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The Identification and Characterization of *ADR6*, a Gene Required for Sporulation and for Expression of the Alcohol Dehydrogenase II Isozyme From *Saccharomyces cerevisiae*

Aileen K. W. Taguchi[1] **and Elton T. Young**

Department of Biochemistry, University of Washington, Seattle, Washington 98195

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ABSTRACT

The alcohol dehydrogenase II isozyme (enzyme, ADHII; structural gene, *ADH2*) of the yeast, *Saccharomyces cerevisiae*, is under stringent carbon catabolite control. This cytoplasmic isozyme exhibits negligible activity during growth in media containing fermentable carbon sources such as glucose and is maximal during growth on nonfermentable carbon sources. A recessive mutation, *adr6-1*, and possibly two other alleles at this locus, were selected for their ability to decrease Ty-activated *ADH2-6^c* expression. The *adr6-1* mutation led to decreased ADHII activity in both *ADH2-6^c* and *ADH2^+* strains, and to decreased levels of *ADH2* mRNA. Ty transcription and the expression of two other carbon catabolite regulated enzymes, isocitrate lyase and malate dehydrogenase, were unaffected by the *adr6-1* mutation. *adr6-1/adr6-1* strains were defective for sporulation, indicating that *adr6* mutations may have pleiotropic effects. The sporulation defect was not a consequence of decreased ADH activity. Since the *ADH2-6^c* mutation is due to insertion of a 5.6-kb Ty element at the TATAA box, it appears that the *ADR6^+*-dependent ADHII activity required *ADH2* sequences 3′ to or including the TATAA box. The *ADH2* upstream activating sequence (UAS) was probably not required. The *ADR6* locus was unlinked to the *ADR1* gene which encodes another *trans*-acting element required for *ADH2* expression.

BAKER'S yeast, *Saccharomyces cerevisiae*, produces at least three different alcohol dehydrogenase (ADH) isozymes to catalyze the interconversion of acetaldehyde and ethanol (LUTSTORF and MEGNET 1968; PAQUIN and WILLIAMSON 1986). The ADHII isozyme (structural gene, *ADH2*) is believed to function in the reutilization of ethanol during oxidative growth via the trichloroacetic acid (TCA) cycle and gluconeogenesis. In the presence of fermentable carbon sources such as glucose, ADHII activity is strongly catabolite repressed. In contrast, ADHII activity undergoes a marked increase or derepression upon transfer of yeast cells to media containing only nonfermentable carbon sources such as glycerol. The stringent carbon catabolite control of ADHII activity has been correlated with changes in the levels of *ADH2* mRNA (WILLIAMSON *et al.* 1983).

Ciriacy isolated allyl alcohol resistant yeast mutants which were no longer able to derepress ADHII activity in the presence of nonfermentable carbon sources (CIRIACY 1975a,b). Some resulting strains contained recessive mutations at the *ADR1* locus. Subsequent studies indicated that *ADR1* codes for a *trans*-acting, positive regulatory element (CIRIACY 1979) required for the production of *ADH2* mRNA (DENIS, CIRIACY and YOUNG 1981). Cloning and sequencing of the *ADR1* gene demonstrated that the wild type gene can code for a 1323 amino acid protein with striking homology to a *Xenopus* transcription factor, TFIIIA (DENIS and YOUNG 1983; HARTSHORNE, BLUMBERG and YOUNG 1986). Deletion analyses of the *ADH2* gene have implicated a 22 bp perfect dyad located at −215 bp from the transcription initiation site in the *ADR1*-dependent derepression of ADHII activity (BEIER, SLEDZIEWSKI and YOUNG 1985; SHUSTER *et al.* 1986). This region has been designated the upstream activating sequence (UAS) of the *ADH2* gene.

Several other loci affecting *ADH2* expression have also been studied. The *ADR4* locus appears to encode a *trans*-acting, negative control element, but recessive *adr4* mutations yielded increased ADHII activity only in the presence of the constitutive *ADR1-5^c* mutation (CIRIACY 1979). Recessive mutations at the *CCR* and *TYE* loci (CIRIACY 1977; CIRIACY and WILLIAMSON 1981; DENIS 1984) led to decreased ADHII activity whereas recessive mutations at the *CRE* loci led to ADHII activity even in the presence of glucose (Denis 1984).

