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Identification of Functional Regions in the Yeast Transcriptional Activator ADR1†

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The transcriptional activator ADR1 from *Saccharomyces cerevisiae* is a postulated DNA-binding protein that controls the expression of the glucose-repressible alcohol dehydrogenase (*ADH2*). Carboxy-terminal deletions of the ADR1 protein (1,323 amino acids in length) were used to localize its functional regions. The transcriptional activation region was localized to the N-terminal 220 amino acids of ADR1 containing two DNA-binding zinc finger motifs. In addition to the N terminus, a large part of the ADR1 sequence was shown to be essential for complete activation of *ADH2*. Deletion of the putative phosphorylation region, defined by *ADR1*^{c} mutations that overcome glucose repression, did not render *ADH2* expression insensitive to glucose repression. Instead, this region (amino acids 220 through 253) was found to be required by ADR1 to bypass glucose repression. These results suggest that *ADR1*^{c} mutations enhance *ADR1* function, rather than block an interaction of the putative phosphorylation region with a repressor molecule. Furthermore, the protein kinase CCR1 was shown to affect *ADH2* expression when the putative phosphorylation region was removed, indicating that CCR1 does not act solely through this region. A functional *ADR1* gene was also found to be necessary for growth on glycerol-containing medium. The N-terminal 506 amino acids of ADR1 were required for this newly identified function, indicating that *ADH2* activation and glycerol growth are controlled by separate regions of ADR1.

Eucaryotic transcriptional regulators tend to be large proteins having molecular masses greater than 70,000 daltons (20, 24, 27, 35). Their large size suggests the presence of multiple domains, each being wholly or partly responsible for imparting one or more biological activities to the protein. Deletion and mutation analyses, together with sequence homology comparisons, have proven useful in identifying and localizing functional regions. These approaches have been notably successful in studies of the simian virus 40 T antigen (19, 32), the glucocorticoid receptor (6, 23, 28), GAL4 (17, 21, 26), and GCN4 (14).

The transcriptional activator ADR1, a protein of 151,000 daltons (12), functions in controlling the expression of the glucose-repressible alcohol dehydrogenase (ADH II; encoded by the *ADH2* gene) from *Saccharomyces cerevisiae* (4). Two functional regions of ADR1 have been identified by sequence comparisons and mutation analysis. One of these regions is highly homologous to the zinc-containing DNA-binding fingers previously identified in transcription factor IIIA of *Xenopus laevis* (29). This region lies between amino acids 99 and 155 of ADR1 (12) and contains two such zinc fingers. Although not yet demonstrated to be a DNA-binding protein, ADR1 is located in the nucleus (1) and has been shown to control *ADH2* expression through a 22-base-pair (bp) segment of dyad symmetry upstream of the *ADH2* gene (31). Deletion analysis indicates that the first 304 N-terminal amino acids of ADR1 are sufficient to cause partial derepression of *ADH2* (10), whereas the first 151 amino acids are not sufficient (12). Furthermore, most mutations that inactivate *ADR1* occur in the zinc finger region (1; C. L. Denis et al., manuscript in preparation). These results confirm the importance of the finger region to ADR1 function.

The second functional region in ADR1 consists of a cAMP-dependent protein kinase phosphorylation recognition site, located between amino acids 227 and 231 (22). Mutations which allow ADR1 to bypass glucose control and activate *ADH2* transcription have been localized to this region (9; J. Cherry et al., manuscript in preparation). These *ADR1*^{c} mutations (constitutive for *ADH2* expression) have been postulated to increase ADR1 function under repressed conditions by decreasing ADR1 phosphorylation (9). This could occur by blocking interaction of ADR1 with a negative effector or by enhancing the ability of ADR1 to activate transcription. It has also been suggested that activation of *ADH2* upon derepression is regulated by a glucose-dependent dephosphorylation of ADR1 (9). The *CCR1* gene, encoding a protein kinase (2, 7) required for *ADH2* derepression (5), may play a role in controlling ADR1 phosphorylation state (8, 9).

We have undertaken a series of progressive carboxy-terminal deletions of ADR1 to obtain a clearer understanding of its structure and function. The questions which we address include the following. (i) What part of ADR1 is required for *ADH2* activation? (ii) Is the putative phosphorylation region the site through which the glucose signal is mediated? (iii) Does CCR1 act through the phosphorylation region of ADR1? We report here that ADR1 contains several functional regions and identify a region specifically required for growth on glycerol. We also present data showing that the putative phosphorylation region, while not being absolutely required for ADR1 activation of *ADH2*, does play a positive role in ADR1 function.

