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Deletion Analysis Identifies a Region, Upstream of the *ADH2* Gene of *Saccharomyces cerevisiae*, Which Is Required for *ADR1*-Mediated Derepression

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Deletion analysis was used to identify sequences upstream of the *ADH2* gene of *Saccharomyces cerevisiae* that are required for its regulation. 5′ and 3′ internal deletions of the *ADH2* control region were created in vitro, and the fragments were ligated adjacent to the *ADH1* promoter and structural gene. Hybrid genes with 3′ deletions extending from −119 to −216 (the start site of *ADH2* transcription is designated +1) were fully repressed and derepressed to high levels. Hybrid genes with 3′ deletions extending from −119 to −257 were repressed but failed to significantly derepress. Hybrid genes lacking the −216 to −257 region also failed to respond to *ADR1-5*^{c}, a mutant allele of the unlinked regulatory gene *ADR1*, which confers constitutive expression on *ADH2*. This implies that the region between these deletion endpoints, which includes a 22-base-pair sequence of dyad symmetry, is required for efficient derepression of an adjacent promoter. Internal deletions extending in the 3′ direction from position −1141 confirmed these results. Deletion mutants lacking the region −1141 to −259 were normally regulated, whereas deletions extending from −1141 to −115 were not derepressible. These results support the hypotheses that the *ADH2* promoter may normally be in an inactive conformation in the yeast chromosome and that derepression of *ADH2* requires positive activation mediated through an upstream activation sequence located between 216 and 257 base pairs 5′ to the start site of *ADH2* transcription. No evidence for a DNA sequence mediating repression was obtained.

Alcohol dehydrogenase II (ADH II) is representative of a class of enzymes which is catabolite repressed: they are absent or less abundant when *Saccharomyces cerevisiae* (bakers' yeast) is grown on a fermentable carbon source. Included in this class of proteins are enzymes that play a role in gluconeogenesis, in the glyoxylate cycle, in the metabolism of sugars other than glucose, and in mitochondrial respiration (25, 26). Decreased activity has been shown to be attributable to enzyme inactivation (19) or to inhibition of gene expression at the transcriptional or posttranscriptional level (6, 20, 34). The molecular mechanism of catabolite repression of gene expression is well understood in *Escherichia coli*, but in yeasts much less is known about this phenomenon. In *S. cerevisiae*, glucose repression does not appear to involve cyclic AMP, a key metabolite in catabolite control of gene expression in *E. coli* (22).

Neither ADH II enzyme activity nor its mRNA is detectable when *S. cerevisiae* is grown on a fermentable carbon source such as glucose (11, 21). The enzyme is derepressed over 100-fold when the fermentable substrate in the medium is exhausted or replaced by a nonfermentable carbon source such as glycerol or ethanol. In addition to the absence of a fermentable carbon source, derepression of *ADH2* requires the function of an unlinked regulatory locus, *ADR1*, which appears to be a positive activator specific for *ADH2* (8, 11). *ADR1* can mutate to *ADR1-5*^{c}, which results in an ADH II-constitutive phenotype. In a strain with an *ADR1-5*^{c} allele, *ADH2* mRNA is synthesized in the presence of glucose and is more highly derepressed than in a strain containing a wild-type allele of *ADR1* (8).

ADH2, the gene which codes for ADH II, has been cloned and sequenced (27, 33). The regulatory region which controls glucose repression of the *ADH2* gene in *S. cerevisiae* has been identified by genetic and molecular approaches (2, 8, 32). Deletion of a 1-kilobase (kb) DNA fragment upstream of the *ADH2* TATA box results in the loss of glucose control of ADH II synthesis (2). This sequence overlaps *ADR3*, a genetically defined, *cis*-acting locus which regulates *ADH2* (8). We have furthermore shown that when these 5′ flanking sequences are placed upstream of *ADH1* and its promoter, they confer glucose repression on that gene, which is normally abundantly expressed when yeasts are grown on glucose-containing media (2). Similar results have been found for the regulatory sequences that affect expression of *GAL10* (16) and *CYC1* (14).

In the experiments described here, we used deletion analysis to characterize the *ADH2* upstream control region to identify more specifically the sequences which play a role in repression and derepression of the adjacent structural gene. Hybrid genes containing deleted fragments of the *ADH2* control region were ligated adjacent to the *ADH1* promoter and structural gene and tested for their ability to direct the synthesis of ADH I. These hybrid genes were also tested in a strain containing a constitutive allele of *ADR1*, *ADR1-5*^{c}, to determine whether a specific site in the *ADH2* regulatory region mediates the effect of the *ADR1* gene product on *ADH2* expression.