We were interested in utilizing allyl alcohol to select for mutations at previously unknown loci which would lead to decreased ADHII activity. In order to avoid

[1] Present address: Department of Biological Sciences, Stanford University, Stanford, California 94305.

isolation of additional *adr1* mutations, we utilized the *ADH2-6^c* allele (CIRIACY 1979; WILLIAMSON, YOUNG and CIRIACY 1981) rather than the wild-type *ADH2* gene. Insertion of a 5.6-kb Ty element in *ADH2-6^c* mutants removes the putative target site for the *ADR1* gene product to an upstream site where the dyad is unable to affect *ADH2* expression (BEIER and YOUNG 1982; WILLIAMSON *et al.* 1983). The Ty element of the *ADH2-6^c* mutation has inserted into the presumptive *ADH2* TATAA box at −105 leading to a precise duplication of the "TATAA" sequence on either side of the inserted Ty element. Since we wished to avoid the isolation of recessive *ccr* (CIRIACY 1977; DENIS 1984), *tye*, and Ty excision mutants (CIRIACY and WILLIAMSON 1981), our selection involved a diploid *ADH2-6^c/ADH2-6^c* strain. Previous work has shown that the *ADH2-6^c* mutation exhibits decreased constitutivity in *MAT***a**/*MATα* diploids due to **a**/α-suppression (TAGUCHI, CIRIACY and YOUNG 1984). Therefore, we used a mating competent *MAT***a**/*MAT***a** *ADH2-6^c/ADH2-6^c* diploid strain which yields a higher degree of constitutivity than mating incompetent *MAT***a**/*MATα* *ADH2-6^c/ADH2-6^c* strains.

We report the isolation and characterization of mutations at the *ADR6* locus which define a previously unknown element required both for expression of the *ADH2* gene and for sporulation. The *ADR6^+* gene appears to mediate transcriptional activation of the *ADH2* gene through a site 3′ to or including the *ADH2* TATAA box.

MATERIALS AND METHODS

Media: Basic yeast medium (YEP) containing 1% (w/v) yeast extract, 2% (w/v) Bacto-Peptone (Difco Laboratories), 0.002% (w/v) adenine, and 0.002% (w/v) uracil was supplemented with 10% (w/v) glucose (YEP-glucose) for glucose-repressing conditions. Derepressed cells were obtained by growth in YEP supplemented with 3% (v/v) glycerol (YEP-glycerol). Cells were maintained in exponential growth for approximately 20 hr at 30° by transfer to fresh medium.

Glycerol allyl alcohol plates, used for mutant selection, contained YEP supplemented with 2% (w/v) glycerol, 20 mM allyl alcohol, and 2% (w/v) agar.

Sporulation of yeast was induced on solid medium (pH 7) containing 2% (w/v) KAc, 0.25% (w/v) yeast extract, 0.1% (w/v) glucose, 2% (w/v) agar, and 0.002% (w/v) each of adenine, leucine, lysine, methionine, tryptophan and uracil.

Yeast strains and general genetic methods: Table 1 lists the *Saccharomyces cerevisiae* strains used in this study. Yeast tetrad analysis procedures have been described (MORTIMER and HAWTHORNE 1969, 1975).

Enzyme activity assays: Alcohol dehydrogenase activity at 25.0° was determined spectrophotometrically by following the kinetics of NAD^+ reduction at 340 nm in the presence of ethanol (DENIS, CIRIACY and YOUNG 1981). Isocitrate lyase activity at 25.0° was determined spectrophotometrically by following the kinetics of formation of glyoxylic acid phenylhydrazone at 324 nm essentially as described by POLAKIS and BARTLEY (1965). Malate dehydrogenase activity at 25.0° was determined spectrophotometrically by following the kinetics of NADH oxidation at 340 nm (POLAKIS and BARTLEY 1965).

Southern analysis: Small amounts of high molecular weight yeast DNA were prepared (DENIS and YOUNG 1983) and cleaved with restriction enzymes (Bethesda Research Laboratories and New England Biolabs) according to the manufacturers' instructions. Electrophoresis of DNA fragments, transfer to nitrocellulose, and hybridization to nick translated YRp7-ADR2-BSa was carried out with the modifications described by WILLIAMSON, YOUNG and CIRIACY (1981).

Protoplast fusion: Ca^{2+}-treated yeast spheroplasts were prepared (BEGGS 1978) and fusion of two spheroplasted haploid strains of the same mating type was allowed to proceed at room temperature for 15 min in 35% (w/v) polyethylene glycol (average molecular weight of 4000) and 10 mM $CaCl_2$. After washing in 1 M sorbitol, the cell mixture was diluted into an overlay solution and spread on a solid minimal medium lacking adenine and tryptophan to select for diploids, much as described by BEGGS (1978).

