MOLECULAR AND CELLULAR BIOLOGY, May 1988, p. 1868–1876
0270-7306/88/051868-09$02.00/0
Copyright © 1988, American Society for Microbiology
Vol. 8, No. 5

Regulation of Expression and Activity of the Yeast Transcription Factor *ADR1*

HAL BLUMBERG,† TOINETTE A. HARTSHORNE,‡ AND ELTON T. YOUNG*
Department of Biochemistry, University of Washington, Seattle, Washington 98195

Received 30 November 1987/Accepted 3 February 1988

Disruption of *ADR1*, a positive regulatory gene in the yeast *Saccharomyces cerevisiae*, abolished derepression of *ADH2* but did not affect glucose repression of *ADH2* or cell viability. The *ADR1* mRNA was 5 kilobases long and had an unusually long leader containing 509 nucleotides. *ADR1* mRNA levels were regulated by the carbon source in a strain-dependent fashion. β-Galactosidase levels measured in strains carrying an *ADR1-lacZ* gene fusion paralleled *ADR1* and *ADR1-lacZ* mRNA levels, indicating a lack of translational regulation of *ADR1* mRNA. *ADH2* was regulated by the carbon source to the same extent in all strains examined and showed complete dependence on *ADR1* as well. The expression of *ADR1* mRNA and an *ADR1*-β-galactosidase fusion protein during glucose repression suggested that the activity of the ADR1 protein is regulated at the posttranslational level to properly regulate *ADH2* expression. The *ADR1*-β-galactosidase fusion protein was able to activate *ADH2* expression during glucose repression but showed significantly higher levels of activation upon derepression. A similar result was obtained when *ADR1* was present on a multicopy plasmid. These results suggest that low-level expression of *ADR1* is required to maintain glucose repression of *ADH2* and are consistent with the hypothesis that *ADR1* is regulated at the posttranslational level.

Many eucaryotic genes are regulated at the transcriptional level. To understand the mechanisms by which this control takes place, it is necessary to determine how regulatory proteins function. It is also important to understand how the activity of regulatory proteins themselves is controlled. Genetic studies have identified a number of regulatory genes in the yeast *Saccharomyces cerevisiae* which have subsequently been isolated by complementation of function (18, 27, 30, 31, 35, 39, 40). Many of these gene products possess sequence-specific DNA-binding activity (3, 22, 26, 28, 39).

A simple system for studying eucaryotic transcription is the glucose-repressible *ADH2* gene in yeast cells. No specific carbon source induces *ADH2* expression; removal of glucose from the growth medium leads to *ADH2* derepression. *ADH2* is transcriptionally regulated (6, 16) and appears to be directly regulated in a positive manner (2). Both positive regulatory genes, *ADR1* (11), *CCR1-3* (12), *CCR4* (14), and *ADR6* (45, 46), and negative regulatory genes, *ADR4* (12), *CRE1*, and *CRE2* (14), are involved in *ADH2* regulation. Deletion analysis of the 5′-flanking region of *ADH2* has identified an upstream activator sequence (UAS) located 230 base pairs (bp) from the transcription start site (2, 43). A 22-bp oligonucleotide containing this UAS confers *ADR1*-dependent activation on a heterologous promoter (J. Yu, submitted for publication).

By genetic criteria, the regulatory gene *ADR1* is proposed to encode the most direct effector of *ADH2* expression (11). *ADR1* can mutate to two different types of alleles: semidominant *ADR1*^{c} alleles, which allow partially constitutive *ADH2* expression in glucose-containing media (13), and recessive *adr1* alleles, which render *ADH2* incapable of derepression (11). The *ADR1* gene and the gene which encodes the *ADR1-5*^{c} allele have been isolated (18). Nucleotide sequence analysis has shown that *ADR1* could encode a protein of 1,323 amino acids, of which the amino-terminal 302 amino acids are sufficient to stimulate *ADH2* transcription (20). A region of *ADR1* between amino acids 98 and 155 shows sequence homology with the repetitive DNA-binding domain of *Xenopus laevis* transcription factor TFIIIA (20), and mutations in this region inactivate *ADR1* (6). Mutation in a presumptive phosphorylation domain creates a hyperactive *ADR1* protein, suggesting that phosphorylation may be involved in regulating the activity or expression of *ADR1* (17). An ADR1-β-galactosidase fusion protein is localized to the nucleus, consistent with its proposed role as a direct activator of *ADH2* transcription (H. Blumberg, Ph.D. thesis, University of Washington, Seattle, 1987).

There are two simple models to explain the role of *ADR1* in expression of the glucose-repressible *ADH2* gene. In the first model, *ADR1* transcription or translation would be glucose repressed. Since the *ADR1* protein is required for transcriptional activation of *ADH2*, no *ADH2* mRNA would be expressed under glucose growth conditions. In the second model, the *ADR1* gene product would be expressed constitutively but would be regulated posttranslationally in a carbon source-dependent manner.

In this report, we demonstrate that some strains regulate *ADR1* mRNA levels and the level of an *ADR1* fusion protein in response to a change in carbon source while other strains do not. Both types of strains regulate *ADH2* expression identically, suggesting that *ADR1* is differentially regulated at the posttranslational level by the carbon source. We also found that overexpression of *ADR1* or expression of an *ADR1-lacZ* gene fusion relieves *ADH2* glucose repression.

MATERIALS AND METHODS

Yeast media. Yeast complete medium contains 10 g of yeast extract, 10 g of Bactopeptone (Difco), 20 mg of adenine, and 20 mg of uracil per liter and is supplemented with 5% glucose (repressed growth) or 3% glycerol and 1% ethanol (derepressed growth). Yeast minimal medium contains 6.7 g of yeast nitrogen base lacking amino acids and 20

* Corresponding author.
† Present address: Department of Biology, Princeton University, Princeton, NJ 08544.
‡ Present address: Department of Genetics, University of California, Berkeley, CA 94720.

TABLE 1. Yeast strains

Strain	Genotype
521-6	*MATa adh1-11 adh3 ura1 trp1 leu2*
521-6-Δ1	*MATa adh1-11 adh3 ura1 trp1 leu2 adr1-Δ1::LEU2*
HB9	*MATa adh1-11 adh3 ura1 leu2 adr1-Δ1::LEU2 trp1*::Yip642ADR1
HB10	*MATa adh1-11 adh3 ura1 leu2 adr1-Δ1::LEU2 trp1*::YIp642ADR1-lacZ
4795-103	*MATa trp1 leu2 ura3 his7 can1 sap3 ade3*
4795-104	*MATα trp1 leu2 ura3 his7 can1 sap3*
4795-105	*MATa/MATα* diploid derived from 4795-103 × 4795-104
HB23-3	*MATa adh1 adh3 trp1 leu2 ura3 his7 can1 sap3 ade3*
HB15	*MATa adh1 adh3 leu2 ura3 his7 can1 sap3 ade3 trp1*::YIp642ADR1-lacZ
HB16	*MATa adh1 adh3 trp1 leu2 his7 can1 sap3 ade3 ura3*::YIp642ADR1-lacZ
HB23-3-Δ1	*MATa adh1 adh3 trp1 leu2 ura3 his7 can1 sap3 ade3 adr1-Δ1::LEU2*
79-72C	*MATα leu2 trp1*
500-11	*MATα adh1-11 adh3 adr1-1 ura1 trp1 leu2*
2150-AA	*MATa adh1 adh3 leu2 ade1* [cir^0]
20B12	*MATα trp1-1 pep4-3*
XV617	*MATa his2 trp1-1 leu2 ura3 ste5*
3482-16-1	*MATa trp1 ura3 his3 leu2 met2* [cir^0]
E8-11C	*MATa leu2 pep4* [cir^0]

mg each of tyrosine, adenine, and uracil per liter. This is supplemented with amino acids except tryptophan and 5% glucose for repressed growth conditions.