MATERIALS AND METHODS

Strains and media. *E. coli* RR1 (5) was used for transformation. *S. cerevisiae* strains used as transformation recipients were 302-21 (*MATα adh1-11 adh2-43 adh3 ADR1 trp1 his4*) (31) and SF6-4A-M4 (*MATa trp1 adh1-11 adh2-43 adh3 ADR1-5*^{c}) (33). Trp$^-$ minimal medium, which is a synthetic complete medium lacking tryptophan (28), was used with either 8% glucose or 3% ethanol as a carbon source.

Yeast transformation. Yeasts were transformed by the

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method of Beggs (1). Transformants were selected by growth on Trp^- minimal medium plates containing sorbitol.

DNA preparation and cloning. Plasmid DNA was prepared from ampicillin-resistant *E. coli* by the method of Birnboim and Doly (3). Quick plasmid preparations were also used for rapid screening by restriction enzyme analysis. Methods for preparative isolation of DNA fragments have been described previously (33). Restriction enzymes (Bethesda Research Laboratories, Inc., and New England BioLabs., Inc.), T4 DNA ligase (Bethesda Research Laboratories), and calf alkaline phosphatase (Bethesda Research Laboratories) were used according to the instructions of the manufacturers.

Construction of hybrid genes with 3′ terminal deletions of the *ADH2* control region. A 1.0-kb *Bam*HI-*Sph*I fragment located 5′ to the *ADH2* structural gene was ligated with pBR322 which had been digested with *Bam*HI and *Sph*I, and ampicillin-resistant transformants of *E. coli* RR1 were isolated. Plasmid DNA containing the 1-kb *Bam*HI-*Sph*I fragment was purified, cut with *Sph*I, and briefly digested with exonuclease BAL 31 (18). The BAL 31-digested plasmid was ligated with phosphorylated *Xho*I molecular recombination linkers and introduced into *E. coli* by transformation to ampicillin resistance. Plasmids containing deletions within the *ADH2* control region were identified by digestion with *Bam*HI and *Xho*I. *Bam*HI-*Xho*I fragments containing deletions of approximately 48 to 650 base pairs (bp) were prepared from each of 10 selected plasmids.

A plasmid had been constructed previously which contains a *Sal*I site 152 bp upstream of the coding sequence of the *ADH1* gene (115 bp upstream of the transcription start site) and a *Bam*HI site 3′ to the gene (2). This *Sal*I-*Bam*HI fragment was purified and ligated separately with each of the *Bam*HI-*Xho*I-deleted *ADH2* control sequence fragments and with a pBC3T1 vector which had been digested with *Bam*HI and treated with calf intestinal alkaline phosphatase to prevent recircularization (pBC3T1 is a yeast-*E. coli* shuttle vector containing the yeast *TRP1* and *CEN3* sequences and pBR322 sequences [2]). DNA from individual transformants was prepared and digested with several restriction enzymes to identify plasmids which had the deleted *ADH2* sequences and the *ADH1* promoter and structural gene present in single copy and in the same orientation as in hybrids we have previously tested.

Construction of hybrid genes with 5′ terminal and internal deletions of the *ADH2* control region. A variant of the pBC3T1 vector was partially digested to generate a linear fragment with *Bam*HI and *Sph*I ends. A 5′*Sal*I-3′*Sph*I fragment containing a hybrid *ADH2-ADH1* gene (2) was partially digested with *Sau*3A, and the fragments were ligated into the *Bam*HI-*Sph*I-digested vector. Plasmids containing deletions corresponding to cleavage at all four *Sau*3A sites in the control region were isolated and designated pΔ5-1 through pΔ5-4, representing cleavage at positions −908, −523, −422, and −259, respectively.

The 5′ terminally deleted DNA fragments of *ADH2* were recloned as internal deletions after preparation of a vector containing the *ADH2* sequences normally present upstream of the *Bam*HI site of the control region. This was done by cloning a 1.7-kb *Bgl*II-*Bam*HI fragment of these upstream sequences into the *Bam*HI site of the pBC3T1 vector. This plasmid was then partially digested with *Bam*HI and *Sph*I, and partial *Sau*3A-*Sph*I-digested hybrid gene fragments were cloned into the vector. These plasmids were designated pΔ5-1B through pΔ5-4B. A 2.7-kb *Bam*HI-*Sph*I fragment containing the *ADH2* control region, promoter sequences, and structural gene was cloned in the same vector (pADH2-B). A polylinker containing a *Bam*HI site was inserted adjacent to the *Sal*I site of pADH1(−115), and a *Bam*HI-*Sph*I fragment containing the *ADH1* structural gene and 115 bp of 5′ sequence flanking its transcription start site was also cloned into this same vector [pADH1(−115)].