Other procedures: Quantitative S1 mapping analysis of *ADH2* mRNA 5′ ends and Northern blot hybridization analysis of Ty RNA were carried out as previously described (TAGUCHI, CIRIACY and YOUNG 1984). Native polyacrylamide gel electrophoresis has been described (WILLIAMSON *et al.* 1980).

RESULTS

Isolation of glycerol + allyl alcohol resistant mutants: The *ADH2-6^c* and *MAT***a** homozygous diploid, 406.6-3, was isolated from strain 406.6-2 (*MAT***a**/*MATα*) following UV-irradiation as described previously (TAGUCHI, CIRIACY and YOUNG 1984). As shown in Table 2, mating competent 406.6-3 undergoes some increase in ADHII activity, from 640 milliunits/mg to 810 milliunits/mg, as the carbon source is switched from glucose to glycerol. In contrast, mating incompetent 406.6-2 shows an aberrant carbon source suppression of the Ty-activated *ADH2-6^c* gene; the glucose-repressed activity (300 milliunits/mg) is higher than the glycerol-derepressed activity (49 milliunits/mg).

Spontaneous mutants of 406.6-3 were selected by demanding growth on solid glycerol medium containing 20 mM allyl alcohol. Following incubation at room temperature for 8–22 days, a total of 31 mutant strains, designated 406.6-m1 through 406.6-m31, were isolated. The mutations were denoted *m1* through *m31*. Our isolation method did not ensure that these mutations were due to independent events. In this work, we report on the characteristics of three mutant strains (406.6-m4, 406.6-m14 and 406.6-m31) which exhibited decreased ADHII activity relative to strain 406.6-3 (Table 2). These three mutant isolates show an unusual ADHII activity pattern; the ADH activity is higher in the presence of the normally repressing carbon source, glucose, than in the presence of glycerol, just as with 406.6-2. The aberrant carbon source response may be due to an effect of the

TABLE 1

Yeast strains

Strain	Genotype[a]	Source
406.6-2	*MAT***a** *ADH2-6*^{c} + *his4* + *trp2* / *MAT*α *ADH2-6*^{c} *his3 his4 ade2 trp2*	This work
406.6-3	*MAT***a**/*MAT***a** derivative of 406.6-2	This work
406.6-14	*MAT*α *ADH2-6*^{c} *his3 ade2 trp2* / *MAT*α *ADH2-6*^{c} + *ade2 trp2*	TAGUCHI, CIRIACY and YOUNG (1984)
406-1b	*MAT*α *ADH2-6*^{c} *trp1 his3*	This work
407-1d	*MAT*α *ADH2*$^+$*-M adr6-1 his3 his4 ura1 ade2*	This work
530-9	*MAT***a** *ADH2*$^+$*-F ADR1-5*^{c} *trp1 leu2 ura1*	DENIS (1984)
m4:3b:1b	*MAT***a** *ADH2-6*^{c}*-F adr6-1 his3 trp2 ade2*	This work
SF2-14A	*MAT*α *ADH2*$^+$*-M his4 ura1*	M. CIRIACY

[a] All strains are *adh1-11 adh3-1 ADR1*$^+$ *ADR6*$^+$ unless otherwise indicated.

TABLE 2

ADHII specific activities of mutant segregants

Strains[a]	ADHII specific activity (milliunits/mg protein)[b] Glucose	Glycerol
406.6-2 (**a**/α)	300 ± 25	49 ± 7.6
406.6-3 (**a**/**a**)	640 ± 33	810 ± 160
406.6-m4 (**a**/**a**)	200	51
406.6-m14 (**a**/**a**)	580	400
406.6-m31 (**a**/**a**)	340	300
406-1b	ND[c]	840 ± 38
M4$^+$ segregants	640 ± 22	1100 ± 60
M4$^-$ segregants	72 ± 16	21 ± 2.1
M14$^+$ segregants	700 ± 61	1100 ± 50
M14$^-$ segregants	95 ± 13	24 ± 3.1
M31$^+$ segregants	770 ± 73	1300 ± 90
M31$^-$ segregants	95 ± 39	19 ± 3.6

[a] The mating type has been specified in parentheses only for diploid strains. All strains have the *ADH2-6*^{c} allele. Segregants were from mating crosses of 406.6-m4, 406.6-m14, or 406.6-m31 to 406.6-14. Additional segregants were obtained from mating crosses of haploid segregants from the above cross to 406-1b.