Yeast strains. The yeast strains used in this work are listed in Table 1. Strain HB23-3 (*adh1 adh3*) was derived from 4795-103 (*ADH1 ADH3*) in two steps. HB23 (*adh1 ADH3*) was isolated on plates containing yeast complete medium, 8% glucose, and 5 mM allyl alcohol. HB23-3 was isolated by plating HB23 on plates containing yeast complete medium, 8% glucose, and 20 mM allyl alcohol.

Yeast transformation. Yeast transformation was performed by the lithium acetate procedure (25). Plasmid Y1p642 *ADR1-lacZ* (*TRP1*) was digested with *Hin*dIII to target integration to the *trp1* locus in strain 521-6-Δ1 to create HB10 and in strain HB23-3 to create strain HB15. Plasmid Y1p642 *ADR1-lacZ* (*URA3*) was digested with *Sma*I to target integration to the *ura3* locus in strain HB23-3 to create HB16.

Plasmid DNA preparation and cloning. Plasmid DNA was prepared from ampicillin-resistant *Escherichia coli* RR1 (7) by the method of Birnboim and Doly (5). Restriction enzymes, calf alkaline phosphatase, and T4 DNA ligase were from Bethesda Research Laboratories, Inc., or New England Biolabs, Inc., and were used according to the manufacturers' instructions.

Plasmid constructions. The number preceding *ADR1* in the plasmid name is the number of amino-terminal residues encoded by the gene. Plasmids pMW5, p411-B, YEp150 ADR1, YEp302ADR1, and YEp1323ADR1 were described previously (20). YEpC1/1 is a high-copy-number plasmid containing the complete 2μm plasmid of *S. cerevisiae* and a partially defective *LEU2* gene as a selectable marker (24). YEp1068ADR1 is a pMW5 derivative containing a 4.8-kb *ADR1* fragment from p411-B. YEpadr1-Δ1 is identical to YEp1068ADR1 except that it contains an *adr1* gene disruption (see Fig. 1). YEp642*ADR1-lacZ* was constructed by inserting a 3.3-kb *Bam*HI *lacZ* fragment from pMCI871 (8) into YEp1068ADR1 digested with *Bgl*II. An 8.1-kb *Bam*HI *ADR1-lacZ* fragment from YEp642ADR1-lacZ was inserted into YRp7 digested with *Bam*HI and *Bgl*II, YIp5 digested with *Bam*HI, and C1/1 digested with *Bam*HI to create YIp642ADR1-lacZ (*CTRP1*), YIp642ADR1-lacZ (*URA3*), and YEpC1/1-642ADR1-lacZ, respectively.

Growth of yeast transformants and enzyme assays. Cells were grown overnight at 30°C in yeast minimal medium containing 5% glucose and lacking tryptophan. One portion of the culture was diluted into fresh minimal medium as described above. Another portion was centrifuged, washed with sterile water, and resuspended in complete yeast medium containing 3% glycerol and 1% ethanol. The cells in both media were then grown for an additional 24 h, until they were both in mid-log-phase growth. Cell extracts and alcohol dehydrogenase (ADH) assays were performed as described (16). β-Galactosidase assays were performed as described (33) with either cell extracts or permeabilized cells. Protein concentrations of cell extracts were determined by the method of Lowry.

RNA analysis. Total yeast nucleic acid was prepared by glass bead disruption of intact cells in the presence of phenol and sodium dodecyl sulfate (42). S1 mapping of the 5′ end of *ADR1* RNA was performed essentially as described by Maniatis et al. (32) with a 1.3-kb *Eco*RI fragment of *ADR1* extending from bp −1300 to bp +45 with respect to the presumptive initiation codon (T. A. Hartshorne, Ph.D. thesis, University of Washington, Seattle, 1986). A temperature of 37°C was used for hybridization in 50% formamide buffer, and S1 digestion was carried out at 30°C for 30 min. After S1 digestion, the S1-resistant hybrids were denatured and analyzed in 4% acrylamide–7% urea gels.

The 5′ end of *ADR1* mRNA was determined by primer extension with an *ADR1*-specific oligonucleotide complementary to a region of *ADR1* from −403 to −384. Total nucleic acid (20 μg) was hybridized to approximately 5×10^7 cpm of ^{32}P-labeled oligonucleotide, and primer extension reactions were carried out (32). Samples were analyzed on DNA sequencing gels adjacent to lanes containing reaction mixes of the same oligonucleotide hybridized to M13 DNA containing the message-sense strand of *ADR1* DNA from the 5′-flanking region of the gene.

The 3′ end of *ADR1* mRNA was determined by using a double-stranded *Hpa*I-*Xba*I fragment extending from position +3571 to +5524 (Hartshorne, thesis) labeled at the 3′ ends by T4 polymerase (36), denatured, and hybridized at 49°C in 40% formamide to 100 μg of total nucleic acid from yeast cells. Samples were treated with 400 U of S1 nuclease at 37 to 40°C (23), precipitated with ethanol, suspended in sample buffer, and electrophoresed on agarose gels. The gels were dried, and autoradiography was performed.

RESULTS

Construction and characterization of a null allele of *ADR1*. A yeast strain carrying a null allele of *ADR1* was constructed for two reasons. First, it was desirable to determine the phenotype of a true null *ADR1* allele and to compare it with existing *adr1* alleles. Second, such a strain would be useful as a recipient strain for analyzing *ADR1* genes mutated in vitro and introduced into yeast cells by transformation. This would allow one to assess *ADR1* structure-function relationships without the possibility of intragenic complementation between two defective *adr1* alleles.