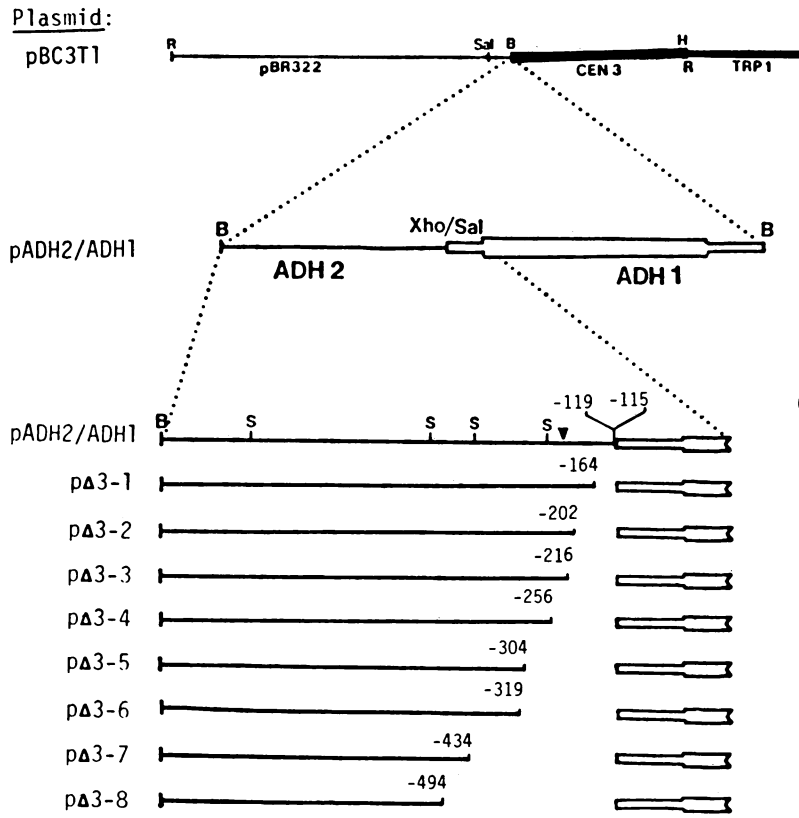
Alcohol dehydrogenase assays. Trp^+ colonies from transformation plates were streaked onto 8% glucose minimal medium plates lacking tryptophan and grown overnight. Cells from these plates were inoculated at a concentration of $\sim 5 \times 10^5$ cells per ml into 10 ml of minimal medium lacking tryptophan and containing 8% glucose. These cultures were grown overnight at 30°C to a concentration of $\sim 2 \times 10^7$ cells per ml. One half of the culture was collected and stored frozen at −70°C. The remaining half was washed once in water and resuspended in 5 ml of minimal medium lacking tryptophan and containing 3% ethanol. These cultures were grown for another 24 h at 30°C and then collected and frozen at −70°C. Glucose- and ethanol-grown cells were thawed and then assayed by the method of Lutsdorf and Megnet (21) as described by Denis et al. (11). Protein concentrations of the cell lysates were determined by the method of Lowry.

RESULTS

3′ deletions of the *ADH2* control region. Since the 1-kb *Bam*HI-*Sph*I fragment normally present upstream of the *ADH2* gene can confer glucose control on an adjacent promoter (2), this fragment was cloned into pBR322 to generate deletions. Deletions of the promoter-proximal (i.e., 3′) end of the control region were constructed by BAL 31 nuclease digestion (see Materials and Methods), starting at an *Sph*I site at position −115 (all deletion endpoints are numbered with respect to the *ADH2* transcription start site at 1). The extent of the deletions of the *ADH2* control region was estimated by electrophoresis on polyacrylamide gels after restriction enzyme digestion with *Bam*HI and *Xho*I. Eight of the *ADH2* control region fragments were ligated adjacent to a fragment containing the *ADH1* structural gene, transcription start site, and promoter (Fig. 1). In the hybrid gene, the *ADH2* deletion endpoints are 20 bp closer to the TATA box than they would be in the *ADH2* gene. The endpoints of six of the shorter 3′ terminal deletions were determined by Maxam-Gilbert DNA sequencing (23). Their positions are indicated in Fig. 2. The *ADH2-ADH1* hybrids were cloned as one fragment into pBC3T1, a yeast-*E. coli* shuttle vector containing *TRP1* and *CEN3* (2). Plasmids containing *CEN3* centromere sequences are mitotically stable and are maintained as approximately single-copy minichromosomes in yeasts (4, 9).