MATERIALS AND METHODS

Yeast strains. Strain 500-16 (*MAT***a** *adh1-11 adh3 adr1-1 ura1 trp1 his4*) was used for yeast transformations. Other strains used in this study are: 521-10 (*MAT***a** *adh1-11 adh3 adr1-1 trp1::YRp7-ADR1-311 ura1 his4*), 205-5d (*MAT*α *adh1-11 adh3 ccr1-1 trp1 ura1*), and 500-16-C3 (same as

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500-16, except that 15 copies of *ADR1-220* are integrated at the *trp1* locus).

ADR1 plasmids. All *ADR1* genes were derived from plasmids YRp7-ADR1-411, YRp7-ADR1-23A, and YRp7-ADR1-311 (9, 10) and were present on YRp7 vectors. Each contains at least 1.2 kilobases of *ADR1* upstream DNA, which is sufficient for normal *ADR1* expression (8). Truncated *ADR1* gene sequences end at the restriction sites and base pair numbers shown in Fig. 1. Each gene is designated by the number of amino acids of ADR1 protein sequence it encodes (e.g., *ADR1-282* encodes the first 282 N-terminal amino acids of *ADR1*). In most cases, non-ADR1 amino acids are present on the carboxy termini of the truncated ADR1 polypeptides; *ADR1-1323* encodes the wild-type coding sequence, *ADR1-1068* encodes an extra 81 amino acids derived from pBR322 sequences from bp 385 to 623, *ADR1-642* encodes an extra 16 amino acids derived from the complementary strand of pBR322 from bp 376 to 326, *ADR1-506* encodes an extra 32 amino acids derived from pBR322 sequences from bp 975 to 1071, *ADR1-304* encodes an extra 7 amino acids from the complementary strand of pBR322 that runs from bp 374 to 353, *ADR1-282* encodes an extra 22 amino acids derived from bp 185 to 251 of the *TRP1* gene, *ADR1-272* encodes 23 extra amino acids that run from bp 2036 to 2105 of pBR322, *ADR1-253* encodes 10 extra amino acids from bp 974 to 1004 of pBR322, *ADR1-220* encodes no extra amino acids, and *ADR1-151* encodes an extra 235 amino acids derived from pBR322 sequences extending from bp 568 to 1273.

Growth conditions, assays, and transformations. For ADH assays, yeast cells were grown to mid-logarithmic phase at 30°C in YEP medium (2% Bacto-Peptone [Difco Laboratories], 1% yeast extract, 20 mg each of adenine and uracil per liter), supplemented either with 8% glucose or 3% ethanol. ADH activity assays were performed as previously described (10), and all values represent the average of at least three separate determinations. Yeast transformations were conducted by either the protoplast (10) or lithium acetate method (16). The methods used for identifying integration of the *ADR1* genes and for determining the number of copies integrated into the genome have been described previously (8). Integration of the YRp7 plasmids carrying the *ADR1* genes was site directed to the *trp1* locus by cutting the plasmids in the interior of the *TRP1* gene with *Mst*II or *Bgl*II.

For transformations conducted with plasmids containing the *ADR1-648* and *ADR1-282* genes, only a single integrant was obtained in each case, carrying 21 and 29 tandem copies, respectively. Isolation of fewer numbers of iterated *ADR1-648* and *ADR1-282* genes per cell was conducted by selecting for deintegration events, as previously described (8). No difference in *ADH2* expression has been observed between strains carrying the same number of *ADR1* genes, whether derived from deintegration or by a primary transformation event (8).

RESULTS

Carboxy-terminal deletions of the *ADR1* gene were used to localize functional regions of the ADR1 protein (Fig. 1). Truncated *ADR1* genes, present either on plasmid vectors or as stable integrants at the *trp1* locus (8), were analyzed. Genes were stably integrated into the genome so that the gene dosage per cell could be accurately determined. This approach allows the *ADR1* dosage dependence of *ADH2* expression to be studied (8). Five criteria were used to compare the functionality of the truncated *ADR1* genes: (i) ability to allow derepression of *ADH2*, (ii) maximal level of *ADH2* expression, (iii) ability to overcome glucose repression, (iv) ability to sustain growth on a nonfermentative carbon source, and (v) insensitivity to a defect in the *CCR1* gene.