Plasmid pADR1-Δ1 was constructed as described in the legend to Fig. 1. An *ADR1* gene disruption was created by

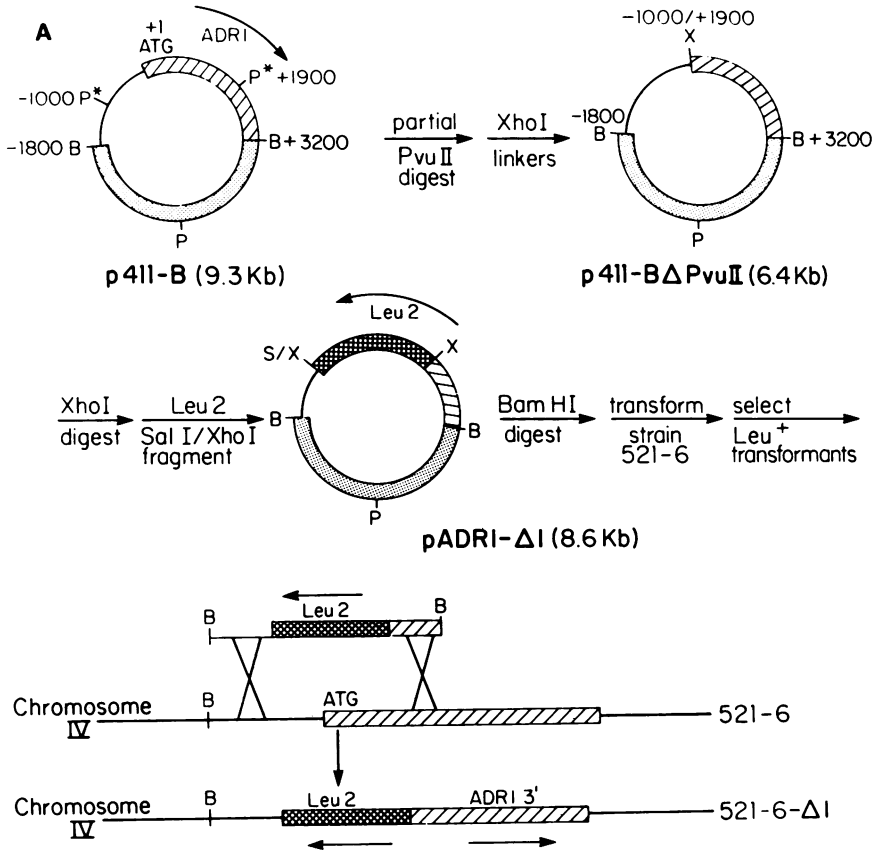

FIG. 1. Construction of an *ADR1* null allele: Plasmid construction. The general procedure for construction of a gene disruption is described by Rothstein (41). Plasmid p411-B is pBR322 containing a 4.8-kb *Bam*HI fragment of *ADR1* (20). p411-B was briefly digested with *Pvu*II, a 6.4-kb fragment was isolated (sites cleaved are marked with an asterisk), *Xho*I linkers were added, and the vector was ligated to give p411-BΔPvuII. This plasmid was digested with *Xho*I, and a 2.2-kb *Sal*I-*Xho*I *LEU2* fragment from YEp13 was inserted (with the direction of transcription opposite to that of *ADR1*) to create pADR1-Δ1. A *Bam*HI fragment of pADR1-Δ1 was used to transform strain 521-6 to leucine prototrophy to create strain 521-6-Δ1. The same procedure was used to transform strains HB23-3 and 4795-105 to leucine prototrophy. Restriction enzyme sites: B, *Bam*HI; P, *Pvu*II; S, *Sal*I; X, *Xho*I. Solid lines, *ADR1* noncoding sequences; hatched bars, *ADR1* coding sequences; stippled bars, PBR322 sequences; crosshatched bars, *LEU2* sequences.

replacing the 5'-flanking region and 2 kb of *ADR1* coding sequence with the yeast *LEU2* gene. Both haploid (521-6) and diploid (4795-105) yeast strains were transformed with a *Bam*HI restriction fragment carrying the *ADR1* gene disruption. Leu^+ transformants were isolated in both haploid and diploid strains, suggesting that *ADR1* is not an essential gene. To demonstrate this more rigorously, Leu^+ diploid transformants were sporulated and subjected to tetrad analysis. The 2:2 segregation of Leu^+:Leu^- spores was observed in all tetrads dissected. Southern analysis confirmed that chromosomal *ADR1* sequences were replaced with *LEU2* (Blumberg, thesis). The Leu^+ segregants could not derepress *ADH2*, whereas the Leu^- segregants could. This is consistent with the $Adr1^-$ phenotype expected of a strain carrying a null allele of *ADR1* (*adr1-Δ1*).

The quantitative effect of an *ADR1* gene disruption on *ADH2* expression was studied in haploid strains 521-6, 521-6-Δ1, HB23-3, and HB23-3-Δ1. Southern analysis indicated that strains 521-6-Δ1 and HB23-3-Δ1 carried the *adr1-Δ1* allele. Strains 521-6 and HB23-3 ($Adr1^+$) (Table 2) derepressed ADHII activity to greater than 1,500 mU/mg, and strains 521-6-Δ1 and HB23-3-Δ1 ($Adr1^-$) had insignificant ADHII activity (less than 20 mU/mg). This result was supported by a comparison of the growth of these strains on plates containing glycerol and allyl alcohol (10). Strains 521-6-Δ1 and HB23-3-Δ1 could grow on these plates, whereas 521-6 and HB23-3 could not (Table 2). Thus, strains carrying an *adr1-Δ1* allele had the same phenotype as previously identified *adr1* strains (11) but lower levels of ADHII enzyme activity (Table 2). Data in Table 2 provide further proof that *adr1-Δ1* is allelic to *ADR1* by demonstrating that the *adr1-Δ1* allele could not complement the $AdhII^-$ phenotype of strain 500-11 (*adr1-1*).

Growth rates of three different isogenic pairs of $Adr1^+$ and $Adr1^-$ strains were measured in complete liquid medium containing either fermentable (glucose) or nonfermentable (ethanol and/or glycerol) carbon sources. All strains had a doubling time of approximately 100 min in glucose-containing medium and a doubling time of about 210 min in medium containing nonfermentable carbon sources (data not shown), indicating that *ADR1* had little or no effect on growth rate in these rich media.

Two positive regulatory genes required for *ADH2* expression, *CCR1* (9, 12; shown to be allelic to *SNF1* [14]) and *ADR6* (45, 46), are also required for sporulation. Therefore, it was of interest to determine whether an a/α diploid strain devoid of *ADR1* activity could sporulate. A strain homozygous for *adr1-Δ1* was constructed and was found to sporulate

TABLE 2. Characterization of an *ADR1* null allele

Strain	*ADR1* allele	Plasmid	ADH activity[a] (mU/mg of protein)		Growth on glycerol-allyl alcohol[b]
			Repressed	Derepressed	
521-5	*ADR1*		<5	2,000	−
521-6-Δ1	*adr1-Δ1*		<5	<20	+
HB23-3	*ADR1*		<5	1,500	−
HB23-3-Δ1	*adr1-Δ1*		<5	20	+
500-11	*adr1-1*		<5	70	+
500-11×521-6	*adr1-1/ADR1*		<5	1,300	−
500-11×521-6-Δ1	*adr1-1/adr1-Δ1*		<5	100	+
500-11	*adr1-1*	YEp1068ADR1	300	5,550	ND[c]
500-11	*adr1-1*	YEpadr1-Δ1	<5	50	ND

[a] Cells were grown to mid-log phase at 30°C in either yeast complete medium plus 5% glucose (repressed) or 3% glycerol and 1% ethanol (derepressed). ADH specific activities were determined at 25°C in cell extracts. The values are the average of triplicate samples and are ± 30%. The values reflect only ADHII activity, as all strains were *adh1 adh3*.

[b] The glycerol-allyl alcohol phenotype was assayed on plates containing 3% glycerol and 5 mM allyl alcohol (10). ADH converts allyl alcohol into the toxic compound acrolein. ADH activities of greater than 100 mU/mg prevent growth in the presence of allyl alcohol.

[c] ND, not determined.

as well as an isogenic **a**/α *ADR1/ADR1* diploid (data not shown).

