Isolation and Identification of Genes Activating UAS2-Dependent *ADH2* Expression in *Saccharomyces cerevisiae*

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ABSTRACT

Two *cis*-acting elements have been identified that act synergistically to regulate expression of the glucose-repressed alcohol dehydrogenase 2 (*ADH2*) gene. UAS1 is bound by the *trans*-activator Adr1p. UAS2 is thought to be the binding site for an unidentified regulatory protein. A genetic selection based on a UAS2-dependent *ADH2* reporter was devised to isolate genes capable of activating UAS2-dependent transcription. One set of UAS2-dependent genes contained *SPT6/CRE2/SSN20*. Multicopy *SPT6* caused improper expression of chromosomal *ADH2*. A second set of UAS2-dependent clones contained a previously uncharacterized open reading frame designated MEU1 (*M*ulticopy *E*nhancer of *U*AS2). A frame shift mutation in *MEU1* abolished its ability to activate UAS2-dependent gene expression. Multicopy *MEU1* expression suppressed the constitutive *ADH2* expression caused by *cre2-1*. Disruption of *MEU1* reduced endogenous *ADH2* expression about twofold but had no effect on cell viability or growth. No homologues of *MEU1* were identified by low-stringency Southern hybridization of yeast genomic DNA, and no significant homologues were found in the sequence data bases. A *MEU1*/β-gal fusion protein was not localized to a particular region of the cell. *MEU1* is linked to *PPR1* on chromosome *XII*.

THE alcohol dehydrogenase 2 (*ADH2*) gene of *Saccharomyces cerevisiae* is a member of a large family of yeast genes that are subject to glucose repression (GANCEDO 1992; JOHNSTON and CARLSON 1992; TRUMBLY 1992). *ADH2* encodes an isozyme, ADHII, whose activity is absent from cells cultured in media containing a fermentable carbon source but derepresses several hundred-fold when cells are cultured in a nonfermentable carbon source (LUTSTORF and MEGNET 1968). *ADH2* expression is controlled at the level of transcription and both positive and negative *trans*-acting regulatory proteins have been identified (CIRIACY 1975a,b, 1979; DENIS 1984; DENIS and MALVAR 1990; KARNITZ *et al.* 1992). Repression of *ADH2* requires *REG1*, a pleiotropic effector of glucose repression, but does not require other generalized repressors of glucose-repressed genes encoded by *HXK2, TUP1*, and *SSN6* (DOMBEK *et al.* 1993). Derepression of *ADH2* expression requires protein kinases encoded by *SNF1/CCR1, SCH9*, and the *TPK1* genes (CHERRY *et al.* 1989; TAYLOR and YOUNG 1990; DENIS and AUDINO 1991). The specific *ADH2* regulatory sequences utilized by these proteins have not been fully worked out. A transcriptional activator, Adr1p, is also required for *ADH2* derepression (CIRIACY 1975a; DENIS *et al.* 1981).

This manuscript is dedicated to the memory of Michael Ciriacy, whose contributions to the study of gene regulation in yeast were marked by clarity and insight.

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Adr1p binds to UAS1, one of two *cis*-acting elements in the *ADH2* promoter (BEIER *et al.* 1985; SHUSTER *et al.* 1986). These *cis*-acting elements act synergistically to control *ADH2* expression (YU *et al.* 1989). UAS1 is a 22-bp palindrome that is bound by two monomers of Adr1p (SHUSTER *et al.* 1986; EISEN *et al.* 1988; THUKRAL *et al.* 1991a; CHENG *et al.* 1993). In the absence of *ADR1*, *ADH2* expression is reduced 100-fold (DENIS *et al.* 1981). Deletion of UAS1 from the *ADH2* promoter resulted in only a 10-fold reduction in *ADH2* expression (SHUSTER *et al.* 1986). These and other observations resulted in the identification of UAS2, a sequence that confers glucose-regulated, *ADR1*-independent expression on a reporter gene (YU *et al.* 1989).

The synergism between UAS1 and UAS2 is lost if UAS2 is inverted or multimerized in the *ADH2* promoter, and efficient synergism depends on the correct helical phasing between UAS1 and UAS2 (DONOVIEL *et al.* 1995). These properties and the proximity of UAS2 to UAS1 (the elements are 9 bp apart) suggest that Adr1p and a UAS2-binding factor interact in an orientation-specific manner. The identity of this putative UAS2-binding factor is not known, furthermore little is known about regulators of *ADH2* expression that function through a UAS2-dependent pathway.

A selection strategy was devised to identify fragments of genomic DNA carried on multicopy-copy plasmids that would enhance UAS2-dependent gene expression. The selection employed an *ADH2* reporter gene that was dependent on a single synthetic UAS2 element. Both UAS2-dependent and -independent trancriptional activators were identified. One gene from each of two

separate classes of UAS2-dependent activators was characterized. One of these encoded *SPT6/CRE2/SSN20,* a previously identified *ADH2* regulator. The second gene, named *MEU1,* has not been previously characterized and appears to function in a UAS2-specific regulatory pathway upstream of the UAS2 binding protein.