ADR1 activating region. Wild-type and deleted *ADR1* genes were stably integrated at the *trp1* locus and assayed for their ability to activate *ADH2* expression (Table 1). *ADR1* genes coding for at least the N-terminal 220 amino acids of ADR1 were capable of allowing *ADH2* derepression. ADR1-220 lacks the putative phosphorylation site implicated in glucose control of ADR1. All functional *ADR1* genes at a single or low dosage conferred an ADH2 phenotype that remained glucose repressed (Table 1). Conversely, no region of ADR1 was identified which, when removed, rendered *ADH2* resistant to glucose repression. ADR1-151, containing only one complete DNA-binding zinc finger, did not allow *ADH2* expression.

Increases in *ADR1* gene size did not show a proportional increase in the ability to activate *ADH2*. This feature is observed most clearly when single dosages of the different genes are compared (Table 1). These variations could be the result of differences in protein or mRNA stability between the various *ADR1* constructs.

To better estimate the relative function of the various-sized *ADR1* genes in a way that would eliminate possible differences in their protein and mRNA stability, the maximal ability of the *ADR1* genes to activate *ADH2* was measured. The maximal activation ability of ADR1 is based on previous observations that high *ADR1* dosages saturate *ADH2* expression during nonfermentative growth in which *ADH2* copy number is the limiting factor (8). By using the value for maximal activation of *ADH2*, a comparison could be made between the functions of different-sized ADR1 proteins that is independent of their relative protein stability. A range of dosages for the truncated *ADR1* genes integrated at the *trp1* locus were obtained, and the resultant ADH II activities were determined. Figure 2A illustrates the effect of varying *ADR1* dosage on *ADH2* derepression for three *ADR1* alleles. Values for maximal ADH II activity were determined by using an Eadie-Hofstee plot and are given in Fig. 2B as a function of ADR1 polypeptide length. For *ADR1-253*, *-304*, *-506*, and *-1068*, the ranges of dosage obtained were not sufficiently broad to allow calculation of maximal ADH II activities. It was observed that by progressively removing parts of the C terminus of ADR1, the ability of ADR1 to maximally activate *ADH2* expression also progressively

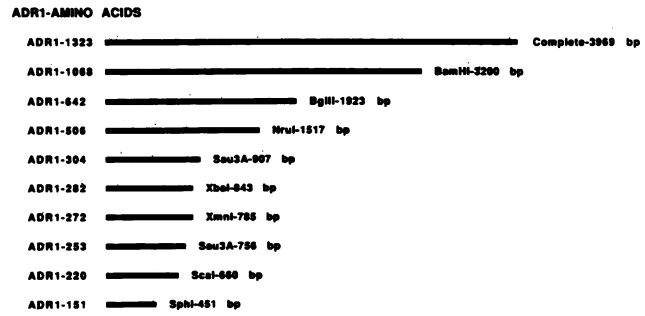

FIG. 1. Comparison of deleted *ADR1* genes. Each *ADR1* construct is designated on the left by the number of amino acids present in its coding sequences. To the right are given the restriction sites and base pair numbers at which *ADR1* DNA sequences end. *ADR1-1323* represents the complete *ADR1* gene as present on YRp7-ADR1-411 (10).

decreased. A major decrease in activity was observed when ADR1 was shortened from 282 to 272 amino acids. These results indicate that a large part, if not all, of the ADR1 protein is required to modulate ADR1 activation of *ADH2*. It should be noted that the K_D, defined as the number of *ADR1* genes required to give half the maximal ADH II activity, was similar for ADR1-1323, -642, -272, and -220 (K_D = 2 to 3 genes), whereas the K_D for ADR1-282 was much greater (K_D = 14 genes). The similarity in K_D for the four polypeptides suggests that their protein stabilities were not significantly different.