Identification and mapping of the *ADR1* mRNA. The *ADR1* mRNA was identified by hybridization of total yeast RNA to strand-specific probes derived from the *ADR1* coding sequence. A 5-kb mRNA species was detected which hybridized to the *ADR1* mRNA-complementary strand but not to the mRNA-identical strand (Hartshorne, thesis). This mRNA was absent in a strain carrying an *adr1-Δ1* allele (Fig. 2, lanes 1 and 2) but was present in RNA isolated from the isogenic *ADR1* parent strain grown on either a repressing or a derepressing carbon source (Fig. 2, lanes 3 and 4), consistent with its origin being the *ADR1* gene.

S1 mapping of the 5′ end of the *ADR1* mRNA indicated that the *ADR1* mRNA start site was about 500 nucleotides upstream of the presumed initiation codon (see Fig. 4). The exact 5′ end of the *ADR1* mRNA, determined by primer extension, corresponded to DNA sequences 509, 500, and 499 nucleotides upstream of the translational initiation codon (Fig. 3A and unpublished data). The 3′ end of the *ADR1* mRNA, determined by S1 mapping, corresponded to positions approximately 420, 590, and 810 nucleotides beyond the end of the *ADR1* open reading frame (Fig. 3B). The distribution of these 3′ termini was not reproducibly different in mRNA isolated from glucose- or ethanol-grown cells. The size of *ADR1* mRNA determined by 5′- and 3′-end mapping agreed with the size estimated by Northern (RNA blot) analysis. *ADR1* (20) lacks the consensus sequence TAC TAACA, which is found in all intron-containing genes transcribed by yeast RNA polymerase II (29), suggesting a lack of introns in the gene.

Carbon source regulation of *ADR1* RNA. The level of *ADR1* mRNA isolated from cells grown on a repressing or depressing carbon source was determined initially by Northern analysis (Fig. 2). In strain 521-6 (lanes 3 and 4 for repressed and derepressed cultures, respectively), there was a small (twofold) increase in *ADR1* RNA content after derepression, but the control RNA, *URA3*, increased to about the same extent after derepression. In strain 79-72C there was a 10- to 20-fold increase in *ADR1* RNA content after 24 h of derepression, compared with the control *URA3* RNA (Fig. 2, lanes 5 and 6). These strains, as well as several others, were assayed by quantitative S1 analysis (Fig. 4). Strains 2150-AA, XV617, and 3482-16-1 (lanes 1 to 6) showed about a 2-fold increase in *ADR1* RNA content after 24 h of derepression; strains 20B12 and HB23-3 revealed a 10- to 20-fold increase after derepression (lanes 7 to 10). RNA from a strain deleted for *ADR1* had no 5′-end signal (lane 11). To determine whether the two times at which RNA samples were isolated were representative of fully repressed and derepressed cultures, RNA was isolated from strain HB23-3 at various times after glucose removal. The time course of derepression indicated that *ADR1* RNA levels in strain HB23-3 showed a rapid 20-fold increase to a maximum at 1 h after glucose depletion (Fig. 5, bottom inset). As a

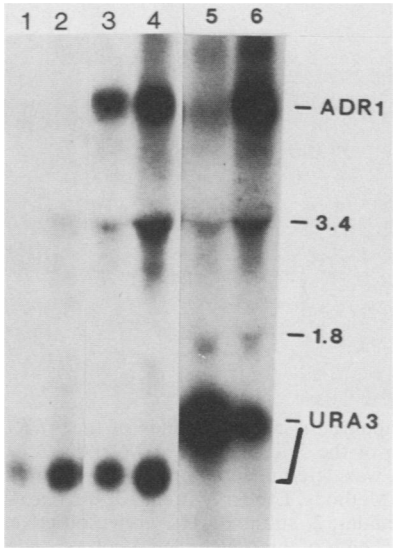

FIG. 2. Identification of *ADR1* mRNA. Total yeast nucleic acid (20 μg) was treated with formaldehyde and formamide, electrophoresed on a gel containing 1.0% agarose and 2.2 M formaldehyde (32), transferred to nitrocellulose, and hybridized to a 1.2-kb *ADR1* *Eco*RI fragment (Hartshorne, thesis) and a 1.2-kb *URA3* *Hin*dIII fragment (1) which were both ^{32}P-labeled by nick translation (32) to a specific activity of 5×10^8 cpm/μg. Lanes: 1, strain 521-6-Δ1 grown on glucose; 2, strain 521-6-Δ1 grown on ethanol; 3, strain 521-6 grown on glucose; 4, strain 521-6 grown on ethanol; 5, strain 79-72C grown on glucose; 6, strain 79-72C grown on ethanol. The bands seen at 3.4 kb and 1.8 kb represent nonspecific hybridization to yeast rRNA. The size of *ADR1* mRNA was approximately 5 kb, as determined on gels containing molecular weight standards (data not shown).

control for RNA content, *DED1* RNA (44) was analyzed and showed little difference between different carbon sources in the same strains (data not shown).

The ADHII enzyme activity was determined in strains 521-6 and HB23-3, which are representative of strains showing little and 20-fold derepression of *ADR1* RNA, respectively. Gene disruptions of *ADR1* were also performed in both strains (Fig. 1 and Table 2). The results indicated that ADHII levels were indistinguishable and *ADH2* expression was completely dependent on *ADR1* in both strains.

Expression of *ADR1-lacZ* gene fusions. The results presented in the previous section demonstrated that *ADH2* expression was not regulated solely via regulation of *ADR1* transcription. *ADR1* expression could be regulated at the translational or posttranslational level. To test the former possibility, a gene fusion was made that would allow the transcription and translation of *ADR1* to be measured by the activity of β-galactosidase.

Strains 521-6-Δ1 and HB23-3 were transformed with integrating plasmids containing an *ADR1-lacZ* gene fusion, creating strains HB10 and HB15, respectively. This gene fusion contains 1.8 kbp of *ADR1* 5′-flanking region and 1.9 kbp of *ADR1* coding sequence (encoding the amino-terminal 642 amino acids of ADR1) fused in-frame to an *E. coli lacZ* structural gene lacking its first seven codons. Transformants were assayed for β-galactosidase activity after growth in glucose-containing medium or after transfer to ethanol-containing medium. β-Galactosidase activities correlated positively with *ADR1* mRNA levels detected in the parent strains by Northern or S1 analysis (Table 3). There was a 2-fold increase in β-galactosidase activity in strain HB10 (parent strain 521-6-Δ1) and a 10- and 20-fold increase in β-galactosidase activity in transformants of strains HB23-3, HB15, and HB16. The latter two strains differ only in the site of integration of the plasmid containing the *ADR1-lacZ* gene fusion. Since their β-galactosidase activities were not detectably different, the site of integration (*trp1* and *ura3*) does not play a significant role in expression of the gene fusion.