MATERIALS AND METHODS

Escherichia coli* and *Saccharomyces* strains:** *E. coli* strain DH5α (HANAHAN 1983) was used for the propagation of all plasmids used in this work. Strain RR1 (BOLIVAR *et al.* 1977) was used to specifically select *LEU2*-containing yeast plasmids. *S. cerevisiae* strains used were MC71-18b *MATa** *adh1 adh3 trp1 ura3 leu2* (M. CIRIACY, personal communication), 147-6d *MAT***a** *adh1-11 adh3 cre2-1 his4 trp1 ura1 leu2* (DENIS 1984), and 521-6 *MAT***a** *adh1-11 adh3 ura1 trp1 leu2* (BLUMBERG *et al.* 1988).

Plasmid transformations: Plasmids were introduced into DH5α or RR1 using frozen competent cells (HANAHAN 1983). Yeast strains were transformed by the lithium acetate method of ITO *et al.* (1983) as modified by GIETZ and SCHIESTL (1991). Transformants were selected by growth on antibiotic-containing or minimal media lacking the appropriate nutrient.

Growth media and culture conditions: Yeast complete medium contained 10 g of yeast extract (Difco), 10 g of bactopeptone (Difco), 20 mg of adenine, and 20 mg of uracil per l supplemented with 5% glucose (YPD, repressing media) or with 3% ethanol (YPE, derepressing medium). Agar (Difco) was added to 2% to make YPD or YPE plates. Yeast synthetic medium, SM, containing the appropriate drop-out solution and supplemented with 5% glucose (SMD) or 3% ethanol (SME) was used (YU *et al.* 1989). Strains were grown at 30°. Repressed cultures were inoculated into YPD or SMD and grown for 12 hr. Cultures were then diluted into fresh media of the same type and grown to an OD_{600} of 0.6–1.0 (Gilford Instruments Model 250 spectrophotometer) before harvesting. Cultures were routinely tested for the presence of glucose at the time of harvest using Diastix (Miles Inc., Diagnostics Division). Derepression was carried out by inoculating cultures into YPD or SMD and growing 12 hr. Cultures were then pelleted and washed with sterile water and resuspended into YPE or SME containing 0.05% glucose. These cultures were then grown to an OD_{600} of 1–1.5 and harvested.

Enzyme and protein assays: Preparation of yeast extracts for ADHII enzyme assay and the assays themselves were carried out as previously described (DENIS *et al.* 1981). β-galactosidase activity was assayed in whole cells as described by MILLER (1972) and modified by GUARENTE (1983). β-galctosidase activity was calculated as follows: $(OD_{420})(1000)(OD_{600}^{-1})$ (min^{-1}) (MILLER 1972). Protein concentration was quantitated by the Coomassie blue dye-binding assay (BioRad, Inc.).

Plasmid constructions: Plasmid p351-*SPT6* was constructed by insertion of a *SPT6*-containing 6-kb *Bgl*II fragment from pCC27 (THUKRAL *et al.* 1991) into *Bam*HI-digested Yep351 (GUTHRIE and FINK 1991). Plasmid pGF27-*SPT6* contains the same *SPT6* fragment inserted into pGF27. Plasmid pGF27 is a derivative of pRS 304 (SIKORSKI and HIETER 1989) with the 2μm-containing 2.2-kb *Eco*RI fragment from YEp24(GUTHRIE and FINK 1991) inserted into an *Aat*I site (G. ZHU, personal communication).

A 5.5-kb fragment of genomic DNA containing *MEU1* was subcloned into pBluescriptSK+ (Stratagene Inc.), creating pbs*MEU1*H5K. This plasmid was used as the source of *MEU1* DNA for further subcloning and the construction of *MEU1*-disruption constructs.

The *MEU1* disruption construct pΔ*MEU1*I was constructed by digesting pbs*MEU1*H5K with *Bst*BI and *Nsi*I followed by incubation with T4 DNA polymerase to create blunt ends. The vector fragment was isolated from an agarose gel and ligated with a 2.2-kb *Sal*I/*Xho*I *LEU2*-containing fragment isolated from YEp13 (GUTHRIE and FINK 1991) that was also made blunt ended with Klenow fragment of DNA polymerase. The other disruption construct, pΔ*MEU1*E, was constructed in two steps. First, the *Spe*I and *Pst*I sites were removed for pBluescript by digestion with *Sca*I and *Eco*RI followed by incubation with T4 DNA polymerase to create blunt ends. The vector fragment was gel purified and religated. The resulting plasmid was digested with *Hin*DIII, and the 5.5-kb *MEU1*-containing *Hin*DIII fragment from pbs*MEU1*H5K was inserted. This plasmid was digested with *Spe*I and *Pst*I followed by incubation with T4 polymerase to create blunt ends. The vector fragment was isolated and ligated with the *LEU2* fragment described above.