The plasmids containing these hybrid genes were introduced by transformation into strain 302-21, a *trp1* yeast strain that contains defective alleles of all of the ADH isozymes. Trp^+ transformants were grown in selective media lacking tryptophan and containing either glucose or ethanol. Cells were harvested and lysed, and ADH enzyme activity in the cell lysates was measured.

Hybrid genes containing deletions of the *ADH2* control regions that play a role in mediating glucose repression would be expected to express high levels of ADH activity even when grown on glucose. Low levels of ADH enzyme activity were found in glucose-grown cells containing hybrid genes in which up to 400 bp of the 3′ end of the *ADH2* control region had been deleted (Fig. 1). Two of the hybrids containing small 3′ deletions reproducibly showed a low but significant constitutive ADH activity. Deletions pΔ3-1 and pΔ3-4 had two- to fourfold higher enzyme activities on



ADH Specific Activity

	ADR1		ADR1-5^c	
	Glucose	Ethanol	Glucose	Ethanol
pADH2/ADH1	30	900	270	2500
pΔ3-1	30	1500	460	4800
pΔ3-2	71	1300	140	3000
pΔ3-3	23	1100	90	1100
pΔ3-4	32	280	60	280
pΔ3-5	100	250	7	270
pΔ3-6	13	270	20	520
pΔ3-7	20	120	30	230
pΔ3-8	20	370	50	530

FIG. 1. Construction and ADH activity of hybrid genes containing 3′ deletions of the *ADH2* control region. Plasmid pBC3T1 is a yeast-*E. coli* shuttle vector that was used for all the constructions described. Plasmid pADH2/ADH1 carries a hybrid gene with an undeleted 1.0-kb *ADH2* control fragment. The construction of pBC3T1 and pADH2/ADH1 [previously designated pADR3/ADC1(−150)] has been described previously (2). Eight hybrid genes containing 3′ deletions of regulatory sequences are depicted. The deletion endpoints are numbered with respect to the 5′ end of the *ADH2* mRNA as +1. The endpoints of the two largest deletions were estimated by restriction enzyme analysis. The remainder were determined by Maxam-Gilbert sequence analysis (23). The *Sau*3A sites used for construction of 5′ terminal deletions are marked with an S. The position of a sequence of dyad symmetry within the *ADH2* control region is marked with an arrow. Plasmids containing hybrid genes were introduced into strains 302-21 (*ADR1*) and SF6-4A-M4 (*ADR1-5^c*) by transformation. Trp$^+$ colonies were picked and assayed for enzyme activity in repressing and derepressing conditions as described in Materials and Methods. Each value represents the average of enzyme activities measured for three to five independent transformants. The average variation of enzyme assays for both strains was less than 42% for glucose-grown cells and less than 22% for ethanol-grown cells.

glucose than did the control plasmid containing the complete *ADH2* control region. Hybrid genes with deletion endpoints that lie between pΔ3-1 and pΔ3-4 were fully repressed, as were hybrid genes with flanking deletion endpoints, suggesting that these effects could have been due to the generation of new sequences created by juxtaposition of *ADH2* deletion endpoints adjacent to the *ADH1* promoter sequence, which might fortuitously have overcome some of the repressing effect of the *ADH2* control region.

Cells carrying a hybrid gene containing an undeleted *ADH2* control region (pADH2/ADH1) were efficiently derepressed when grown on medium containing only ethanol as a carbon source. These cells contained 900 mU of ADH activity per mg, which was a 30-fold increase over the amount found after growth on glucose-containing medium. Hybrid genes in which sequences required for derepression had been deleted should fail to show a large increase in the amount of activity compared with that found under repressing conditions. Hybrid genes with deletions ending 3′ to position −217 were derepressed to >1,000 mU/mg of protein (Fig. 1). This represented a 20- to 60-fold increase over ADH levels found in glucose-grown cells. In contrast, deletions that removed sequences 3′ to position −257 derepressed to less than 300 mU/mg, a significant but not total loss of derepression. These results suggested that the nucleotide sequences found between positions −217 and −257 were required to fully derepress an adjacent gene when cells were grown on ethanol-containing medium.

Hybrid genes containing the *ADH2* control sequences are constitutively expressed in a strain with the regulatory allele *ADR1-5^c* (2). If the hybrid genes lacked the *ADR1-5^c* target site, they should not be constitutively expressed. Strain SF6-4A-M4 (*ADR1-5^c*) was transformed with the hybrid genes containing 3′ deletions of the *ADH2* control region, and Trp$^+$ colonies were tested for ADH activity after growth on glucose- or ethanol-containing medium (Fig. 1).