Region of ADR1 required to bypass glucose repression. Glucose repression of *ADH2* can be overcome by increased dosage of the *ADR1* gene (8, 15; Table 1). To define the region of ADR1 required to bypass glucose repression, plasmids bearing the *ADR1* genes were transformed into *S. cerevisiae*, and ADH II enzyme activities were determined after growth on medium containing both glucose and the respiratory inhibitor antimycin A. Antimycin A restricts growth to those cells capable of fermentation and, in this case, the cells which are expressing the *ADH2* gene. Because *ADR1* concentration is limiting for *ADH2* expression when cells are grown on glucose (8), the presence of the inhibitor selects for cells carrying copy numbers of *ADR1* plasmid sufficient to bypass glucose repression. *ADR1-151*, which was incapable of activating *ADH2* under derepressed conditions, did not allow growth on medium containing antimycin A (Table 2). *ADR1-220* resulted in poor growth in the presence of antimycin A (data not shown) because of the low levels of ADH II enzyme activity expressed (8 mU/mg; Table 2). *ADR1-220* was, however, capable of activating *ADH2* during derepression (Table 1). Increased expression of all ADR1 polypeptides longer than ADR1-220 bypassed glucose repression and allowed for increased ADH II levels (greater than 80 mU/mg; Table 2). The 7- to 10-fold difference in ADH II activity under glucose-repressed conditions between the *ADR1-1323* and the *ADR1-304*, *-282*, *-272*, and *-253* alleles is most likely a result of a comparable 5- to 8-fold decrease in transcriptional activation ability, as seen under derepressed conditions (Table 1). ADR1-220 appears, therefore, to lack part of the region required to bypass glucose repression.

The above results obtained using plasmid-bearing *ADR1*

TABLE 1. Effect of truncated *ADR1* genes on *ADH2* expression

Gene[a]	Copies	ADH II activity (mU/mg) on:	
		Glucose[b]	Ethanol[c]
ADR1-1323[d]	1	5	2,400
	96	680	7,400
ADR1-1068	1	2	8
	37	2	530
ADR1-642	1	8	1,400
	21	160	4,400
ADR1-506	1	5	120
	12	22	1,000
ADR1-304	1	5	800
ADR1-282	5	5	680
	29	28	1,800
ADR1-272	1	2	370
	20	56	1,000
ADR1-253	1	2	220
	5	7	760
ADR1-220	1	1	120
	15	5	360
ADR1-151	6	2	10
	Plasmid[e]	ND[f]	10
adr1-1	1	2	10

[a] The number after the *ADR1* designates the number of *ADR1*-encoded amino acids present. All strains are isogenic to 500-16, except for *ADR1-304*, as determined by Denis and Young (10).

[b] Yeasts were pregrown on YEP plates supplemented with 8% glucose before inoculation into YEP medium containing 8% glucose.

[c] Yeasts were pregrown on YEP plates supplemented with 2% glucose before inoculation into YEP medium containing 3% ethanol.

[d] As determined by Denis (8).

[e] The *ADR1-151* gene was retained on the YRp7 plasmid.

[f] ND, Not done.