A cen-containing *MEU1* plasmid, pCEN-*MEU1,* was constructed by isolating the *MEU1*-containing fragment from pbs*MEU1*H5K that had been digested with *Sma*I and *Sal*I and ligating this fragment with pRS315 (SIKORSKI and HIETER 1989) that had been digested with the same two enzymes and gel purified. A 2μ-containing *MEU1* plasmid, p2μ-*MEU1,* was constructed by inserting the same *Sal*I//*Sma*I *MEU1* fragment used in pCEN-*MEU1* into *Sma*I/*Sal*I-digested pGF28 that had been gel purified. The pGF28 plasmid was made by inserting a 2.2-kb *Eco*RI fragment from YEp24, that contains the 2μ sequences, into the *Aat*I site of pRS305 (SIKORSKI and HIETER 1989; ZHU *et al.* 1993). A similar construct containing a frame shift mutation in *MEU1,* p2μ-*MEU1*Δ*Bst*BI, was made by inserting a *Sma*I/*Sal*I fragment from pbs*MEU1*Δ*Bst*BI into *Sma*I/*Sal*I-digested, gel purified pGF28. The pbs*MEU1Bst*BI construct was made by digesting pbs*MEU1*H5K with *Bst*BI followed by incubation with Klenow fragment of DNA polymerase to create blunt ends. The resulting plasmid was religated and a *Bst*BI-negative clone was sequenced to confirm the presence of the expected frame-shift mutation.

The *MEU1*/*lacZ* fusion gene construct, p*MEU1Z,* was made by digestion of pbs*MEU1*H5K with *Bst*XI, treating with T4 DNA polymerase to create blunt ends, and subsequently digesting with *Eco*RI. The *Eco*RI/(*Bst*XI) fragment was gel purified and ligated to pGF29 [a 2μ version of pRS306 (SIKORSKI and HIETER 1989) constructed in the same manner as pGF28] that had been digested with *Bam*HI, treated with Klenow fragment of DNA polymerase, and then digested with *Eco*RI. The resulting intermediary plasmid was digested with *Not*I treated with Klenow fragment of DNA polymerase. This product was ligated to a *lacZ*-containing *Bam*HI fragment from pMC1871 (TAI *et al.* 1988) that had also been made blunt-ended with Klenow. This ligation was predicted to yield an in-frame fusion between *MEU1* and *lacZ. E. coli* transformants giving a light blue colony color in the presence of X-gal (no IPTG) were tested for the predicted restriction enzyme digestion pattern, a clone with the expected restriction map was sequences through *MEU1* and into *lacZ* to confirm that the genes were indeed in-frame.

Gene disruptions: Disruption of the *MEU1* locus on chromosome *XII* was carried out by the single step method as described (GUTHRIE and FINK 1991). Plasmids containing the disruption cassettes were digested with *Nco*I and *Sna*BI, and DNA fragments containing the cassettes were gel purified. The isolated fragments were used to transform a diploid version of MC71-18b. The diploid was made by HO-mediated mating type switching of MC71-18b MAT**a,** followed by isolation of a MATα strain, that was in turn crossed with MC71-18b MAT**a.** The structure of the genome at the *MEU1* locus of three resulting Leu^+ prototrophs was analyzed by PCR and Southern blot. PCR was carried out with two sets of primers, one for the endogenous allele and one for the disrupted alleles. PCR reactions were carried out on whole yeast cells by dispersing a small colony in 10 μl of water and using 1 μl

of the suspended cells in a 50 μl PCR reaction. A Leu$^+$ diploid shown to be heterozygous (*MEU1/meu1*Δ::LEU2) was sporulated and tetrads were dissected and analyzed.

Chromosomal localization: *S. cerevisiae* chromosomes (Bio-Rad Inc. Cat. No. 170-165) were separated through a 1% agarose (Boehringer Mannhiem Cat. No. 1240 609) gel using a CHEF DRII Megabase DNA Pulsed Field Electrophoresis system (BioRad Inc. Cat. No. 170-3612). Total run time was 24 hr at 200 V with 15 hr at a 50-sec switch time followed by 9 hr at a 90-sec switch time. Gels were run at 16°. Gels were then treated for 5 min with 0.25 N HCl and then denatured in 0.5 N NaOH for 30 min. DNA was transferred to Hybond N+ (Amersham Cat. No. RPN203N) under alkaline conditions as recommended by the manufacturer. Hybridization was carried out using random prime labeled probes in 2× SCC, 1% sodium dodecylsulfate (SDS), 10% polyethylene glycol (average MW 8000), and 0.1 mg/ml denatured salmon sperm DNA. Blots were hybridized overnight at 68° and then washed twice for 1 hr in 2× SCC, 0.1% SDS followed by two 1-hr washes in 0.2× SCC, 0.1% SDS. All washes were carried out at 68°.

DNA sequence analysis: All DNA sequencing was carried out using double-stranded templates by the dideoxy method of SANGER (SANGER *et al.* 1977; MANIATIS *et al.* 1982). Primers were synthesized on an A.B.I. model 380 automated DNA synthesizer. For all reported DNA sequences at least two independent reactions were carried out for both strands. The nucleotide sequence containing *MEU1* has been assigned the accession number X90564 (SAVILLE *et al.* 1995).