[b] ADHII specific activities were measured with one to five trials. For each segregant type, four to 32 strains were examined. The values in parentheses indicate the range of values obtained when three measurements were taken. For four or more measurements, the mean ± the standard error of the mean (BEVINGTON 1969) is presented.

[c] Not determined.

adjacent Ty element (Ty RNA appears to exhibit a similar carbon source response) (TAGUCHI, CIRIACY and YOUNG 1984; ELDER *et al.* 1980) or to some as yet unknown regulatory element which is sensitive to carbon source.

Southern blot analysis of DNA isolated from 406.6-3 and from the mutants showed that Ty excision or major DNA rearrangement in the *ADH2* region is not the cause of the mutant phenotypes (TAGUCHI 1986). Northern blot analysis showed that total cellular Ty RNA levels are unaffected by the *m4*, *m14* and *m31* mutations (TAGUCHI 1986). The mutations did not affect Ty regulation in general and were candidates for *ADH2* regulatory mutations.

Are the mutant phenotypes due to single mutations? The *MAT***a**/*MAT***a** *ADH2-6*^{c}/*ADH2-6*^{c} mutant strains (406.6-m4, 406.6-m14 and 406.6-m31) were mated to a *MAT*α/*MAT*α *ADH2-6*^{c}/*ADH2-6*^{c} diploid, 406.6-14. The resulting tetraploids were subjected to two successive sporulations to yield haploid spores.

In order to determine if the M4$^-$, M14$^-$ and M31$^-$ phenotypes were each due to single mutations or to multiple mutations, one mutant haploid strain from each of the four crosses described above was mated to 406-1b, an *ADH2-6*^{c} haploid strain. As expected for a single mutation, the *m4*, *m14* and *m31* crosses yielded 2:2 segregation of mutant:wild type in 9–10 tetrads. Each of these three mutations were due to either single mutations or to two tightly linked mutations (<5.5 cM apart).

Table 2 displays the ADHII activities of the haploid segregants. The *m4* mutation appeared to be dominant since the original mutant diploid isolate, 406.6-m4, as well as the haploid segregants have low ADHII activities (however, subsequent studies detailed in the following section showed that *m4* is a recessive mutation). *m14* and *m31* appeared to be semidominant mutations. In other respects, the *m4*, *m14* and *m31* mutations appeared quite similar. The ADHII activities of the haploid mutant segregants were within a standard deviation of each other and were higher under glucose repression than under glycerol derepression. The apparent dominance or semidominance was more pronounced in the presence of glycerol. Our subsequent studies concentrated on the *m4* mutation. Because *M4*$^+$ is required for ADHII activity, we will refer to it as *ADR6*$^+$ (Alcohol *D*ehydroge-

TABLE 3

***adr6-1* is a recessive mutation**

Construction method	Pertinent genotype[a]	ADHII specific activity (milliunits/mg)[b]	
		Glucose	Glycerol
UV-induced mitotic recombination	$ADR6^+/ADR6^+$	ND[c]	1300 ± 110
	$ADR6^+$/adr6-1	ND	930 ± 180
	adr6-1/adr6-1	ND	10 ± 2.6
Protoplast fusion	$ADR6^+/ADR6^+$	520 (480–570)	860 (820–900)
	$ADR6^+$/adr6-1	450 (380–530)	980 (340–1200)
	adr6-1/adr6-1	11 (0–32)	3.7 (0.0–8.0)

[a] All strains are homozygous at their mating type loci and homozygous for *ADH2-6*[c].

[b] ADH activities for *MAT* homozygous strains constructed by UV-induced mitotic recombination, are presented as the mean of measurements from four strains ± the standard error of the mean. The average ADH activities for two to five strains constructed by protoplast fusion are presented, followed by the observed range of measurements in parentheses.

[c] Not determined.

nase *R*egulator *6*) and the *m4* mutation as *adr6-1* in the following sections.