The time course of derepression of the *ADR1-lacZ* gene fusion mRNA and β-galactosidase activity were determined for strain HB10. As shown in the top curve of Fig. 5, β-galactosidase activity increased rapidly after removal of glucose to a level twofold higher than during repression. The RNA levels (top inset) behaved differently. They showed a transient 5- to 10-fold increase by 5 h after glucose removal and then a decrease, so that at 22 h the level was about twice that during glucose growth. In strain HB15 (middle curve), β-galactosidase activity increased more slowly to a level about 30-fold higher than during glucose repression, as shown in Table 3. The total *ADR1* RNA measured by S1 analysis in this strain represented both the gene fusion RNA and *ADR1* RNA derived from the normal gene. There was a very low level of total *ADR1* RNA during repressed growth (inset), indicating that the gene fusion was expressed at a low level at that time, as was the wild-type *ADR1* RNA (bottom inset). Northern analysis of *ADR1* and *ADR1-lacZ* RNA isolated from repressed and derepressed cells corroborated the conclusion that these two genes are regulated in a similar fashion (Hartshorne, unpublished). Since very little *ADR1* RNA was present during glucose growth of either strain HB23-3 or HB15, the *ADR1-lacZ* gene fusion appears to be as efficiently glucose-repressed as the wild-type *ADR1* gene. Thus, there is no evidence for translational control of expression of the gene fusion mRNA during glucose repression.

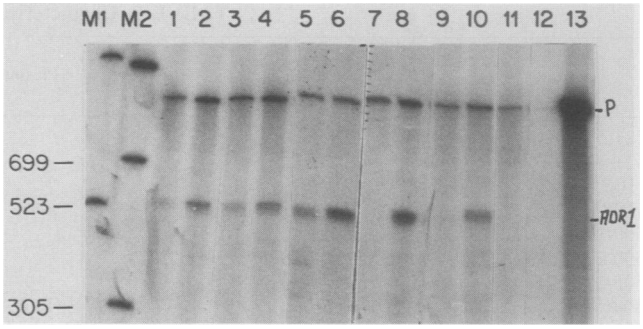

FIG. 4. Quantitation by S1 mapping of the *ADR1* RNA from different strains of *S. cerevisiae*. Total nucleic acids were isolated from various yeast strains and analyzed by S1 mapping of the 5′ end as described in Materials and Methods. Total yeast RNA (80 μg) was used. Lanes M1 and M2, molecular weight markers (nucleotides); lanes 1 and 2, strain 2150AA; lanes 3 and 4, strain XV617; lanes 5 and 6, strain 3482-16-1; lanes 7 and 8, strain 20B12; lanes 9 and 10, strain HB23-3; lane 11, strain 521-6-Δ1; odd-numbered lanes, 1 to 9, RNA from glucose-repressed cultures; even-numbered lanes 2 to 10 and lane 11, RNA from derepressed cultures; lane 12, *E. coli* tRNA replaced the yeast RNA; lane 13, no RNA and no S1 treatment. The position of *ADR1* RNA protected from S1 is indicated; P indicates the position of the intact 1.3-kb probe.

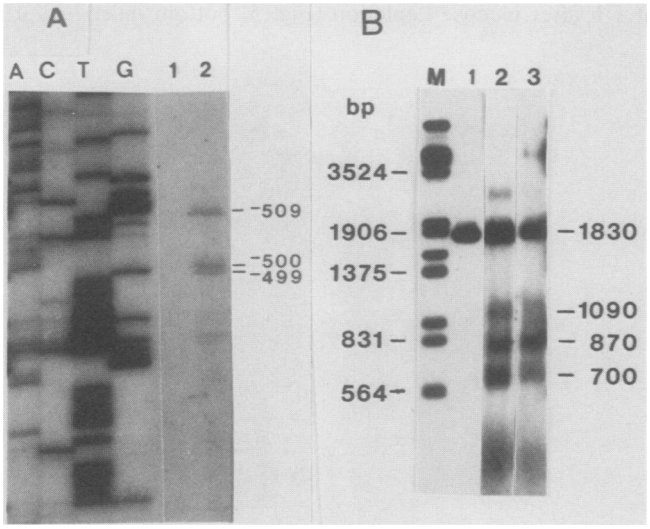

FIG. 3. Mapping the 5′ and 3′ ends of the *ADR1* mRNA. (A) Determination of the 5′ end of the *ADR1* mRNA. The 5′ end of *ADR1* mRNA was determined by primer extension as described in Materials and Methods. Lanes: 1, strain 521-6-Δ1 grown on glucose-containing medium; 2, strain E8-11C grown on ethanol-containing medium. The 5′ ends of the *ADR1* mRNA corresponded to positions −509, −500, and −499 nucleotides upstream of the translation initiation codon. (B) Determination of the 3′ end of the *ADR1* mRNA. The 3′ end of *ADR1* mRNA was determined by S1 mapping as described in Materials and Methods. Total nucleic acid (100 μg) was used. Lanes: 1, probe alone, no S1; 2, strain 7972-C ($Adr1^+$) grown on glucose-containing medium; 3, strain 7972-C ($Adr1^+$) grown on ethanol-containing medium. No protected fragments were observed when total nucleic acid derived from strain 521-6-Δ1 was used (data not shown) or when total nucleic acid was treated with RNase prior to hybridization with the probe. Protected fragments of 1,090, 870, and 700 nucleotides correspond to RNA 3′ ends 810, 590, and 420 (+20) nucleotides beyond the *ADR1* open reading frame (20). Increasing the amount of S1 to 1,500 U did not alter the pattern of fragments obtained.

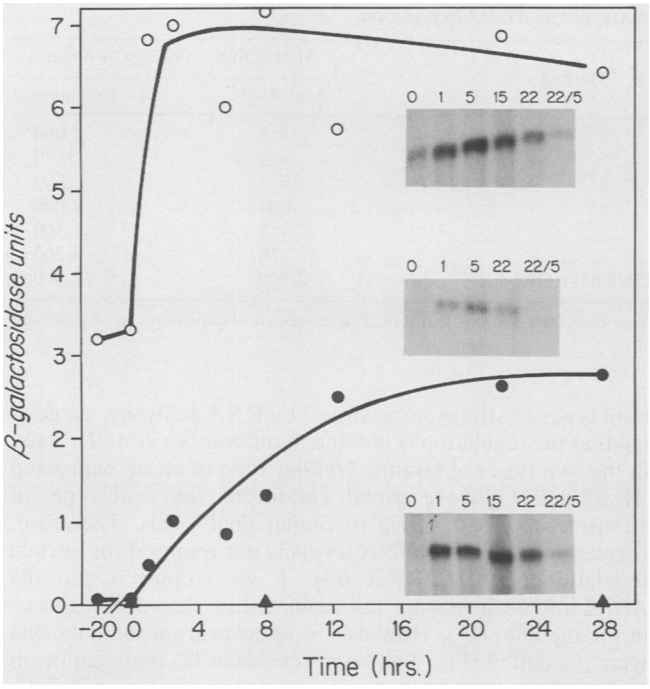

FIG. 5. Activity and expression of ADR1-β-galactosidase fusion proteins in strains HB10, HB15, and HB23-3. Strains HB10 and HB15, derived from strains 521-6-Δ1 and HB23-3, respectively, contain an *ADR1-lacZ* gene fusion integrated in single copy at the *trp1* locus (Blumberg, thesis). The gene fusion encodes 642 amino acids of ADR1 fused to amino acid 8 of β-galactosidase (Blumberg, thesis). The cultures were grown in YEP medium containing 5% glucose to an A_{600} of 1, centrifuged, and resuspended in YEP containing 3% glycerol and 2% ethanol. Samples were withdrawn for β-galactosidase assays at the indicated times. The insets adjacent to each curve show the amount of *ADR1* mRNA 5′ end at the times indicated (in hours) as revealed by S1 analysis (Materials and Methods). Strain HB15 contains two *ADR1* genes: a copy of the wild-type gene at the normal chromosomal locus and a copy of the *ADR1-lacZ* gene fusion at the *trp1* locus. RNA (80 μg) was analyzed for each time point shown; in addition, 16 μg was analyzed at 22 h (22/5) to demonstrate that the DNA probe was in excess. β-Galactosidase is expressed as the slope, A_{420}/min., normalized to the A_{600} units assayed, multiplied by 1,000. Symbols: ○, HB10; ●, HB15; ▲, HB23-3 (no *ADR1-lacZ* gene fusion). Zero time represents the time that glucose was removed.