Hybrid genes with 3′ deletions ending at or before −217 responded to the *ADR1-5^c* allele. That is, they synthesized ADH even when grown under repressing growth conditions, and with the exception of pΔ3-3, they showed the excessive derepression characteristic of *ADR1-5^c*. Hybrid genes with larger 3′ deletions showed no significant difference in ADH activities in the *ADR1* wild-type and the *ADR1-5^c* strains after growth on either glucose- or ethanol-containing medium. Transformants containing pΔ3-1, -2, and -3 showed a progressively decreasing response to the *ADR1-5^c* allele, suggesting that DNA sequences essential for mediating the *ADR1-5^c* effect lie between positions −164 and −257.

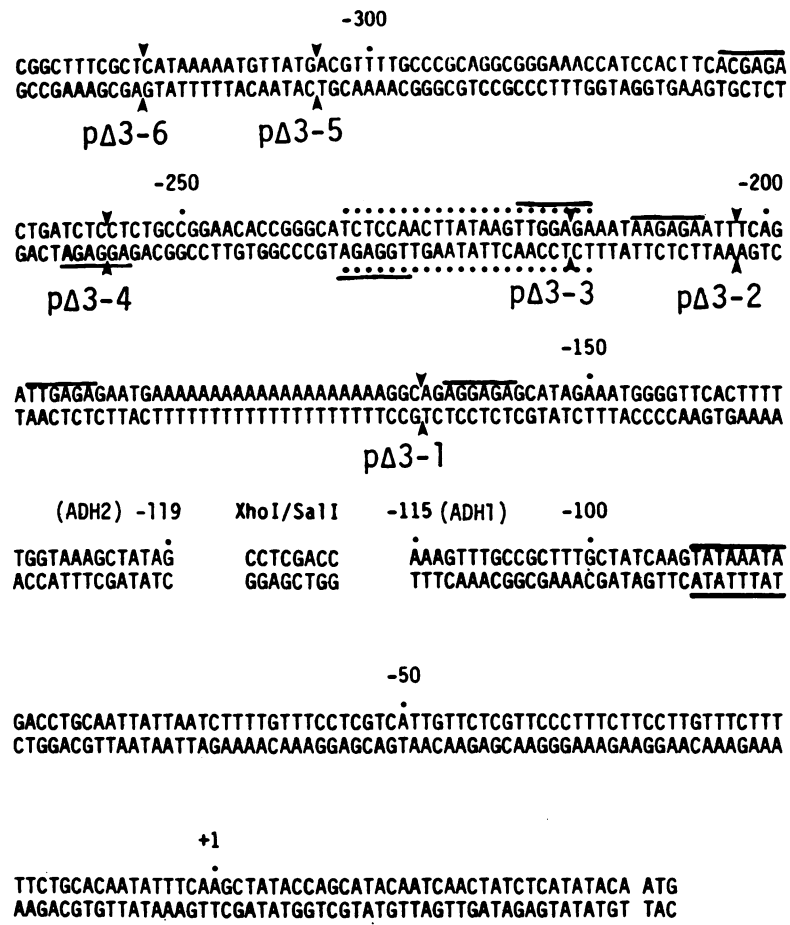


FIG. 2. Sequence of the *ADH1* promoter and the 3′ terminal portion of the *ADH2* control region. DNA sequence of a hybrid pADH2/ADH1 gene showing the 3′ terminal 215 bp of the *ADH2* control region, the *Xho*I-*Sal*I linker sequences, and the 5′ flanking *ADH1* sequences, including the TATAA box (heavy solid lines), the major transcription start site (+1), and the *ADH1* protein translation start site. The endpoints of six 3′ deletions of the regulatory sequences are marked with arrowheads. A 22-bp sequence of dyad symmetry in the *ADH2* control region is marked by dotted lines. A repeated element with the consensus sequence A/TGGAGA is indicated by solid lines. The *Sau*3A site used for the construction of the pΔ5-4 plasmid is at −259.