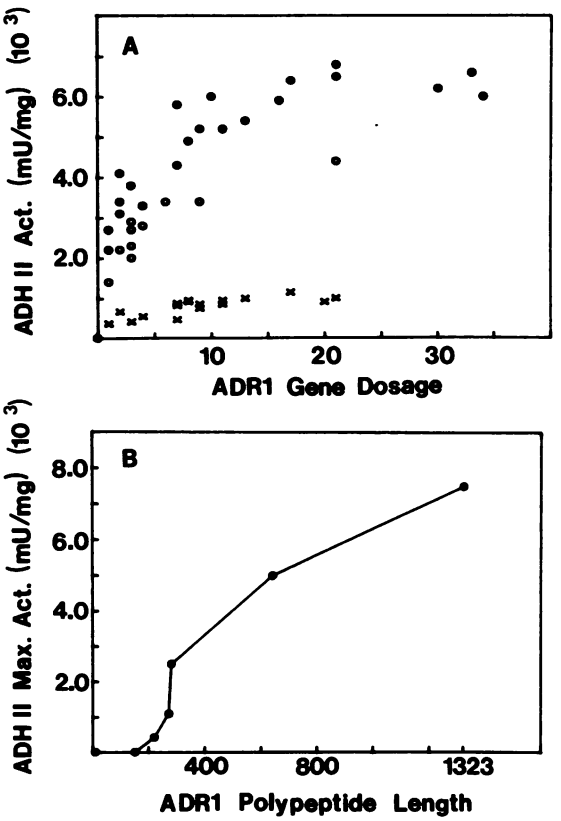

FIG. 2. Effect of *ADR1* dosage on *ADH2* expression and maximal ADH II activity as a function of ADR1 polypeptide length. (A) ADH II enzyme activity (Act.) is given as a function of *ADR1* dosage. ADH II activity was determined after growth on ethanol-containing medium as described in footnote *c* of Table 1. Values represent the average of at least three determinations. *ADR1* dosages were determined as described in Materials and Methods. The values for *ADR1-1323* are as determined by Denis (8). Symbols: ●, *ADR1-1323*; ○, *ADR1-642*; ×, *ADR1-272*. (B) Maximal ADH II activity (Max. Act.) is given as a function of ADR1 polypeptide length. Maximal ADH II activity was obtained from an Eadie-Hofstee plot of ADH II enzyme activity under derepressed conditions as a function of *ADR1* dosage. The polypeptide lengths (in amino acids) that are plotted, from left to right, are as follows: 0, 151, 220, 272, 282, 642, and 1,323.

TABLE 2. ADR1 regions involved in bypassing glucose control and in growth on nonfermentative carbon sources

Gene	ADH II activity (mU/mg)[a] on glucose and antimycin A	Relative growth[b] on:		ADH II activity (mU/mg) on ethanol
		Glycerol	Ethanol	
ADR1-1323	760	+	+	2,400
ADR1-1068	100	+	+	530
ADR1-642	530	+	+	1,400
ADR1-506	210	+	+	1,000
ADR1-304	100	−	+	800
ADR1-282	78	−	+	1,800
ADR1-272	81	−	+	1,000
ADR1-253	96	−	+	760
ADR1-220	8	−	+	360
ADR1-151	NG	−	−	10
adr1-1	NG	−	−	10
adr1-1 ADH2-S6	200	−	+	2,200

[a] Growth was in YEP medium supplemented with 8% glucose and 0.1 mg of antimycin A per ml. All *ADR1* genes were carried on YRp7 plasmids in strain 500-16 (*adr1-1*) with the following exceptions: *adr1-1* was strain 500-16 without a plasmid, and *adr1-1 ADH2-S6* (strain SPH6, generously provided by J. Cherry) was strain 500-16 carrying an integration at the *trp1* locus of the *ADH2* gene derived from plasmid YRp7-ADH2-Bsb (37), whose control sequences upstream of −176 bp have been deleted. After growth on medium containing antimycin A, strains were checked to ensure that antimycin A growth was dependent on plasmid sequences and not due to mutations that had arisen in genomic sequences. NG, No growth.

[b] Growth was in YEP medium supplemented with either 3% glycerol or ethanol. All strains contained integrated *ADR1* genes. For each *ADR1* allele, the dosage tested was that which resulted in an ADH II activity during growth on ethanol as near as possible to 1,500 mU/mg, as indicated in the last column, to minimize differences in *ADH2* expression. Growth to densities of 2×10^8 to 5×10^8 cells per ml (+) and growth to 1×10^7 to 3×10^7 cells per ml (−) are indicated.

genes to bypass glucose repression are confirmed when a comparison is made of the relative abilities of known dosages of integrated *ADR1* genes to bypass glucose repression (Table 1). Fifteen copies of *ADR1-220* allowed 1/10 the level of ADH II activity under conditions of growth on glucose as a comparable dosage of ADR1-272, whereas under derepressed conditions, *ADR1-220* allowed about 1/2 to 1/3 the maximal *ADH2* expression as allowed by *ADR1-272* (Table 1; Fig. 2). In contrast, although 21 copies of *ADR1-642* under repressed conditions allowed three times as much ADH II activity as did 20 copies of *ADR1-272*, this is probably due to decreased transcriptional activation by *ADR1-272* relative to *ADR1-642*. *ADR1-642* allowed four times as much maximal ADH II activity under derepressed conditions as did *ADR1-272* (Table 1; Fig. 2). These results, therefore, are consistent with the interpretation that the ADR1-220 polypeptide, relative to larger ADR1 polypeptides, lacks sequences which are required to bypass glucose repression.

Site of CCR1 interaction with ADR1. The ADR1-220 protein, which was capable of allowing *ADH2* gene expression, lacks a putative phosphorylation site believed to be the site through which the protein kinase CCR1 acts, albeit indirectly (8, 9). By the proposed model, *ADH2* expression elicited by ADR1-220 would be expected to be unresponsive to defects in the *CCR1* gene if indeed *CCR1* acted through this site. To test this hypothesis, strains containing *ADR1-220*, *ADR1-304*, and *ADR1-1323* genes were constructed which carried the *ccr1-1* allele (5). *ADH2* derepression was blocked in each case by the *ccr1-1* allele (Table 3). These results indicate that CCR1 does not transmit its signals solely through amino acids 220 through 1323 and suggest that the putative phosphorylation site at amino acid 230 is not the only requirement for CCR1 control.