Function of *ADR1-lacZ* gene fusion. We previously showed that *ADR1-lacZ* encodes a bifunctional protein. When present on a centromere-containing plasmid, this gene fusion produces an activator of *ADH2* expression that partially overcomes glucose repression (6). The present data (Table 4) confirmed this observation and showed that the ability of the fusion protein to activate *ADH2* during repressed growth conditions was strain dependent. In strain HB16, *ADH2* expression was barely detectable during glucose growth conditions, and 150-fold derepression occurred during growth on a nonfermentable carbon source. In strain HB10, which contains the same gene fusion, constitutive *ADH2* expression was observed, and there was 15-fold derepression. Presumably, *ADR1-lacZ* gave rise to partially constitutive *ADH2* expression in strains derived from 521-6 but not in those derived from strain HB23-3 because higher levels of ADR1-β-galactosidase were produced during glucose growth in the former (Fig. 5 and Table 3). In further support of this interpretation, increasing the *ADR1-lacZ* copy number by means of a high-copy-number plasmid led to even higher levels of ADHII activity (Table 4). This strain had 10-fold higher levels of β-galactosidase activity than strains containing a single copy of the gene fusion (Table 3).

It was surprising that a single copy of the gene fusion led to partially constitutive *ADH2* expression in strain HB10 since an isogenic *ADR1* parent strain (521-6) lacking the gene fusion was completely glucose repressed. The constitutive expression could occur because ADR1-β-galactosidase is missing the carboxy-terminal region of ADR1 or because of fusion to LacZ. To distinguish between these possibilities, a plasmid carrying the same fragment of *ADR1* not fused to *lacZ* was integrated at the *trp1* locus in strain 521-6-Δ1. *ADH2* expression in this strain (HB9) was glucose repressed and derepressed to about 10% of the level observed in strain HB10 (Table 4). This indicates that fusion of the amino-terminal 642 amino acids of ADR1 to β-galactosidase is responsible for constitutive expression and enhanced derepression of *ADH2* in strain HB10.

Increased *ADR1* gene dosage leads to partially constitutive *ADH2* expression. We studied the effects of increased *ADR1* gene dosage on *ADH2* expression under glucose and ethanol growth conditions. Increased *ADR1* gene dosage, due to a multicopy plasmid containing the entire *ADR1* gene, led to partially constitutive *ADH2* expression and higher levels of *ADH2* derepression than in a strain with a single-copy *ADR1* gene (Table 5). A truncated gene which encoded the amino-terminal 302 amino acids of ADR1 also caused partially constitutive *ADH2* expression, while a truncated gene en-

TABLE 3. Expression of *ADR1-lacZ* gene fusions

Strain[a]	*ADR1* allele	Plasmid[b]	β-Galactosidase activity[c] (U/mg of protein)	
			Repressed	Derepressed
521-6-Δ1	*adr1-Δ1*		<0.1	<0.1
HB10	*adr1-Δ1 trp1*::YIp642ADR1-lacZ		5.5	9.3
HB23-3	*ADR1*		<0.1	<0.1
HB16	*ADR1 ura3*::YIp642ADR1-lacZ		0.5	4.7
HB15	*ADR1 trp1*::YIp642ADR1-lacZ		0.3	6.1
2150-AA	*ADR1*	YEpC1/1-642ADR1-lacZ	50	40

[a] Strains are listed in Table 1. The parent strain of HB10 is 521-6-Δ1; that of strains HB16 and HB15 is HB23-3.
[b] ADR1-lacZ integrating plasmids and episomal plasmids contain a 7.0-kb *ADR1-lacZ Bam*HI fragment as described in the text.
[c] Cells carrying plasmids were grown as described in Materials and Methods. Cells not carrying a plasmid were grown as described in Table 2, footnote *a*. β-Galactosidase specific activities were determined as described (29) in cell extracts. The values are the average of at least four determinations in separate extracts and are ± 50%.

TABLE 4. *ADR1-lacZ* encodes an activator of *ADH2* expression

Strain	*ADR1* allele	Plasmid	ADH activity[a] (mU/mg of protein)	
			Repressed	Derepressed
521-6	*ADR1*		<5	2,000
521-6-Δ1	*adr1-Δ1*		<5	<20
HB9	*adr1-Δ1 trp1*::YIp642ADR1		<5	600
HB10	*adr1-Δ1 trp1*::YIp642ADR1-lacZ		600	9,000
HB23-3	*ADR1*		<5	1,500
HB16	*ADR1 ura3*::YIp642ADR1-lacZ		20	3,200
2150-AA	*ADR1*	YEpC1/1-642ADR1-lacZ	2,500	12,000

[a] Cells were grown as described in Table 2, footnote *a*. ADR specific activities were determined in cell extracts. The values are the averages of at least four determinations and are ± 30%.

coding the amino-terminal 150 amino acids of ADR1 did not (Table 5). The relief of *ADH2* glucose repression required the *ADH2* UAS (data not shown), indicating that overexpression of *ADR1* leads to *ADH2* expression by the normal *ADH2* activation pathway (2, 43).

DISCUSSION

ADR1 is predicted from its nucleotide sequence to encode a protein of 1,323 amino acids. The amino-terminal 302 amino acids are sufficient to restore a partial Adh2$^+$ phenotype to an *adr1* strain (18, 20). The carboxy-terminal 1,000 amino acids may play an auxiliary but nonessential role in derepression of *ADH2*. An essential role for the carboxy-terminal portion of the protein in mitotic growth or sporulation is apparently ruled out by the studies with a strain disrupted for *ADR1*. Since the disrupted and 5′-deleted *ADR1* gene lacks a promoter, it seems unlikely that the remaining 3′ portion of the gene still present in the disrupted strain could be expressed. The only abnormal phenotype of the strain disrupted for *ADR1* detected was an inability to express *ADH2*. This suggests that *ADR1* has no repressor function and that it acts primarily as a positive regulatory factor in *ADH2* expression, consistent with genetic (11) and in vivo titration (24) data.