5′ deletions of the *ADH2* control region. To further characterize the *ADH2* control region, 5′ terminal deletions were constructed by partial *Sau*3A digestion of the hybrid gene (see Materials and Methods for details). Plasmids containing deletions corresponding to digestion at each one of the four *Sau*3A sites in the *ADH2* control region were identified by restriction enzyme digestion. Hybrid genes were recloned into a vector which contained 1.7 kb of yeast sequence normally found upstream of the 5′ end of the *ADH2* control region. The hybrid genes containing the 5′ deletions were present in the same position and orientation in pBC3T1 as in the previously tested constructions. Both the *ADH1* gene [i.e., the ADH1(−115) fragment] and a 2.7-kb fragment of yeast sequence containing the *ADH2* structural gene, promoter, and undeleted control region were also cloned into this vector. Plasmids containing these deletions were introduced into strain 302-21 by transformation. Transformed cells were grown on glucose- or ethanol-containing medium, and cell lysates were assayed for ADH enzyme activity (Fig. 3). The control plasmid, pADH2-B, was glucose repressed and derepressed as expected. The hybrid *ADH2-ADH1* gene containing the intact *ADH2* control regions was repressed and derepressed to the same extent as the *ADH2* gene. The hybrid genes containing deletions to the four *Sau*3A sites in the *ADH2* control region at positions −908, −523, −422, and −259 were also repressed fully on glucose medium and derepressed to the same extent as the hybrid gene containing an intact control region. Only hybrid gene *ADH2-ADH1*(−115)B, which lacked the *ADH2* control region, failed to derepress. Since a hybrid gene retaining sequences 3′ to position −259 (pΔ5-4B) was derepressed but a hybrid gene lacking the sequences between −259 and −119 [pADH2/ADH1(−115)] was not derepressed, these sequences are apparently required for derepression. This result is consistent with the results of 3′ deletion analysis which showed that loss of sequences in the −257 to −217 region resulted in loss of derepressibility. As in the 3′ deletion analysis, a site mediating repression was not revealed. All of the 5′ deletions were completely glucose repressed.

The ability of the plasmids containing 5′ deletions to synthesize ADH I constitutively in response to the *ADR1-5*^{c} allele was tested. Strain SF6-4A-M4 (*ADR1-5*^{c}) was transformed to Trp^{+}, and the ability to grow on plates containing glucose and antimycin A was determined. This test requires that the cells contain at least 200 mU of ADH activity per mg. Plasmids containing deletions to the four *Sau*3A sites were antimycin A resistant, whereas pADH2/ADH1(−115)B