TABLE 3. Effect of *ccr1* mutation in conjunction with *ADR1* genes

Relevant genotype	ADH II activity (mU/mg) on ethanol
ADR1[a]	2,500
ADR1 ccr1[a]	50
ADR1-304[b]	800
ADR1-304 ccr1[c]	6
ADR1-220[b]	360
ADR1-220 ccr1[d]	5

[a] As determined by Denis (8).
[b] See Table 1.
[c] Average of four segregants from diploid: 521-10 × 205-5d.
[d] Average of three segregants with 15 copies of *ADR1-220* from diploid: 500-16-C3 × 205-5d.

ADR1 domain required for growth on glycerol as a carbon source. Each of the *ADR1* genes capable of activating *ADH2* allows cells to grow on ethanol-containing medium (Table 2). However, this was not found to be the case for growth on glycerol-containing medium. Yeast cells containing a complete *ADR1* gene grew rapidly to an initial cell density of 1×10^7 to 2×10^7 cells per ml on glycerol-containing medium (Fig. 3). This initial growth was due to the use of carbon sources other than glycerol in the YEP medium, since similar initial growth rates were observed in YEP medium lacking glycerol (Fig. 3). Further growth, to densities 3×10^8 to 5×10^8 cells per ml, required the presence of glycerol and an *ADR1* gene having at least 506 N-terminal amino acids (Fig. 3; Table 2). Strains carrying an *adr1-1* allele but also having ADH II enzyme activity due to the *ADH2-S6* allele were unable to grow on glycerol-containing medium (Table 2), indicating that growth on glycerol was independent of ADH II activity levels. Reinoculation of cells grown to 3×10^8 to 5×10^8 cells per ml on glycerol-containing medium into fresh glycerol-containing medium resulted in growth patterns identical to those described above, indicating that glycerol growth is not due to mutation or adaptation. Glycerol kinase and glycerophosphate dehydrogenase, two enzymes responsible for glycerol metabolism (33), were assayed in cells containing the *ADR1* or the *adr1-1* allele, but no differences were observed (data not shown).

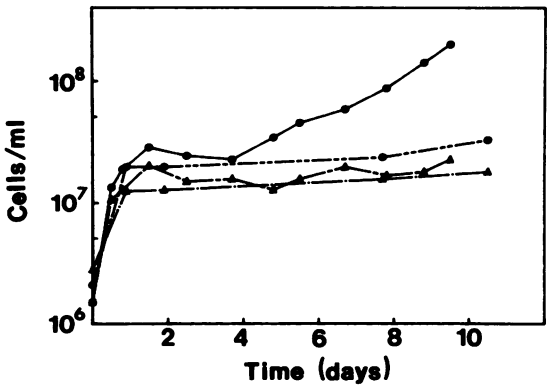

FIG. 3. Growth curves for cells grown on glycerol-containing medium. Symbols: ●, strain 411-40 (one copy of *ADR1-1323* as described in the legend to Fig. 1; also Table 1) grown on YEP medium supplemented with 3% glycerol; ▲, strain SPH6 (*adr1-1 ADH2-S6*) grown on YEP medium supplemented with 3% glycerol; ○, strain 411-40 grown on YEP medium; △, strain SPH6 grown on YEP medium. Strain SPH6 is described in footnote *a* of Table 2.