We found that yeast strains differed in their regulation of *ADR1* expression. In some yeast strains *ADR1* mRNA accumulation was strongly glucose repressed (20- to 30-fold), while in other strains the RNA was approximately equally abundant during repressed and derepressed growth conditions. However, in one such strain, transient derepression occurred. The major difference in terms of *ADR1* expression was a 20-fold-lower level of *ADR1* mRNA during glucose growth conditions in strains such as HB23-3, 20B12, and 79-72C than in strains such as 521-6, 2150-AA, XV617, and 3482-16-1. This glucose repression of *ADR1* was evident both in RNA analyses and from assays of an ADR1-β-galactosidase fusion protein. A cloned *ADR1-lacZ* gene fusion behaved the same way as the resident *ADR1* gene in both types of strains, as assessed by RNA analyses, suggesting that the regulation is not due to differences in *ADR1* itself in the two types of strains. Neither type of strain expressed *ADH2* under glucose growth conditions, and both types of strain derepressed *ADH2* to similar final levels. Therefore, regulation of *ADR1* mRNA levels is not required for normal regulation of *ADH2*. Recently, it was reported that the *ADR1* mRNA levels are not regulated by the carbon source in the medium (17). However, it appears from the data that were presented (Fig. 3 and 4 in reference 17) that significant derepression of the *ADR1* mRNA occurred in strain 79-72C, as we found by Northern analysis.

Since regulation of *ADR1* mRNA levels is not necessary for proper expression of *ADH2*, we wished to determine whether *ADR1* was regulated at the translational level. We used the β-galactosidase activity derived from an *ADR1-lacZ* gene fusion as a measure of translation of *ADR1* mRNA. There was a positive correlation between the levels of *ADR1* mRNA, *ADR1-lacZ* mRNA, and β-galactosidase activity in all strains studied, which indicates that *ADR1-lacZ*, and presumably *ADR1* itself, is not translationally regulated.

The data suggest that neither transcriptional nor translational regulation of *ADR1* can account for the proper expression of *ADH2* in all yeast strains. It seems likely that the activity of the ADR1 protein is regulated in a carbon source-dependent manner, at least in strains which do not regulate *ADR1* mRNA levels. In strains which downregulate *ADR1* mRNA levels on glucose-containing medium, a second posttranslational mechanism may not be needed. Alternatively, these strains may regulate *ADR1* expression both transcriptionally and posttranslationally to provide two independent mechanisms to ensure glucose repression of *ADH2*.

The interpretation that *ADR1* is regulated posttranslationally is complicated by the fact that ADR1-β-galactosidase activated *ADH2* transcription during glucose growth conditions. However, there was still a 15-fold derepression of

TABLE 5. Increased *ADR1* gene dosage causes constitutive *ADH2* expression

Strain	*ADR1* allele	Plasmid	ADH activity (mU/mg of protein)		Antimycin phenotype[a]
			Repressed	Depressed	
521-6-Δ1	*adr1-Δ1*	pMW5	<5	25	S
		YEp150ADR1	<5	20	S
		YEp302ADR1	210	1,400	R
		YEp1323ADR1	230	6,500	R
521-6	*ADR1*	pMW5	<5	2,000	S

[a] The antimycin phenotype was determined on YPD plates containing antimycin A (1 μg/ml). S, Sensitive; R, resistant.

ADH2, suggesting that ADR1-β-galactosidase is still subject to some form of post-translational regulation. The hyperactivity of the ADR1-β-galactosidase fusion protein on both repressing and derepressing medium requires the β-galactosidase part of the fusion protein, but how it does so is not known. The β-galactosidase protein could, for example, interfere with the ability of ADR1 to be modified or to interact with another protein.

Increasing the gene dosage of *ADR1* caused partially constitutive *ADH2* expression during glucose growth conditions and led to higher than normal levels of *ADH2* derepression during growth on nonfermentable carbon sources (15, 24; Blumberg, thesis). We presume that increased expression of *ADR1* overrides a regulatory mechanism which normally keeps *ADR1* inactive during growth on glucose. If this is true, low-level *ADR1* expression would be required for maintenance of *ADH2* glucose repression. Increased gene dosage of *ADR1* mimics the effect of the *ADR1-lacZ* gene fusion and of *ADR1*^{c} mutations on *ADH2* expression. It is unclear whether increased *ADR1* gene dosage, *ADR1-lacZ*, and *ADR1*^{c} mutations act by the same mechanism to increase *ADH2* expression. Recently, the *ADR1-5*^{c} mutation has been identified (17) as a single-amino-acid change in one of three putative phosphorylation sites in *ADR1* (20). Since this mutation is in the coding part of the gene and the *ADR1-5*^{c} RNA is expressed at normal levels (17; Hartshorne, thesis), the simplest explanation for its effect is that it alters the activity of the protein, an interpretation consistent with our conclusion that *ADR1* is regulated posttranslationally.

There are many posttranslational mechanisms one could imagine as being involved in regulating *ADR1* activity. Among the possible mechanisms are protein-protein interaction between ADR1 and a negative regulatory protein, covalent modification of ADR1, regulation of ADR1 protein half-life, and competition with other factors for binding the *ADH2* UAS. All of these models must take into account the observation that overexpression of *ADR1*, synthesis of an ADR1-β-galactosidase fusion protein, and mutation to *ADR1*^{c} only partially relieve *ADH2* glucose repression.

ACKNOWLEDGMENTS

We thank Nancy Hollingsworth for constructing plasmid pADR1-Δ1, Josie Yu for performing some of the experiments involving the *ADR1* gene disruption in diploids, Kevin Struhl for the *DED1* oligonucleotide, and Darlene Alarcon and Ruth Kaspar for preparing this manuscript.

Support for this research was provided by Public Health Service grants GM26079 and 5-T32-GM-07270 from the National Institutes of Health.