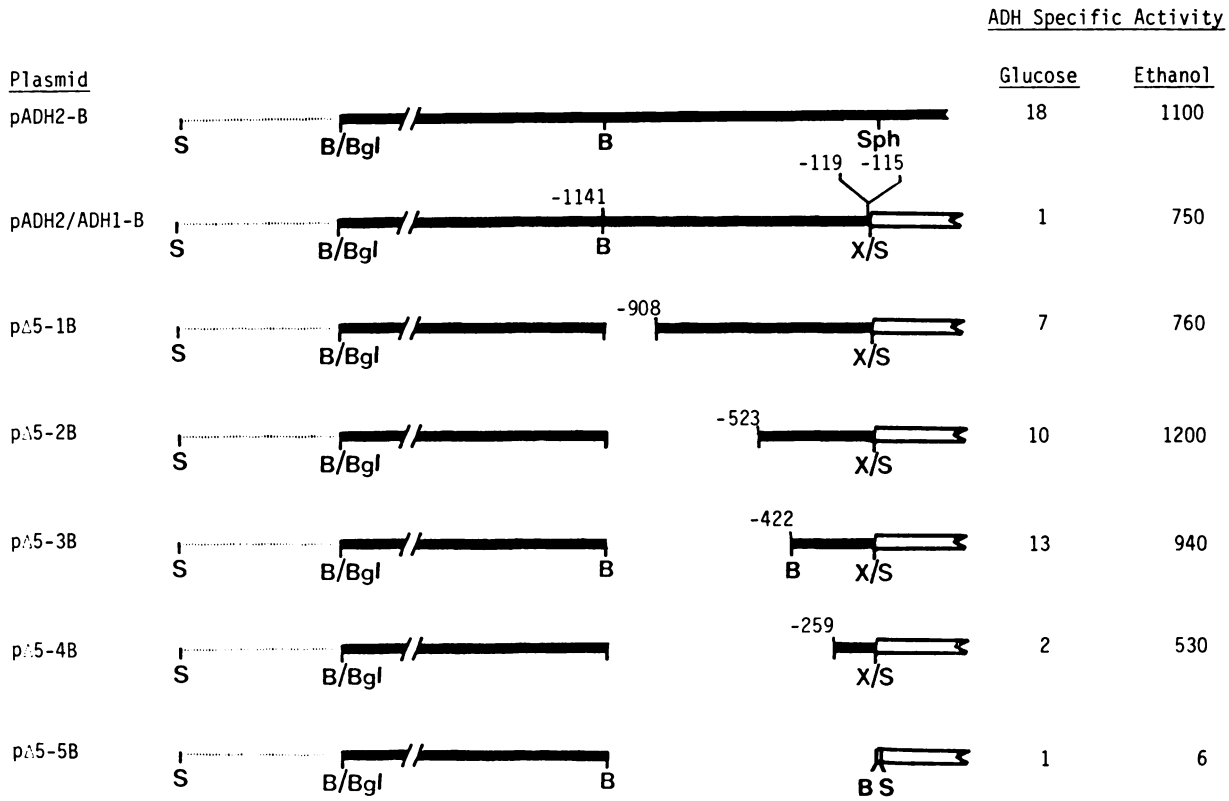


FIG. 3. ADH activity of hybrid genes containing 5′ deletions of the *ADH2* control region. All of the constructions depicted are cloned in the pBC3T1 vector described in the text. Plasmid pADH2-B contains the undeleted *ADH2* control region and structural gene. Plasmid pADH2/ADH1-B carries a hybrid gene containing an undeleted 1.0-kb *ADH2* control fragment and the *ADH1* promoter and structural gene. The positions of the four *Sau*3A sites used for 5′ deletion constructions (pΔ5-1 through pΔ5-4) are at −908, −523, −422, and −259 bp upstream of the *ADH2* mRNA start site. The *ADH2* upstream *Bam*HI site is at −1141 bp. The deletion junction in pΔ5-3B recreates a *Bam*HI site, which is marked. All of the plasmids also have a 1.7-kb fragment of yeast DNA that is normally present upstream of the *Bam*HI site located 5′ to the *ADH2* gene. It is present in the plasmids in the same position and orientation with respect to *ADH2* sequences as in the chromosome. The restriction endonuclease cleavage sites are: S, *Sal*I site; B, *Bam*HI site; X/S, *Xho*I-*Sal*I junction; Sph, *Sph*I site; and B/Bgl, *Bam*HI-*Bgl*II junction. Dotted lines are pBR322 sequences, solid lines are *ADH2* sequences, and open boxes are *ADH1* sequences.

was not, indicating that sequences 3′ to position −259 are sufficient for response to the constitutivity caused by the *ADR1-5*^{c} allele (data not shown). Thus, there is a good correspondence between the sequences which confer the ability to be derepressed on ethanol-containing medium and the sequences which allow ADH to be synthesized on glucose-containing medium under the control of *ADR1-5*^{c}.

The complete absence of ADH activity in cells carrying pADH1(−115)B was unexpected, since ADH I is abundantly expressed when the *ADH1*(−115) fragment was ligated adjacent to vector sequences (2). To ensure that pADH1(−115)B contained a functional *ADH1* structural gene, the plasmid was recovered from *S. cerevisiae*, and the *ADH1* gene was excised and ligated into a new vector. This plasmid was introduced into strain 302-21 by transformation and tested for ADH activity. The transformants contained 2,400 mU of ADH activity per mg when grown on glucose, proving that the *ADH1* promoter and structural gene derived from the pADH1(−115)B plasmid were functional (data not shown).

DISCUSSION

These experiments did not identify a unique site in the *ADH2* control region whose deletion alleviated glucose repression. Instead, deletions in the control region of *ADH2* identified a *cis*-acting region necessary for activation of the *ADH1* promoter. This region is located between positions −217 and −257 with respect to the *ADH2* RNA initiation site. Hybrid genes containing the −217 to −257 region are repressed and derepressed under the appropriate growth conditions and are also responsive to *ADR1-5*^{c}, which confers constitutive expression on *ADH2*. Hybrid genes lacking this region have low activity on glucose medium, fail to derepress ADH enzyme activity normally after growth on ethanol, and do not respond to *ADR1-5*^{c}. Since this region is upstream of the TATAA box and the transcriptional start site, the simplest interpretation is that this region affects transcription initiation rather than posttranscriptional events, such as capping, processing, transport, or mRNA stability. The region that we have identified seems to be analogous to the upstream activation sites that have been identified in 5′ flanking sequences of several yeast genes, including *GAL10* (16), *CYC1* (15), *HIS3* (30), and *HIS4* (13). Our previous interpretation of experiments in which the upstream *ADH2* region was shown to confer glucose regulation on *ADH1* was that this region contained a glucose repression site. This was based primarily on the difference in activities between plasmids in which either *ADH2* upstream sequences or pBR322 sequences were placed upstream of the *ADH1* TATA box. When pBR322 sequences were upstream of *ADH1*, the *ADH1* gene was equally active on both repressing and derepressing carbon sources, and its activity