Indirect immunofluorescence: Fixation and staining of cells was carried out as described (GUTHRIE and FINK 1991). The primary antisera was a monoclonal anti β-galactosidase IgG (Boehringer Mannheim #1083 082). The secondary antibody was fluorescein isothiocyanate (FITC)-conjugated goat anti mouse IgG (Boehringer Mannheim #605 240). Fluorescence was observed on a Zeiss Axioplan microscope using a 100× objective and filters for 4′,6-diamidino-2-phenylindole and FITC recommended by the manufacturer.

RESULTS

Selection of activators enhancing expression of a UAS2-dependent reporter gene: Two observations suggested a genetic approach that could be utilized to identify genes capable of increasing UAS2-dependent gene expression when present in cells at high copy-number. First, it was found that multimerization of synthetic UAS2 elements driving expression of an *ADH2* reporter gene resulted in increased levels of *ADH2* gene expression (DONOVIEL *et al.* 1995). Specifically, UAS2 multimerization allowed a strain, lacking endogenous ADH genes, to grow fermentatively. Fermentation-positive colonies can be selected on media containing an inhibitor of oxidative phosphorylation such as Antimycin A (At) (Figure 1). The second observation is of a more general nature. That is, enhanced levels of transcriptional activators frequently induce higher levels of expression from genes containing their cognate binding site. For example, overexpression of Adr1p activates glucose-insensitive expression of *ADH2* (DENIS 1987; BLUMBERG *et al.* 1988) and of *CTA1,* another gene regulated by Adr1p (SIMON *et al.* 1991, 1992). In this case, overexpression of *ADR1* phenotypically mimics multimerization of UAS1 (its cognate binding site) in a reporter gene (DENIS 1987; KARNITZ *et al.* 1992; DONOVIEL *et al.* 1995). Based on these two observations, it seemed likely that the selection strategy outlined in Figure 2 would identify genes that enhance levels of UAS2-dependent gene expression. Specifically, overexpression of a UAS2-dependent *trans*-activator would yield enhanced levels of *ADH2* expression that could be detected using Antimycin A to select for fermentation-positive cells.

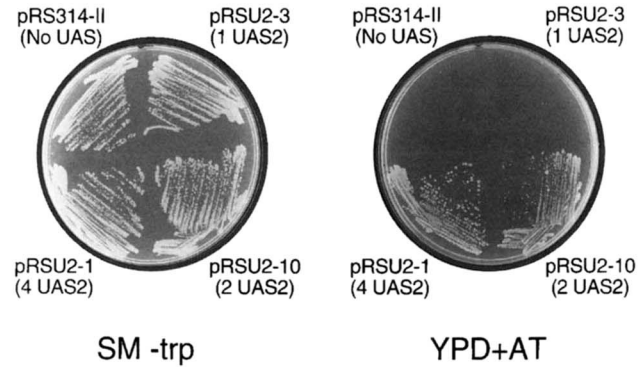

FIGURE 1.—The growth phenotype of a series of adh-null yeast strains containing reporter genes in which *ADH2* expressed from its native basal promoter was dependent on synthetic UAS2 elements. The reporter pRS314-II contains no UAS2; pRSU3-3 contains a single UAS2 element; pRSU2-10 contains two UAS2 elements; and pRSU2-1 contains four UAS2 elements. Individual reporter genes were introduced into strain *MDY10 α adh1-Δ1 ADH2Δ1::URA3 adh3 ura3 trp1 leu2.* Growth was tested on plates lacking (SM −trp) and containing (YPD +At) antimycin A. Plates were incubated for 5 days at 30°.

A yeast gene bank carried on the high copy-number vector YEp351 (*LEU2*) (HILL *et al.* 1986) was introduced into MDY10, which already contained the UAS2/*ADH2* reporter gene (*TRP1*). The UAS2/*ADH2* reporter represents the only source of ADH activity in MDY10. Approximately 8500 Trp$^+$ Leu$^+$ transformants were replica plated to media containing At. On the replica plates 94 At^r colonies grew within 5 days. Of these, 67 regrew when plated again on trp$^-$leu$^-$At media. In addition to genes that act as positive regulators of UAS2-dependent gene expression, it was expected that other types of genes would also come through this selection. In particular genes encoding the ADH isozymes could bypass the reporter, yielding At^r cells. Genes involved in drug transport through the cell membrane, genes effecting plasmid copy number, and genes that, when present at high copy-number, result in transcriptional abnormalities might also be picked up by this selection scheme. Consequently, secondary screens for UAS2-dependence of the At^r phenotype were carried out.