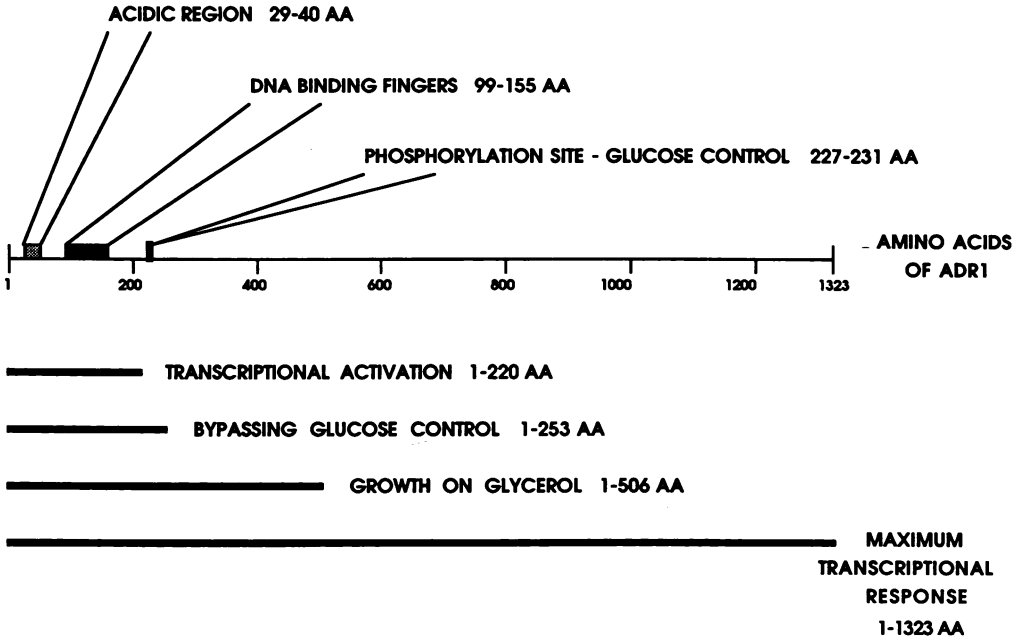

FIG. 4. Functional regions of the ADR1 protein. Solid lines beneath the ADR1 polypeptide indicate regions which have been identified as important in ADR1 function. Regions identified by sequence homology comparisons and mutation analysis are indicated above the line representing the ADR1 polypeptide. The numbers after the lines refer to the amino acid regions indicated.

DISCUSSION

The large size of the transcriptional activator ADR1 suggests that it contains multiple domains, each potentially having one or more functions. Several of the regions which have been identified are depicted in Fig. 4. The deletion analysis experiments described in this paper confirm the importance of the putative DNA-binding region of ADR1 (amino acids 99 through 155) to ADR1 function. Deletion into this region resulted in an ADR1 polypeptide unable to allow *ADH2* derepression. While this result is consistent with a previous deletion analysis of *ADR1* (12), the possibility remains that such an observation could be the result of decreased levels of ADR1-151 protein in the cell, rather than intrinsic inactivity of ADR1-151. However, this is probably not the case. When an ADR1-151–β-galactosidase fusion protein was expressed to the same levels as longer ADR1-β-galactosidase fusions, as measured by β-galactosidase activity, the ADR1-151–β-galactosidase protein was unable to activate *ADH2*, whereas the longer fusion polypeptides were able to (R. Vallari and J. Cherry, personal communication). Our results thus support the hypothesis that ADR1-151 is inactive as a result of its lack of two complete zinc finger regions. In this regard, it is interesting to note that all proteins which share homology to the DNA-binding fingers of transcription factor IIIA contain at least two such fingers (3, 29, 30, 36), implying that multiples of such regions are required for function.

Although we have not been able to show directly that ADR1 binds to *ADH2* DNA, it is probable that it does bind DNA on the basis of its homology to transcription factor IIIA. If this were the case, the first 220 amino acids of ADR1 must contain at least the recognition sites for binding and the ability to activate transcription. Transcriptional activation regions have been mapped relative to the DNA-binding regions for several other eucaryotic regulators. For the yeast GCN4 (14) and GAL4 (25) activators, the two regions are physically distinct. For the glucocorticoid receptor, however, it appears that both regions may occur in the same or overlapping segments of the protein (13, 28). The GCN4 and GAL4 activation regions coincide with an acidic part of the polypeptide that has been postulated to be a contact point with RNA polymerase II or another transcriptional component (34). ADR1 also contains a single acidic region, amino acids 29 through 40 (50% acidic), within the first 220 amino acids (Fig. 4).