was independent of orientation in the vector. That is, a specific vector sequence was not responsible for activating transcription. When *ADH2* upstream sequences were placed adjacent to the same *ADH1* fragment, *ADH1* expression was regulated in the same manner as *ADH2*, with respect to both carbon source and mutations in regulatory genes affecting *ADH2* expression, a consequence we attribute to positive activation mediated through the upstream activation sites. As judged by the results obtained when the *ADH1* promoter was placed adjacent to vector sequences, *ADH2* upstream sequences had a repressing effect on glucose. The more likely explanation, in view of the present results, is that pBR322 sequences had a stimulatory effect on the *ADH1* promoter, independent of growth conditions. A similar effect was seen in the present studies when more extensive 3′ deletions of the *ADH2* control region were analyzed or when the 5′ deletions were terminal, that is, adjacent to vector sequences, rather than internal (data not shown). A vector effect on cloned *HIS3* alleles has been noted previously (29). There is a distinction, however, from the cloned *HIS3* alleles. The His^+ character of *HIS3* alleles containing 100 bp or less of 5′ flanking *HIS3* DNA showed an orientation dependence, whereas the *ADH1* DNA did not. Similar to the effect seen here, additional 5′ flanking yeast sequences protected *HIS3* from an orientation-dependent-position effect.

The alternative to an activation site is the possibility that the observed loss of ADH I enzyme activity is a consequence of moving an upstream inhibitory sequence closer to the *ADH1* promoter and is not the result of the deletion of an activator sequence. This possibility seems less likely in light of the evidence that a small increment in the size of the 3′ deletion of the control region (from 100 to 142 bp) resulted in a large effect. Furthermore, deletion of genomic sequences in the region containing the activation site resulted in a non- or poorly derepressible phenotype (J. Shuster, personal communication), whereas deletions in adjacent sequences either upstream or downstream of this region did not affect derepression. This result makes it unlikely that the phenotype is a result of altering the distance between inhibitory sequences and the promoter. It also indicates that this region is important for derepression of *ADH2* at its normal chromosomal site.

The region that is required for *ADH2* activation contains a 22-bp sequence of perfect dyad symmetry normally located 215 to 236 bp 5′ to the mRNA initiation site. Since domains of protein recognition in procaryotic regulatory regions often have elements of dyad symmetry, it is tempting to speculate that the dyad sequence in the *ADH2* activation region represents a recognition site for *ADR1*-mediated activation of *ADH2*. Recent evidence indicates that an *ADH2* allele lacking just the dyad (7) can be derepressed only 10 to 20% as well as a wild-type *ADH2* allele (D. Cox, personal communication).

Examination of the region implicated in *ADH2* regulation by the deletion analysis reported here and by Shuster reveals a short, repeated element with the consensus sequence A/TGGAGA. This sequence or a slight variant of it is repeated seven times in the region between −158 and −266 (Fig. 2). It is present at both ends of the dyad sequence noted above, as part of another 11-bp inverted repeat sequence, GGCAGAGGAGA, that is located at positions −157 and −250, and as part of directly repeated sequences, AGAGAAT, at positions −189 and −204. Regulatory sequences that function in both orientations with respect to an adjacent promoter have been noted in several genes, including herpes simplex virus thymidine kinase gene (24) and the yeast *HIS4* gene (17). Mutation Δ3-4, which has the most severe effect on derepression and is unresponsive to *ADR1-5*^{c} retains only two of these repeated sequences, that one most distal to the promoter and another which is part of a longer inverted repeat. Mutations Δ3-2 and Δ3-3, which are normally derepressed but are only partially responsive to *ADR1-5*^{c}, retain five and three of the repeats, respectively.

The deletion of the poly(dA-dT)$_{20}$ tract (−168 to −187) had little if any effect on expression of the adjacent *ADH1* promoter. This result has been confirmed with an integrated *ADH2* allele which lacks only the poly(dA-dT)$_{20}$ tract (7; D. Cox, personal communication). Extension of this tract to 54 or 55 A residues in the *ADH2-4*^{c} and *ADH2-5*^{c} mutants disrupts normal glucose repression of *ADH2* (8, 27). These mutants are still responsive to mutations at the *ADR1* locus, however, indicating that the signals which facilitate positive activation are still present.

Genetic analyses have not identified a gene that behaves as a classical repressor locus for *ADH2*, whereas several recessive mutations that render *ADH2* nonderepressible have been found (8). This result is consistent with the presence of an upstream activation sequence as reported here and the apparent absence of an upstream negative regulatory site. While several genetic loci have been identified which allow partially constitutive ADH II activity, they usually have no more than 10% of the fully derepressed level of activity (10; M. Irani, personal communication). Genetic analysis suggested that these loci may act indirectly by affecting positive activation of *ADH2* (10).

Positive activation, mediated by the region identified by deletion analysis, appears to be a sufficient explanation for derepression of *ADH2*. That is, glucose repression of *ADH2* could result from modulation of the activity or amount of positive effectors such as that encoded by *ADR1*. It will be possible to test this hypothesis by using the cloned *ADR1* gene (12) to study *ADR1* mRNA levels and to generate *ADR1*-specific antibody probes for the *ADR1* protein synthesized in vivo.

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