LITERATURE CITED

1. **Bach, M.-L., F. Lacroute, and D. Botstein.** 1979. Evidence for transcriptional regulation of orotidine-5'-phosphate decarboxylase in yeast by hybridization of mRNA to the yeast structural gene cloned in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **76**:386–390.
2. **Beier, D. R., A. Sledziewski, and E. T. Young.** 1985. Deletion analysis identifies a region, upstream of the *ADH2* gene of *Saccharomyces cerevisiae*, which is required for *ADR1*-mediated derepression. Mol. Cell. Biol. **5**:1743–1749.
3. **Bender, A., and G. F. Sprague.** 1987. MATα1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. Cell **50**:681–691.
4. **Berk, A. J., and P. A. Sharp.** 1978. Spliced early mRNAs of simian virus 40. Proc. Natl. Acad. Sci. USA **75**:1274–1278.
5. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7**:1513–1523.
6. **Blumberg, H., A. Eisen, A. Sledziewski, D. Bader, and E. T. Young.** 1987. Two zinc fingers of a yeast regulatory protein shown by genetic evidence to be essential for its function. Nature (London) **7**:443–445.
7. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. C. Betlach, H. L. Heynecker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
8. **Casadaban, M. J., A. Martinez-Arias, S. K. Shapria, and J. Chou.** 1983. β-Galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100**:293–308.
9. **Celenza, J. L., and M. Carlson.** 1984. Cloning and genetic mapping of SNF1, a gene required for expression of glucose-repressible genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **4**:49–53.
10. **Ciriacy, M.** 1975. Genetics of alcohol dehydrogenase in *Saccharomyces cerevisiae*. I. Isolation and genetic analysis of adh mutants. Mutat. Res. **29**:315–325.
11. **Ciriacy, M.** 1975. Genetics of alcohol dehydrogenase in *Saccharomyces cerevisiae*. II. Two loci controlling synthesis of the glucose-repressible ADHII. Mol. Gen. Genet. **138**:157–164.
12. **Ciriacy, M.** 1977. Isolation and characterization of yeast mutants defective in intermediary carbon metabolism and in carbon catabolite derepression. Mol. Gen. Genetics **154**:213–220.
13. **Ciriacy, M.** 1979. Isolation and characterization of further cis- and trans-acting regulatory elements involved in the synthesis of glucose-repressible alcohol dehydrogenase (ADHII) in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **176**:427–431.
14. **Denis, C. L.** 1984. Identification of new genes involved in the regulation of yeast alcohol dehydrogenase II. Genetics **108**:833–844.
15. **Denis, C. L.** 1987. The effects of ADR1 and CCR1 gene dosage on the regulation of the glucose-repressible alcohol dehydrogenase from *Saccharomyces cerevisiae*. Mol. Gen. Genet. **208**: 101–106.
16. **Denis, C. L., M. Ciriacy, and E. T. Young.** 1981. A positive regulatory gene is required for accumulation of the functional messenger RNA for the glucose-repressible alcohol dehydrogenase from *Saccharomyces cerevisiae*. J. Mol. Biol. **148**:355–368.
17. **Denis, C. L., and C. Gallo.** 1986. Constitutive RNA synthesis for the yeast activator *ADR1* and identification of the ADR1-5^c mutation: implications in posttranslational control of *ADR1*. Mol. Cell. Biol. **6**:4026–4030.
18. **Denis, C. L., and E. T. Young.** 1983. Isolation and characterization of the positive regulatory gene *ADR1* from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **3**:360–370.
19. **Gold, L., D. Pribnow, T. Schneider, S. Shinedling, B. S. Singer, and G. Stormo.** 1981. Translational initiation in prokaryotes. Annu. Rev. Microbiol. **35**:365–403.
20. **Hartshorne, T. A., H. Blumberg, and E. T. Young.** 1986. Sequence homology of the yeast regulatory protein *ADR1* with Xenopus transcription factor TFIIIA. Nature (London) **320**: 283–287.
21. **Hinnebusch, A. G.** 1984. Evidence for translational regulation of the activator of general amino acid control in yeast. Proc. Natl. Acad. Sci. USA **81**:6442–6446.
22. **Hope, I. A., and K. Struhl.** 1985. GCN4 protein, synthesized in vitro, binds HIS3 regulatory sequences: implications for general control of amino acid biosynthetic genes in yeast. Cell **43**:177–188.
23. **Inoue, T., and T. R. Cech.** 1985. Secondary structure of the circular form of the Tetrahymena rRNA intervening sequence: a technique for RNA structure analysis using chemical probes and reverse transcriptase. Proc. Natl. Acad. Sci. USA **82**:648–652.
24. **Irani, M., W. E. Taylor, and E. T. Young.** 1987. Transcription of the *ADH2* gene in *Saccharomyces cerevisiae* is limited by positive factors that bind competitively to its intact promoter region on multicopy plasmids. Mol. Cell. Biol. **7**:1233–1241.
25. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153**:163–168.

26. **Johnson, A. D., and I. Herskowitz.** 1985. A repressor (MATα2 product) and its operator control expression of a set of cell type specific genes in yeast. Cell **42**:237–247.
27. **Johnston, S. A., and J. E. Hopper.** 1982. Isolation of the yeast regulatory gene GAL4 and analysis of its dosage effects on the galactose/melibiose regulon. Proc. Natl. Acad. Sci. USA **79**: 6971–6975.
28. **Keegan, L., G. Gill, and M. Ptashne.** 1986. Separation of DNA binding from the transcription-activating function of a eukaryotic regulatory protein. Science **231**:699–704.
29. **Langford, C. J., F. Klinz, C. Donath, and D. Gallwitz.** 1984. Point mutations identify the conserved, intron-contained TAC TAAC box as an essential splicing signal sequence in yeast. Cell **36**:645–653.
30. **Laughon, A., and R. F. Gesteland.** 1982. Isolation and preliminary characterization of the GAL4 gene, a positive regulator of transcription in yeast. Proc. Natl. Acad. Sci. USA **79**:6827–6831.
31. **Losson, R., R. P. P. Fuchs, and F. Lacroute.** 1983. *In vivo* transcription of a eukaryotic regulatory gene. EMBO J. **2**:2179–2184.
32. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Miller, J.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Mueller, P. P., and A. G. Hinnebusch.** 1986. Multiple upstream AUG codons mediate translational control of *GCN4*. Cell **45**: 201–207.
35. **Nasmyth, K. A., and K. Tatchell.** 1980. The structure of transposable yeast mating type loci. Cell **19**:753–764.
36. **O'Farrell, P. H., E. Kutter, and M. Nakanishi.** 1980. A restriction map of the bacteriophage T4 genome. Mol. Gen. Genet. **179**:421–435.
37. **Penn, M. D., G. Thireos, and H. Greer.** 1984. Temporal analysis of general control of amino acid biosynthesis in *Saccharomyces cerevisiae*: role of positive regulatory genes in initiation and maintenance of mRNA derepression. Mol. Cell. Biol. **4**:520–528.
38. **Perlman, D., and J. E. Hopper.** 1979. Constitutive synthesis of the GAL4 protein, a galactose pathway regulator in *Saccharomyces cerevisiae*. Cell **16**:89–95.
39. **Pfeifer, K., B. Arcangioli, and L. Guarente.** 1987. Yeast HAP1 activator competes with the factor RC2 for binding to the upstream activation site UAS, of the CyC1 gene. Cell **49**:9–18.
40. **Pinkham, J. L., and L. Guarente.** 1985. Cloning and molecular analysis of the *HAP2* locus: a global regulator of respiratory genes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **5**:3410–3416.
41. **Rothstein, R. J.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
42. **Schultz, L. D.** 1978. Transcriptional role of yeast deoxyribonucleic acid dependent ribonucleic acid polymerase III. Biochemistry **17**:750–758.
43. **Shuster, J., J. Yu, D. Cox, R. V. L. Chan, M. Smith, and E. Young.** 1986. *ADR1*-mediated regulation of *ADH2* requires an inverted repeat sequence. Mol. Cell. Biol. **6**:1894–1902.
44. **Struhl, K.** 1985. Nucleotide sequencing and transcriptional mapping of the yeast pet56-his3-ded1 gene region. Nucleic Acids Res. **13**:8587–8601.
45. **Taguchi, A. K. W., and E. T. Young.** 1987. The identification and characterization of ADR6, a gene required for sporulation and for expression of the alcohol dehydrogenase II isozyme from *Saccharomyces cerevisiae*. Genetics **116**:523–530.
46. **Taguchi, A. K. W., and E. T. Young.** 1987. The cloning and mapping of ADR6, a gene required for sporulation and for expression of the alcohol dehydrogenase II isozyme from *Saccharomyces cerevisiae*. Genetics **116**:531–540.
47. **Thireos, G., M. D. Penn, and H. Greer.** 1984. 5′ untranslated sequences are required for the translational control of a yeast regulatory gene. Proc. Natl. Acad. Sci. USA **81**:5096–5100.