Whereas the first 220 amino acids of ADR1 were sufficient to cause at least some derepression of *ADH2*, no shortened ADR1 protein was as functional as ADR1-1323 (Fig. 2B). Progressive carboxy-terminal deletions caused progressive decreases in the ability of ADR1 to maximally activate *ADH2*. These differences in maximal activation of *ADH2* are not due to differences in protein stability, indicating that a large part, if not all, of ADR1 is required for normal modulation of its transcriptional function. These findings are in contrast to the results of a previous study using plasmid *ADR1* gene constructs, which suggested that an ADR1-506 polypeptide retained wild-type function (12). The interpretation of the earlier results was complicated, however, by the fact that not all cells contained plasmids and that *ADR1* dosage per cell was not known. Although we are unable to define the function of amino acids 220 through 1323 of ADR1 as they pertain to *ADH2* control, it is possible that this region provides contact points to itself or other regulatory proteins that improve the efficiency of ADR1 activation but are not required for transcriptional activation. It is also possible that parts of the C terminus are required to ensure proper folding of the N terminus. Without the whole original C terminus, the N-terminal domain, although partially active, would form a functionally impaired structure.

A second region of importance to ADR1 function has been defined by characterization of $ADR1^c$ mutations that allow *ADR1* to bypass glucose control. These mutations occur within the cAMP-dependent protein kinase phosphorylation recognition sequence (22) between amino acids 227 and 231

(9; Cherry et al., in preparation). *ADR1*^{c} mutations could bypass glucose repression, either by enhancing ADR1 transcriptional activity or by blocking ADR1 interaction with a negative effector that acts at the putative phosphorylation region. The ADR1-220 protein lacking the site of the *ADR1*^{c} mutations was shown to be capable of activating *ADH2* under derepressed conditions, indicating that the putative phosphorylation site is not absolutely required for ADR1 function. However, ADR1-220 still conferred a glucose-repressed ADH2 phenotype. Therefore, our studies do not support the hypothesis that ADR1 is inactivated under repressed conditions by the binding of a repressor at the putative phosphorylation region.

Instead, the effect of increased *ADR1* dosage on glucose-repressed ADH II activity supports the model that the putative phosphorylation region plays a positive role in ADR1 function. Increased levels of ADR1-220 as compared with ADR1-253 and larger ADR1 polypeptides displayed diminished ability (at least 10-fold) to bypass glucose control. In contrast, under derepressed conditions, ADR1-220 was only two- to threefold less functional than ADR1-253 and ADR1-272 (Table 1; Fig. 2B). Also, the K_D values for ADR1-220 and ADR1-272 were similar, suggesting that the difference in function under repressed conditions was not a result of a difference in protein stability. On the basis of these results, the region between amino acids 220 and 253 appears to be required for increased dosages of *ADR1* to activate *ADH2* under glucose conditions. The fact that ADR1-220 is partly active under derepressed conditions may indicate that the transcriptional state of *ADH2* during derepression is sufficiently enhanced by other induced transcriptional factors to allow a shortened ADR1 to be sufficient for *ADH2* expression. Under repressed conditions, however, these other factors would be expected to be less active, and a larger ADR1 polypeptide would be required to bypass glucose repression.

We believe, therefore, that the *ADR1*^{c} mutations bypass glucose control by enhancing ADR1 activity. Such a mechanism could be envisioned to occur by increased DNA binding, by augmented contacts with other regulatory proteins or transcriptional components, or by counteracting a negative domain in the N-terminal 220 amino acids. The control of ADR1 should be compared with that proposed for the control of yeast transcriptional activator GAL4, in which GAL4 is inactivated by an interaction with the negative effector GAL80 (18, 26). The region of GAL4 to which GAL80 binds, however, is also required by GAL4 for transcriptional activation. Similarly, for ADR1 we propose that phosphorylation would inactivate ADR1 by interfering with a required function of the phosphorylated region.

The protein kinase CCR1 has been suggested to act through the putative phosphorylation region of ADR1 in its positive control of *ADH2* (9). However, deletion of the ADR1 phosphorylation region did not render *ADH2* derepression independent of the *CCR1* mutation (Table 3). This result implies that CCR1 may control ADR1 through the first 220 amino acids at other potential phosphorylation sites or that CCR1 does not act through ADR1 at all.

The requirement of ADR1 for growth on glycerol implicates ADR1 as a more global regulator of nonfermentative growth than had previously been thought. However, the identity of the factor through which ADR1 controls growth on glycerol is unknown. A factor important to growth on glycerol, other than the enzymatic activities of glycerol kinase and glycerophosphate dehydrogenase, could be the process of transporting reducing equivalents or other molecules across the mitochondrial membrane (5, 11). Amino acids 304 through 506 of ADR1 are required for growth on glycerol, though they are not essential for *ADH2* activation. These results suggest that ADR1 control of growth on glycerol is modulated by as yet undefined regulators which require this region of the ADR1 protein.

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