***adr6-1* is a recessive mutation:** The original isolation of *adr6-1* in the diploid strain suggested that it was a dominant mutation. To determine whether this was true or not, three types of diploids were constructed—*ADR6*$^+$/*ADR6*$^+$, *ADR6*$^+$/*adr6-1* and *adr6-1/adr6-1* strains, each with *ADH2-6*^{c}/*ADH2-6*^{c}. Since **a**/α-suppression of *ADH2-6*^{c} would lead to low ADHII activity in all three types of diploids, two methods were used to produce *MAT* homozygous diploids. First, mating type homozygous strains were isolated from **a**/α strains following UV-induced recombination. The second method involved protoplast fusion of two strains with the same mating type. The ADHII activities of the resultant strains showed that *adr6-1* is a recessive mutation since only the *adr6-1/adr6-1* diploids exhibited a reduction in ADHII activity (Table 3). 406.6-m4 may have contained both *adr6-1* and at least one additional modifying mutations which allowed the Adr6$^-$ phenotype to be expressed in the diploid. Alternatively, 406.6-m4 may have simply been homozygous for *adr6-1*.

***adr6-1, m14* and *m31* lead to a sporulation defect:** An attempt was made to assign *adr6-1, m14* and *m31* to complementation groups. A simple *cis-trans* test was not attempted since the *ADH2-6*^{c} allele in our strains undergoes **a**/α-suppression which is not easily distinguishable from the Adr6$^-$, M14$^-$ and M31$^-$ phenotypes. Instead, we tried to determine whether these mutations segregated away from each other or not. This attempt failed, however, when it was observed that diploids containing two mutations in a heterozygous condition (*adr6-1/m31* and *m14/m31* diploid strains) did not sporulate. Diploids homozygous for a single mutation (*adr6-1/adr6-1, m14/m14* and *m31/m31* strains) also did not sporulate. However, diploids heterozygous for a single mutation (*adr6-1/ADR6*$^+$, *m14/M14*$^+$ and *m31/M31*$^+$ strains) and homozygous wild-type diploid controls did complete sporulation. The observation that the doubly heterozygous diploids were similar to the homozygous diploids with respect to lack of sporulation ability suggested that the sporulation defect was probably due to the *adr6-1, m14* and *m31* mutations rather than to some other adventitious mutation. The observation of this recessive sporulation defect and the carbon source-dependent pattern of ADHII activity decrease seen with these three mutations support the hypothesis that *adr6-1, m14* and *m31* are in the same complementation group.

ADHII activity is probably not required for sporulation on KAc sporulation medium. We have observed that an Adh1$^-$ Adh2$^-$ Adh3$^-$ diploid can successfully sporulate (Taguchi 1986). Therefore, it seems likely that *ADR6*$^+$ is required for some process involved in sporulation as well as for stimulation of ADHII activity.

The mutations do not affect isocitrate lyase and malate dehydrogenase activity: To determine whether other carbon catabolite-sensitive enzymes are also affected by these mutations, we assayed isocitrate lyase (ICL), a glucose-repressible glyoxylate shunt enzyme found in the cytoplasm, and malate dehydrogenase (MDH) which is involved in the glyoxylate pathway and the TCA cycle. The cytoplasmic isozyme of MDH is glucose-inactivated and the mitochondrial isozyme undergoes glucose-repression (Hagele, Neeff and Mecke 1978). Both ICL and ADHII activity have been observed to decrease in *ccr1* strains (Ciriacy 1977). Table 4 demonstrates that ICL activity and MDH activity are unaffected by the *adr6-1* mutation in the presence of the nonfermentable carbon source, glycerol. The *m14* and *m31* mutations also had no effect on ICL activity (Taguchi 1986).

TABLE 4

Isocitrate lyase activity and malate dehydrogenase activity are unaffected by *adr6-1*

Exp. No.	Pertinent genotype[a]	Enzyme specific activity (milliunits/mg)		
		ADHII	ICL	MDH
1[b]	*ADR6*$^+$	1200 ± 120	15 ± 0.90	ND[c]
1	*adr6-1*	18 ± 3.4	12 ± 1.8	ND
2[d]	*ADR6*$^+$	1600 ± 240	ND	5400 ± 190
2	*adr6-1*	39 ± 7.3	ND	4100 ± 450

[a] Tetrads from the cross, m4:3b:1b (*ADH2-6*^{c} *adr6-1*) × 406-1b (*ADH2-6*^{c} *ADR6*$^+$), were grown in YEP-glycerol and assayed. The mean activity ± the standard error of the mean is presented.

[b] Six to ten segregants were assayed with one to two trials.

[c] Not determined.

[d] Six segregants of each genotype were assayed.

As expected, both Adr6$^+$ and Adr6$^-$ segregants underwent stringent glucose-repression of ICL activity and some glucose-repression of MDH activity (TAGUCHI 1986). These results indicate that the *adr6-1* mutation does not pleiotropically affect all glucose-repressible enzymes.