To identify the ADH isozyme present in each of the 67 AT^r strains, cell extracts were made and proteins were electrophoresed through a nondenaturing polyacrylamid gel. ADH activity was visualized *in situ,* and the ADH isozyme present was identified by its specific mobility in the gel (WILLIAMSON *et al.* 1980). The results

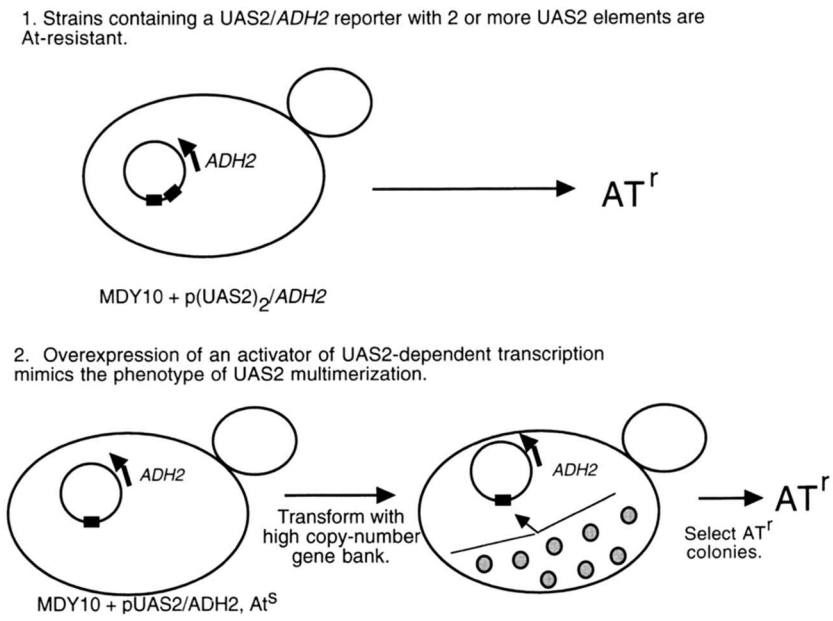

FIGURE 2.—A schematic representation of the selection used to isolate high copy-number activators of UAS2-dependent reporter gene expression. A budding yeast cell is represented by two tangential ovals. The UAS2-dependent, *ADH2*-expressing reporter genes are shown as large circles within the yeast cells. UAS2 elements are shown as small black boxes on these circles. A clone from a high copy-number gene bank is represented by many smaller shaded circles within the cell. (1) An adh-null yeast cell containing an *ADH2*-expressing reporter gene, p(UAS2)$_2$/*ADH2*, with two synthetic UAS2 elements. Such a cell is At-resistant (Figure 1). (2) An isogenic yeast strain containing an *ADH2*-expressing reporter gene, pUAS2/*ADH2*, with only one UAS2 element is At^s. The selection involved transformation of this At^s strain with a gene bank carried on a high copy-number plasmid and identifying strains converted to At^r. The conversion to At^r was predicted to occur due to overexpression of activators of UAS2-dependent expression. Such activators might include the UAS2 binding factor or other proteins functioning upstream of a DNA binding activity.

showed that 11/67 At^r clones contained ADHI activity, 42/67 contained ADHII activity, and 6/67 contained ADHIII activity. The remaining eight At^r clones had no detectable ADH activity and likely were At^r due to the presence of a drug-resistance gene. The 42 strains containing ADHII activity were tested further for dependence of the At^r phenotype on the presence of the UAS2/*ADH2* reporter gene.

Eight of the 42 strains containing ADHII activity were discarded because they were found to contain cloned *ADH2* sequences. The remaining 34 plasmids selected from the gene bank reproduced the At^r phenotype only when cotransformed into MDY10 with the UAS2/*ADH2* reporter gene. This pool of selected plasmids likely contained genes that were acting through a UAS2-specific *trans*-activation pathway and were studied further.

Characterization of high-copy activators of UAS2-dependent gene expression: Three techniques were used to group the clones into classes that likely contained the same gene of interest before a detailed analysis was carried out. First, restriction mapping of the isolated clones showed that clone 74 was identical to 75, and that 67, 72, and 77 were identical. The remaining clones were dissimilar and could not be conclusively grouped based on restriction digestion alone.

It seemed likely that different genes present in the pool of clones would reside on different chromosomes. Thus, the genomic DNA present on each of the cloned plasmids was mapped to a particular chromosome. This was done by hybridizing probes made from isolated genomic DNA fragments to blots of intact yeast chromosomes. Fourteen different chromosomes were identified, indicating that at least 14 different genes were present in the pool of 34 UAS2-dependent clones.

The third method used to group these clones was a phenotype analysis using several different reporters of promoter activity. These included a chromosomal copy of *ADH2*, a UAS-less *CYC1*/lacZ reporter, UAS1/ and UAS2/CYC1/*lacZ* reporters, and the UAS2/*ADH2* reporter used in the original selection. Three distinct classes of clones resulted from this analysis (Table 1). Class A had an unexpected phenotype. Plasmids in this class increased expression of UAS2-containing reporters, the UAS-less reporter, and chromosomal *ADH2*. Class A plasmids did not increase expression of the UAS1-dependent reporter. Both class A plasmids mapped to chromosome *IX*. Plasmids in classes B and C were UAS2-dependent but differed from each other in that class C plasmids converted a strain containing chromosomal *ADH2* to At^r while class B plasmids did not. Class C contained three members (67, 72, 77) that were identical by restriction mapping and hybridized to chromosome *VII/XV*. The remaining 29 plasmids made up class B. Class B clones yielded β-galactosidase activities from the UAS2/CYC1/*lacZ* reporter (YU *et al.* 1989), which ranged from 5 to 65 Miller units.

Plasmid clones in classes B and C were clearly UAS2-dependent and therefore of interest. Since it is likely

TABLE 1

Three classes of cloned genomic plasmids are defined by growth phenotype

Class	No.	At growth phenotype[a]			β-galactosidase activity[b]	
		No UAS[c]	UAS2[d]	*ADH2*[e]	(UAS2)2/*lac Z*[f]	(UAS1)2/*lacZ*[g]
A	2	At^r	At^r	At^r	5	<0.5
B	29	At^s	At^r	At^s	5–65	<0.5
C	3	At^s	At^r	At^r	50–60	<0.5

[a] Growth phenotype of strains transformed with plasmids isolated as high copy-number activators of UAS2-dependent gene expression and a reporter gene (c-g) on plates containing Antimycin A (At). Growth represents ability to ferment glucose due to ADH expression. At^r denotes growth in the presence of At while At^s denotes no growth, *i.e.*, sensitivity to At. Strains were partitioned into class A (two members), class B (29 members), or class C (three members) based on their At phenotype in the presence of *ADH2*-expressing reporters (c, d) or the *ADH2* gene (e).

[b] Range of reporter gene activities expressed in Miller Units for strains grouped into classes A, B, or C. All values represent the mean of at least three independent transformants and the standard error of these means was below 10% in all cases.

[c] This construct (pRS314-II) contains the *ADH2* basal promoter and structural sequences but no UAS elements.

[d] A construct (pRSU2-3) derived from pRS314-II containing a single synthetic UAS2 element.

[e] Chromosomal ADH2.

[f] A β-gal-expressing construct (pM2T); *lacZ* expression is driven from the *CYC1* basal promoter by two synthetic UAS2 elements.

[g] Identical to pM2T (f) except that the UAS2 elements have been replaced by two synthetic UAS1 elements.

that all three members of class C contain the same genomic DNA fragment, only clone 67 was selected for further study. The exact number of genes present in class B remained uncertain. However, clone 74 was chosen for further study because it increased levels of UAS2/*CYC1*/*lacZ* reporter gene to a greater extent than most other class B clones, it mapped to chromosome *XII*, in common with several other class B clones, and it was identical to clone 75, indicating that the same genomic DNA fragment had been isolated at least twice.

Isolation of clone 67 and 74 cDNAs: To simplify the identification of genes within the genomic DNA fragments of clones 67 and 74, probes were made from these plasmids and used to identify related cDNA clones. Probes specific to the genomic DNA sequences of clones 67 and 74 were used to screen a yeast cDNA library carried in λYES (ELLEDGE *et al.* 1991). Fourteen positive plaques were identified from 10^6 plated using clone 67-specific probes while ~1000 positives were identified using clone 74-specific probes. This result suggests that gene(s) present on clone 74 are transcribed into more abundant mRNA species that gene(s) present on clone 67. A plaque was purified from each group of positive clones. The corresponding plasmid form of each λYES clone was isolated, and the cDNA inserts were removed as *Xho*I fragments that were inserted into pBluescriptSK+, yielding pbs67c and pbs74c. These plasmids were used for DNA sequencing and further manipulations.

The cDNA inserts from pbs67c and pbs74c were radiolabeled and used to reprobe the same filters used originally to screen the λYES cDNA library. Comparison of the autoradiograms from the two sets of hybridizations demonstrated that, for both clone 67 and clone 74, the genomic and cDNA probes identified an identical set of plaques. This result demonstrates that for each set of genomic DNA probes a single species of cDNA molecules was identified, suggesting that a single region of each genomic DNA fragment is being transcribed.

Clone 67 contains *SPT6/CRE2/SSN20*: Comparison of DNA sequence obtained from pbs67c to the yeast sequence database showed 100% identity with *SPT6/CRE2/SSN20*, a gene known to regulate expression of *ADH2* (DENIS 1984; DENIS and MALVAR 1990) and *SUC2* (CARLSON *et al.* 1984; NEIGEBORN *et al.* 1987; WINSTON and CARLSON 1992), and effect expression from genes containing Ty insertions (CLARK-ADAMS and WINSTON 1987; WINSTON and CARLSON 1992).

Sequences encoding the C-terminal region of *SPT6* were missing from the genomic DNA fragment present on clone 67. Was this deletion responsible for the UAS2-specific activation phenotype of clone 67? To test this possibility, a 2μ-based plasmid containing the entire *SPT6* gene, p351-*SPT6*, was created. Yeast strains carrying p351-*SPT6* had UAS2-specific phenotypes similar to strains containing clone 67 when tested with the same panel of reporters used in Table 1 (data not shown). This indicates that truncation of *SPT6* is not responsible for the activation of UAS2-dependent gene expression.

The suggestion that overexpression of *SPT6* could enhance chromosomal *ADH2* activity was investigated in more detail because it has been reported that a recessive mutation in *SPT6/CRE2, cre2-1*, but not *SPT6* overexpression, bypassed glucose repression of *ADH2* expression (DENIS 1984; DENIS and MALVAR 1990). A multicopy plasmid containing *SPT6* was introduced into two pairs of isogenic *ADR1* and *adr1* strains. Multicopy *SPT6* expression allowed strain 521-6(*ADR1*) to grow fermen-

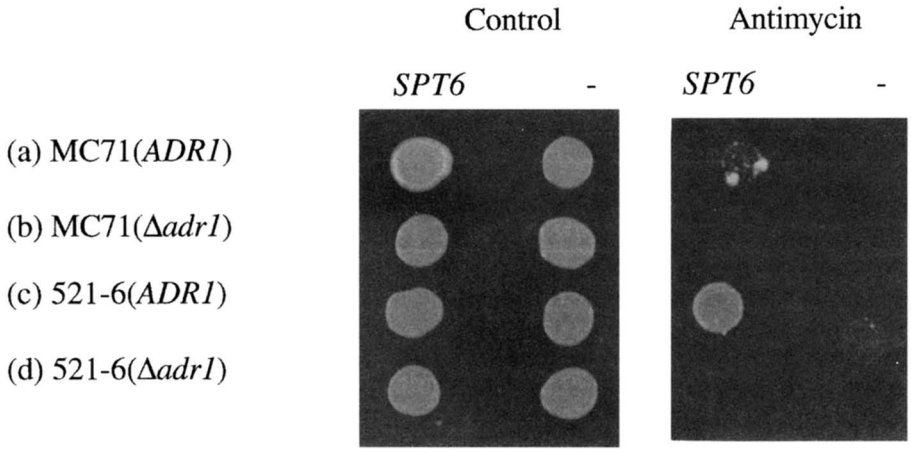

FIGURE 3.—*SPT6* on a multicopy plasmid bypasses glucose repression of *ADH2* expression in an *ADR1*- and strain-dependent manner. A 2μm-based plasmid, marked with *TRP1*, containing *SPT6(SPT6)* or vector only (−) was introduced into strains MC71-18b(*ADR1*) (a), KDY29(*adr1Δ1::LEU2*) (b), 521-6(*ADR1*) (c), and 521-6(*adr1Δ1::LEU2*) (d). Cultures of each transformant were diluted and spotted onto SD trp$^-$ plates (control) or plates containing the respiratory inhibitor Antimycin A (At). Only cells containing ADH activity, in this case derived from the *ADH2* gene, are able to ferment and grow in the presence of At.

tatively, indicating that in this strain *SPT6* overexpression bypassed glucose repression of *ADH2*. Its isogeneic *adr1Δ1::LEU2* derivative could not grow in the presence of At. Strain MC71-18b containing multicopy *SPT6* grew much more poorly in the presence of At, irrespective of its *ADR1* genotype (Figure 3).

ADHII activity in extracts made from all four strains was quantitated (Table 2). The effects on repressed *ADH2* expression confirmed the phenotypes determined by testing growth in the presence of Antimycin A. The effects of multicopy *SPT6* were both *ADR1*- and strain-dependent; ADH activity was highest in strain 521-6(*ADR1*). The effect of multicopy *SPT6* was also evident in derepressed cells of both strains in the absence of *ADR1*, being elevated about five- to 10-fold over the vector control. Thus, it is clear that overexpression of *SPT6* affects *ADH2* expression, as it affects *his4–9delta* and *SUC2* expression (CARLSON *et al.* 1984; CLARK-ADAMS and WINSTON 1987; NEIGEBORN *et al.* 1987; WINSTON and CARLSON 1992). That is, increased *SPT6* copy-number overcomes a repressing influence allowing expression from genes under conditions when they are normally transcriptionally inactive.

TABLE 2

ADHII activity in *ADR1* and *adr1Δ1* strains overexpressing *SPT6*

Strain (*ADR1* genotype)	ADHII activity[a]			
	Vector[b]		SPT6[c]	
	r	dr	r	dr
MC71-18B (*ADR1*)	0	1200	10	1200
KDY29 (*adr1Δ1*)	0	60	5	320
521-6 (*ADR1*)	5	1500	35	1400
521-6 (*adr1Δ1*)	2	30	10	270

[a] ADHII enzyme activity is expressed in milliunits per mg of protein. The average error of the mean was ~25%. r, cultures grown in repressing glucose-containing growth medium; dr, cultures grown in derepressing growth conditions, *i.e.*, in the absence of glucose.

[b] Vector refers to cells containing the base plasmid, pGF27.

[c] *SPT6* refers to cells containing the multicopy plasmid pGF27-*SPT6*.

Multicopy *SPT6* activated UAS2-dependent reporter genes and chromosomal *ADH2*, which is UAS1- and UAS2-dependent, but not UAS1-dependent reporters (Table 1). A recessive mutation in *SPT6/CRE2, cre2-1*, activates *ADH2* expression in an *ADR1*-independent manner (DENIS 1984). The effect of *cre2-1* on UAS1- and UAS2-dependent reporters was assessed to determine whether both UAS elements were influenced by reduction in Spt6p activity. In a *cre2-1* strain activity from a UAS2-dependent reporter was decreased about twofold compared to activity in a wild-type strain. In the same pair of strains activity of a UAS1-dependent reporter remained unchanged (data not shown). Thus, reduction of Spt6p activity affects a UAS2-dependent reporter to a greater extent than a UAS1-dependent reporter, which is also *ADR1*-dependent.

Clone 74 contains a previously unidentified gene: The sequence data from either end of the pbs74c insert showed no homology to known yeast genes, although a potential open reading frame (ORF) was identified. Sequencing of the entire cDNA insert of pbs74c (1.1 kb) identified a single ORF that appeared to be incomplete. Further sequencing of the genomic DNA fragment of pbs74H5k completed the ORF. Conceptual translation of this ORF yielded a protein of 337 amino acids. The ORF was named *MEU1* for *M*ulticopy *E*nhancer of *U*AS2. *MEU1* is contained within the DNA sequence of a 7.8-kb region of chromosome *XII*, containing *PPR1*, that has been submitted to EMBL, GenBank, and DDBJ databases under accession number X90564 (SAVILLE *et al.* 1995). *MEU1* corresponds to ORF3 within the 7.8-kb DNA fragment. Four other ORFs are present on the 7.8-kb fragment, not including *MEU1* and *PPR1* (SAVILLE *et al.* 1995).

Two experiments demonstrated that *MEU1* encoded the UAS2-activating function of clone 74. First, a subclone of the genomic fragment *Eco*RI/*Pst*I fragment containing *MEU1*, present on a high copy-number plasmid, was able to activate UAS2-containing reporters to the same extent as clone 74 (Table 3). Second, a mutation was made in *MEU1* to demonstrate that an intact *MEU1* ORF was required for the *trans*-activation phenotype. A high copy-number plasmid containing an allele

TABLE 3

Effect of *MEU1* gene dosage on *ADH2* expression

Plasmids[d]	ADHII activity in mU/mg[a]			
	UAS2/*ADH2*[b]		$(UAS2)_2$/*ADH2*[c]	
	r	dr	r	dr
None	20	80	50	170
Clone 74	50	220	280	760
p2μm*MEU1*Δ*Bst*BI	20	80	40	130
p2μm*MEU1*RI/PstI	50	200	ND	ND
p2μm*MEU1*	60	280	250	680
pCEN-*MEU1*	30	110	90	270

[a] ADHII activity from adh-null strain MDY10 transformed with reporter genes (*b*, *c*) and plasmids (*d*) indicated. All values represent the mean of at least three transformants, and the standard error of the mean was in all cases <12% of the mean. r and dr represent values from cultures grown in the presence or absence of glucose, respectively.

[b,c] These reporters were derived from pRS314-II that contains the *ADH2* basal promoter and structural sequences but lacks any UAS. UAS2/*ADH2* (pRSU2-3) contains a single synthetic UAS2 element. $(UAS2)_2$/*ADH2* (pRSU2-10) contains two synthetic UAS2 elements.

[d] Plasmids containing *MEU1:* None indicates a strain transformed with the 2 μm base plasmid lacking any *MEU1* sequences; Clone 74 is the *MEU1*-containing plasmid isolated during the selection; the Δ*Bst*BI contains a *MEU1* allele with a frame-shift mutation disrupting the ORF; the RI/*Pst*I construct contains a smaller 2.3-kb *Eco*RI/*Pst*I subclone from clone 74 containing *MEU1;* p2μm*MEU1* contains a 5.5-kb *Hin*dIII subclone of clone 74 containing *MEU1;* pCEN-*MEU1* is a CEN6-containing construct carrying the 5.5-kb *Hin*dIII *MEU1* fragment from clone 74.

of *MEU1* with a frame-shift mutation at the codon for putative amino acid 29, p2μm-*MEU1*Δ*Bst*BI, was introduced into yeast strains containing UAS2-dependent reporters. The frame shift mutation was predicted to result in termination of translation six codons from the mutation site due to multiple stop codons. Strains containing p2μm-*MEU1*Δ*Bst*BI and UAS2-dependent reporters showed no increase in *ADH2* expression over strains containing the reporter genes alone (Table 3). In the same experiment an intact *MEU1* allele was able to activate UAS2-dependent reporters to the same degree as clone 74. This result demonstrates that the clone 74 ORF represents a new gene, *MEU1,* and that it is this gene and not some other feature of the clone 74 plasmid that is required for increased *trans*-activation of UAS2-dependent gene expression.

To determine whether homologues of *MEU1* exist, which could provide clues as to *MEU1* function, a homology search of the sequence databases was performed. A 17-amino acid sequence in the N-terminal region of Meu1p is 93% identical to the N-terminal portion of an uncharacterized protein, the product of ORF2 in the *PetC* region of the *Rhodospirillum rubrum* genome (Majewski and Trebst 1990). Meu1p and the ORF2 protein share an additional 60% identitiy through a 70-amino acid region (Meu1p amino acids 57–127 and amino acids 46–116 of the ORF2 prodict). