating ADH2 under derepressing conditions (25).

Effect of Overproduction of Adr1 on Glucose Repression of ADH2—To study the relationship between Adr1 levels and ADH2 transcription more directly, isogenic strains expressing different levels of Adr1 were created by integrating multiple copies of ADR1 at the LEU2 locus (see “Experimental Procedures”). Fig. 2 and other data show that Adr1 levels in repressed conditions increased with ADR1 copy number and were about 30-fold higher in the multicopy strain JSY14 than in wild-type strain JSY11. The Adr1 levels were also higher than normal in the multicopy strains under derepressing conditions, although by a smaller factor than under repressed conditions. The high ADR1 expression in the transformants under glucose growth conditions suggests that the integrated copies of ADR1 were not subject to glucose repression. This result could be due to effects on the ADR1 promoter from the adjacent vector sequences or to position effects at the site of integration.

Fig. 3A shows that ADHII enzyme activity was not detectable in extracts from the three ADR1 multicopy strains grown under repressed conditions, suggesting that ADH2 expression was still repressed despite the high level of Adr1. ADHII levels were comparable in extracts from all five strains after growth on glucose. Under derepressing conditions, the multicopy strains contained greater ADHII activity than the wild-type strain, indicating that the excess Adr1 was active in the multicopy strains.

The effect of high Adr1 levels on ADH2 expression was examined directly by Northern blot analysis of ADH2 mRNA (Fig. 3B). Under glucose growth conditions, ADH2 mRNA was virtually undetectable in all of the strains. Under derepressing conditions, ADH2 mRNA accumulated to levels in the Adr1-overproducing strains JSY12, JSY13, and JSY14 that were 2.7-, 5.5-, and 6-fold higher, respectively, than in the wild-type strain.2

A more sensitive and quantitative assay of ADH2 expression was provided by an ADH2/lacZ gene fusion. When the reporter was present on an episomal plasmid, β-galactosidase activity was low in all of the strains in repressed conditions (Table II).
wild-type strain. In the absence of ADR1, ADH2/lacZ derepression occurred after a lag period and proceeded slowly, reaching a level 100-fold lower than in the presence of ADR1 (note that Fig. 5 shows the activity on a log scale). The ADR1-independent activity is due to UAS2, a second regulated UAS element in the ADH2 promoter (42).

**Fig. 4.** Time course of ADH2 mRNA accumulation following the transfer of cells from repressing to derepressing medium. Northern blot analysis was performed with total RNA isolated from samples of JSY11, JSY12, JSY14, and HHY13 that were taken from derepressing cultures at the indicated time points. All lanes were loaded with 20 μg of RNA. The membrane was hybridized with a radiolabeled probe specific for ADH2 mRNA.

| Strain | Copies of ADR1 | β-Galactosidase activity |
|--------|----------------|-------------------------|
|        |                | Repressed | Derepressed |
| HHY13  | 0              | 0.8        | 100         |
| JSY11  | 1              | 1.8        | 1470        |
| JSY12  | 2              | 9          | 2240        |
| JSY13  | 3              | 76         | 2300        |
| JSY14  | 4              | 118        | 2640        |

**TABLE III**

Effect of ADR1 overproduction on expression of a CYC1/lacZ reporter containing UAS1 alone

| Strain | Copies of ADR1 | β-Galactosidase activity |
|--------|----------------|-------------------------|
|        |                | Repressed | Derepressed |
| HHY13  | 0              | 0.2        | 0.3         |
| JSY11  | 1              | 0.2        | 10          |
| JSY12  | 2              | 0.4        | 180         |
| JSY14  | 4              | 0.8        | 660         |

that sequences other than UAS1 in the ADH2 promoter are responsible for its repression in the presence of high levels of Adr1.

ADH2 Derepression Does Not Require de Novo Protein Synthesis—The rapid appearance of ADH2 mRNA and ADHII/β-galactosidase activities in strains with high levels of Adr1 suggests that the activator was rapidly converted to an active form. To determine whether this change in its activity requires de novo protein synthesis, derepression was carried out in the presence of cycloheximide, an inhibitor of protein synthesis. ADH2 derepression could be detected at 1 h in cycloheximide-treated cells when high levels of Adr1 were present (Fig. 6, lanes 9 and 12), but not in wild-type cells or in cells lacking Adr1 (lanes 3 and 6). Thus, de novo protein synthesis is not required for derepression of ADH2 expression. Cycloheximide appeared to allow superderepression of ADH2 expression in the high copy strains (compare lanes 8 and 11 with lanes 9 and 12).

Surprisingly, cycloheximide treatment of cells maintained in glucose led to ADR1-independent derepression of ADH2 (Fig. 6B).

Glucose Repression of ADH2 Expression Is Mediated by the DNA Binding Domain of Adr1—The results described above...
suggest that Adr1 is regulated post-translationally. The domain responsible for this regulation appears to lie in the amino terminus of Adr1, since mini-Adr1, consisting of the first 172 amino acids of Adr1 and transcription activation domain III (amino acids 420–462 of Adr1) is active and confers glucose-regulated expression on ADH2 and other UAS1-containing genes (12). However, transcription activation domain III is not glucose-regulated when fused to GAL4, suggesting that it is not the target of post-translational regulation (11, 12). The regulatory region thus includes the DNA binding domain (9) and the nuclear localization signal (44). However, it is possible that transcription activation domain III confers regulation specifically on the Adr1 DNA binding domain. To test this possibility, we fused the first 172 amino acids of Adr1 to a heterologous transcription activation domain. We used the viral VP16 activation domain, since it is very active in yeast (45). The gene fusion was driven by the ADR1 promoter carried on a low copy plasmid.

Fig. 7 shows that ADH2 expression, as monitored by ADH activity gels, is tightly glucose-repressed and strongly derepressed by this hybrid regulatory protein. An UAS1-containing reporter gene also showed stringent glucose repression. The β-galactosidase activity increased 110-fold after glucose was removed from cultures carrying the ADR1-VP16 activator. Thus, a hybrid Adr1 activator containing only the first 172 amino acids of Adr1 conferred tight glucose repression on its target promoters, and it was as active after derepression as wild-type Adr1. ADR1-VP16 is present and competent for DNA binding in extracts of glucose-repressed cells, so differential stability is not responsible for its inactivity.

Fig. 8 shows that ADH2 expression, as monitored by ADH activity gels, is tightly glucose-repressed and strongly derepressed by this hybrid regulatory protein. An UAS1-containing reporter gene also showed stringent glucose repression. The β-galactosidase activity increased 110-fold after glucose was removed from cultures carrying the ADR1-VP16 activator. Thus, a hybrid Adr1 activator containing only the first 172 amino acids of Adr1 conferred tight glucose repression on its target promoters, and it was as active after derepression as wild-type Adr1. ADR1-VP16 is present and competent for DNA binding in extracts of glucose-repressed cells, so differential stability is not responsible for its inactivity.

ADR1 is important for expression of genes required for glycerol metabolism and for peroxisome biogenesis. We tested ADR1-VP16 for its ability to complement an adr1-null strain for growth on glycerol or oleate. As shown in Fig. 8, ADR1-VP16 complemented this growth defect as well as wild-type ADR1 did. The DNA binding domain of Adr1 without an activation domain (ADR1Δ172) was unable to complement the mutant.

Thus, derepression of genes required for glycerol growth or for peroxisome induction does not require ADR1-specific activation domains.

To test whether Adr1-VP16 is subject to the same regulatory genes as wild-type Adr1, we measured ADH2 derepression in snf1 and reg1 mutant strains. ADH2 expression was dependent on SNF1 for derepression when Adr1-VP16 was the activator and constitutive ADH2 expression was observed in a reg1 mutant strain. Thus, the Adr1/VP16 activator responds to the same regulatory signals as Adr1 itself. These data show that the amino-terminal 172 amino acids of Adr1, fused to a non-yeast activation domain, is sufficient to carry out all of the known functions of ADR1.
that Adr1-GFP is nuclear in repressed conditions and yet is inactive. To determine whether the glucose regulation mediated by Adr1 can be conferred on a heterologous DNA binding domain, we fused most of Adr1 to the DNA binding domain (GBD) of GAL4. The fusion protein, consisting of amino acids 1–147 of Gal4 and 20–1323 of Adr1, was driven by the ADR1 promoter and was expressed from a low copy, centromere-containing plasmid, pJSL93. pJSL93 was introduced into two strains, one containing a GAL4-responsive lacZ reporter and another containing an ADR1-responsive lacZ reporter. The GAL4-responsive reporter was derepressed only 2-fold in the absence of glucose, whereas the ADR1-responsive reporter was derepressed over 100-fold (Table IV). Western blotting showed similar levels of intact fusion protein in both strains in both growth conditions. The data demonstrate that GBD-Adr1 is present and functional in the nucleus of glucose-repressed cells and that the activity of Adr1 fused to Gal4 is not glucose-regulated when bound to UAS$_G$.

**DISCUSSION**

**ADH2** expression remains strongly glucose-repressed in the presence of high levels of Adr1, indicating that the concentration of Adr1 is not the limiting factor for **ADH2** expression during glucose repression. Thus, glucose repression of **ADH2** expression is not regulated in a manner analogous to glucose repression of **GAL4**-dependent genes, where a 5-fold increase in **GAL4** expression is sufficient to overcome glucose repression.

Our results and conclusions differ from those of a previous study (54). In that study, **ADR1** copy number was varied by integrating multiple copies of **ADR1**. ADHII activity in glucose growth conditions increased in proportion to the copy number of **ADR1** present. However, Adr1 itself was not measured, and it is possible that the integrated copies of **ADR1** were more active than the single endogenous gene, as we observed in our study. In support of this interpretation, high **ADR1** copy number decreased the growth rate in the previous study, while we did not observe a detrimental effect of additional copies of **ADR1**.

As with many transcription factors, activity of Adr1 is regulated post-translationally, as demonstrated by derepression of **ADH2** expression in the absence of protein synthesis. The most common form of post-translational modification involved in...
changing the activity of a transcription factor is phosphorylation. Although Adr1 can be phosphorylated in vitro by PKA (55), there is no evidence that this phosphorylation is important for glucose repression (25). Mini-Adr1 lacks the site of phosphorylation by PKA, Ser230, yet it is regulated in the same manner as ADR1 itself. Thus, this phosphorylation site cannot be important for glucose repression, either as a target of a protein kinase (19, 24, 25) or as a binding site for a repressor (11). Phosphorylation in vivo within the amino-terminal 180 amino acids was not detected, so if it occurs, it must be transient or occurring only on a small population of Adr1.

Other data do indicate an important role for phosphorylation in regulating the derepression of ADH2. Glc7, a type 1 protein phosphatase, and its regulatory subunit, Reg1, are involved in glucose repression of ADH2 expression in an ADR1-dependent manner. The Snf1 protein kinase is necessary for ADH2 derepression. Thus, post-translational regulation of Adr1 and of Adr1/VP16 requires phosphorylation/dephosphorylation reactions catalyzed by these proteins. The substrate for these presumed reactions could be Adr1 itself or an unidentified protein that interacts with Adr1.

It was surprising that ADH2 mRNA could be detected in the absence of protein synthesis during glucose repression in the absence of Adr1, since ADH2 expression is normally dependent on Adr1. One possibility is that continued DNA synthesis in the absence of protein synthesis leads to loss of nucleosomes at the ADH2 promoter (56, 57), and that this allows promiscuous transcription. Although we cannot rule out an effect of cycloheximide on the stability of ADH2 mRNA in these experiments, we consider this unlikely, since ADH2 mRNA was about 500-fold reduced in glucose-grown cells compared with derepressed cells.

Fusion of the amino-terminal 172 amino acids of Adr1 to a constitutive transcription activation domain, that of the Herpesvirus VP16 gene, created a stringently glucose-regulated transcription factor. This region of Adr1 contains a nuclear localization signal and the DNA binding domain. Nuclear localization of Adr1/GFP was not glucose-regulated as is the Mig1 repressor. Thus, the regulatory region of Adr1 appears to be the DNA binding domain. Adr1 isolated from glucose-grown cells is able to bind UAS1 (26) as is the Adr1/VP16 fusion protein studied here. Thus, it seems unlikely that the DNA-binding activity is modified to an inactive form in these conditions. However, we have been unable to detect Adr1 bound to UAS1 in either repressed or derepressed cells, so we do not know if Adr1 exists in a transcriptionally inactive form when it is bound to DNA in repressed conditions. However, the transcription-activation function of Adr1 is not inherently inactive in glucose-repressed cells, since a Gal4/Adr1 fusion protein was not subject to glucose repression when assayed at a GAL4-dependent promoter.

The discovery that the DNA binding domain of Adr1 or its binding site, UAS1, is the target of glucose repression appears to be unique among glucose-regulated transcription factors. Many glucose-repressed genes in yeast are regulated by altering the activity of the DNA-binding repressor, Mig1. The region of Mig1 responsible for glucose repression lies outside of the DNA-binding region of Mig1 (45), and it is nuclear entry, rather than DNA binding, that is glucose-regulated (18). A putative glucose-sensitive region of Gal4 lies outside of its DNA binding domain as well (58). Cat8, another glucose-sensitive transcription factor, can confer glucose regulation through a heterologous DNA binding domain, unlike Adr1 (21).

We envision two alternative models for regulation of Adr1 activity. In the first model, Adr1 is prevented from binding to UAS1. This could be due to competition between Adr1 and a repressor for binding to UAS1. There are several other zinc finger proteins in yeast, some of which are predicted to have the same binding specificity as Adr1. One or more of these zinc finger proteins or an unrelated protein could act as a repressor by binding to UAS1. The putative repressor could be displaced by high levels of Adr1, and activation of transcription could ensue even in the presence of glucose. The putative repressor(s) could be inactivated after glucose removal, or its synthesis could be glucose-dependent. This could explain the more rapid derepression of ADH2 expression that is observed in the presence of high levels of Adr1 and the apparent “super-induction” seen in the absence of protein synthesis. However, evidence against this model is that UAS1 does not appear to act as an operator site when inserted into a constitutive promoter.

In a second model, protein-protein interaction is responsible for lack of activity of Adr1 in repressed conditions. These interactions could be negative, preventing Adr1 from binding or activating transcription, or they could be positive but absent in the presence of glucose. For example, a transcription component essential for Adr1 activity could be absent or modified to an inactive form in glucose-growth conditions.

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