***ADR6*$^+$ is required for *ADH2*$^+$ expression:** *adr6-1* was isolated in an *ADH2-6*^{c} mutant strain in which a Ty element insertion has occurred in the 5′ region of the *ADH2* gene. To determine whether *adr6-1* also affects the wild type *ADH2* gene, an *adr6-1 ADH2-6*^{c}*-F* strain (m4:3b:1b) was mated to an *ADR6*$^+$ *ADH2*$^+$*-M* strain (SF2-14A) and tetrad analysis was carried out on the resulting spores. The two *ADH2* alleles were distinguished on the basis of the electrophoretic mobilities of the native ADHII enzymes. *ADH2-F* codes for a fast mobility form of ADHII and *ADH2-M* codes for an intermediate mobility form of ADHII. The genotypes of the spores were easily assigned following native gel analysis and determination of the ADHII activities. Out of four tetrads examined, three were tetratype and one was a nonparental ditype with respect to *ADR6* and *ADH2*. The ease of tetratype recovery showed that *ADH2* and *adr6-1* segregated independently and that the two genes are not closely linked.

The ADHII activities of the four tetrads are shown in Table 5. In the presence of the derepressing carbon source, glycerol, *adr6-1* leads to a decrease in *ADH2*$^+$ activity (15-fold decrease), much as it does with *ADH2-6*^{c} (34-fold decrease). Under glucose-repression, ADHII activity is normally quite low in *ADH2*$^+$ strains and it is not clear whether *adr6-1* leads to a further decrease in repressed activity or not.

***ADR6* and *ADR1* are two different loci:** A recessive mutation, such as *adr6-1*, which leads to decreased ADHII activity could be due to a lesion in a gene which normally codes for an activator of the *ADH2* gene. Recessive *adr1* mutations lead to a similar decrease in ADHII activity. The *ADR1*$^+$ gene encodes a protein which appears to be a positive transcriptional element specific for *ADH2*. To determine whether the *ADR6* locus is equivalent to *ADR1*, an *ADR1*$^+$ *adr6-1 ADH2*$^+$ strain (407-1d) was mated to an *ADR1-5*^{c} *ADR6*$^+$ *ADH2*$^+$ strain (530-9). Tetratype asci were easily recovered from this mating cross, indicating that *ADR1-5*^{c} and *adr6-1* are not allelic. The ADHII activities of the resulting segregants (Table 6) indicate that the *ADR1-5*^{c} allele can almost completely overcome the effect of *adr6-1* on *ADH2*$^+$. Thus, *ADR1-5*^{c} appears partially epistatic to *adr6-1* in the presence of *ADH2*$^+$.

TABLE 5

***adr6-1* affects *ADH2*$^+$**

Genotype[a]		ADHII specific activity (milliunits/mg)[b]	
ADH2	*ADR6*	Glucose	Glycerol
6^c	+	690 (520–1200)	910 (310–2900)
6^c	−	170 (93–210)	27 (19–32)
+	+	9.5 (6.6–13)	3200 (1500–4100)
+	−	3.2 (0.0–8.0)	220 (98–340)

[a] Three tetratype and one nonparental ditype asci from the cross m4:3b:1b (*ADH2-6*^{c}*-F adr6-1*) × SF2-14A (*ADH2*$^+$*-M ADR6*$^+$) were examined.

[b] The average ADHII activity from three to five segregants of the pertinent genotype is presented. The range of observed values is shown in parentheses.

TABLE 6

***adr6-1* and *ADR1-5*^{c} are not allelic**

Genotype[a]			ADHII specific activity (milliunits/mg)[b]	
ADH2	*ADR1*	*ADR6*	Glucose	Glycerol
+	+	+	5.2 (0.86–10)	1,800 (1,100–2,900)
+	+	−	1.7 (0.0–3.8)	72 (55–83)
+	5^c	+	340 (200–480)	10,000 (7,900–13,000)
+	5^c	−	190 (130–280)	2,700 (2,000–3,200)

[a] These strains are segregants from the cross, 530-9 × 407-1d. The two spores of each tetrad which had substantial ADHII activity in the presence of glucose were assigned the *ADR1-5*^{c} allele. Assignment of the *adr6-1* mutation to two spores of each tetrad was confirmed by mating these segregants to a known *adr6-1* strain and looking for the Adr6$^-$-related sporulation defect.

[b] The average ADHII activity from three strains of each genotype is shown. The range of observed values is presented in parentheses.

ADR6*$^+$ is required for normal levels of *ADH2* mRNA:** We next asked whether the *ADR6*$^+$ gene product was required for normal levels of *ADH2* mRNA. Quantitative S1 mapping of *ADH2* mRNA 5′ ends was carried out (Fig. 1). RNA isolated from the original wild type parental strain, a *MATa**/*MAT***a** *ADH2-6*^{c}/*ADH2-6*^{c} diploid, grown in the presence of glycerol was run in lanes 1 and 2. Lane 3 shows that *ADH2* mRNA from a glycerol-derepressed *adr6-1* mu-

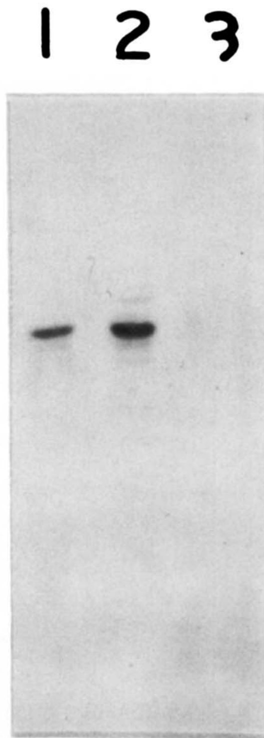

FIGURE 1.—S1 mapping analysis of *ADH2* mRNA 5′ ends in *ADR6*$^+$ and *adr6-1* strains. A no-S1 control had a single band higher up in the autoradiogram corresponding to full length probe and a no-RNA control yielded no bands (data not shown). Lane 1, 10 μg RNA from 406.6-3 (*MAT***a**/*MAT*α *ADH2-6*^{c}/*ADH2-6*^{c} *ADR6*$^+$/*ADR6*$^+$), 850 milliunits/mg ADHII activity. Lane 2, 20 μg RNA from 406.6-3. Lane 3, 10 μg RNA from m4:1b (*MAT***a** *ADH2-6*^{c} *m4*), 18 milliunits/mg. All lanes contained samples from cells grown in YEP-glycerol.

tant strain was not detected using ten μg of total RNA. We estimate that there is at least a 10-fold difference in *ADH2* mRNA level between the wild-type and *adr6-1* strains. The ADHII activities of the *ADR6*$^+$ and *adr6-1* strains were 850 and 18 milliunits/mg, respectively. These results indicate that *ADR6*$^+$ is required for normal levels of *ADH2* mRNA to be observed.

DISCUSSION

This paper describes the selection and properties of the *adr6-1* (*m4*), *m14* and *m31* mutations. Since each of these mutations leads to decreased ADHII activity, higher ADHII activity in glucose-containing medium than in the presence of glycerol in an *ADH2-6*^{c} background, and a recessive sporulation defective phenotype, it is possible that these mutations are allelic. Also, the three mutations have no effect on Ty RNA levels, isocitrate lyase activity, and malate dehydrogenase activity.

***ADR1-5*^{c} and *adr6-1* epistasis:** *ADH2*$^+$ gene expression apparently requires at least two *trans*-activating elements, defined by the *ADR1* and *ADR6* genes. The *ADR1* locus codes for a constitutively synthesized mRNA (DENIS and GALLO 1986) which can code for a 1323 amino acid protein (HARTSHORNE, BLUMBERG and YOUNG 1986). The *ADR1-5*^{c} mutation used in our epistasis study was due to a lesion in the protein-coding portion of the gene (DENIS and GALLO 1986). Since the *ADR1-5*^{c} mRNA was not present in greater abundance than *ADR1* mRNA, it appears likely that the *ADR1-5*^{c}-encoded protein has an enhanced ability to activate *ADH2* (DENIS and GALLO 1986).

We observed that *ADR1-5*^{c} was partially epistatic to *adr6-1*. A simple linear regulatory sequence in which either the *ADR6* gene product directly activates *ADR1* transcription or the *ADR1* gene product directly activates *ADR6* transcription is unlikely since in both of these cases, *adr6-1* would have been fully epistatic to *ADR1-5*^{c}.

A similar example of partial epistasis has been observed with mutations in the *GAL80*$^+$ and *gal81*$^+$ genes which are involved in the regulation of the galactose utilization enzymes (OSHIMA 1982). *GAL80* encodes a repressor function for the GAL4 transcription activator protein. *gal81*$^+$ has been mapped to a site within the *GAL4* gene. *GAL80*^{s} (super-repressible) *GAL81* (super-active GAL4 protein) double mutants show variable epistasis ranging from epistasis to partial epistasis to hypostasis depending on the alleles examined (DOUGLAS and HAWTHORNE 1972; NOGI *et al.* 1977). A recent model for the regulation of the galactose utilization enzymes involves protein-protein interactions between the GAL80 repressor and the GAL4 activator (OSHIMA 1982). In the absence of inducer, GAL80 repressor binds to the GAL4 protein via the *gal81*$^+$ site leading to repression of GAL4 activator function. In the presence of inducer, the interaction is reversed and the GAL4 protein is free to activate transcription of galactose utilization genes.

The behavior of our possibly analogous *adr6-1* *ADR1-5*^{c} double mutants could be explained if *ADR6* is required for activation of ADR1 protein function and if the *ADR1-5*^{c} mutation can partially overcome this requirement. The *ADR1-5*^{c} mutation leads to an arginine to lysine conversion within a possible cAMP protein kinase phosphorylation site (DENIS and GALLO 1986). It is tempting to speculate that *ADR1-5*^{c} defines the site of *ADR6* action. However, other characteristics of the *ADR1* and *ADR6* genes which are outlined in the following section argue against models involving ADR6-activation of ADR1 function. It seems most likely that the *ADR1* and *ADR6* genes stimulate *ADH2* expression through two somewhat independent pathways, and that the *ADR1-5*^{c} allele has gained the ability to partially replace the normal *ADR6* requirement.

***ADH2* sequences involved in *ADH2* mRNA production:** Many *trans*-acting yeast regulatory factors are believed to act at UAS (*U*pstream *A*ctivating *Se*-

quence) elements which are located 5′ to the presumptive TATAA boxes of regulated genes (GUARENTE *et al.* 1984; MILLER, MACKAY and NASMYTH 1985). Sequence-specific DNA-binding activity has been demonstrated for several factors (BRAM and KORNBERG 1985; GINIGER, VARNUM and PTASHNE 1985; HOPE and STRUHL 1985; JOHNSON and HERSKOWITZ 1985).

A second type of factor is exemplified by the *PPR1* gene product. *PPR1* has been shown to require *URA1* and *URA3* sequences either at or 3′ to the TATAA box (LOSSON, FUCHS and LACROUTE 1985). LOSSON and colleagues (1985) suggest that the required sequences are likely to occur 5′ to the translation start site.

Another set of factors encoded by the *ADR6* and *TYE* loci may mediate *ADH2* expression through sequences 3′ to the *ADH2* UAS. Mutations at the *ADR6* and *TYE* loci affect both the Ty insertion *ADH2-6^c^* allele and the *ADH2^+^* gene. The *ADH2-6^c^* Ty element is inserted at about −107 from the *ADH2* transcription initiation site (WILLIAMSON *et al.* 1983). The presumed TATAA box (−105) is intact in the *ADH2-6^c^* mutant allele, but the upstream activating sequence (UAS), which is required for ADR1-dependent *ADH2* expression, has been moved 5.6 kb further 5′ from its original site. Therefore, *ADR6*-dependent ADHII activity may not require the UAS, but may require the TATAA box or sequences 3′ to it. It is not clear whether the required regions are DNA or RNA sequences. The *ADR6*, *TYE* and *PPR1* gene products may act in an analogous fashion.

The *ADH2* sequence requirements for *ADR1* action and *ADR6* action appear to differ, suggesting independent modes of action for these two elements. *ADR1*-dependent *ADH2* derepression requires the 22 bp dyad region of the *ADH2* UAS (−215) located 5′ to the *ADH2* TATAA box (−105) (BEIER, SLEDZIEWSKI and YOUNG 1985; SHUSTER *et al.* 1986; J. YU, personal communication), whereas *ADR6* appears to require *ADH2* sequences located 3′ to or including the TATAA box. Thus, the *ADR1* gene product may function in the regulation of *ADH2* gene transcript initiation. The ADR1 protein may act at the UAS to facilitate entry of RNA polymerase II. The *ADR6* gene product may act at some subsequent step, but prior to the translation of *ADH2* mRNA. A role for the *ADR6* gene product in facilitation of *ADH2* RNA transcription, processing or maturation of the *ADH2* mRNA, or stabilization of the *ADH2* mRNA is possible.

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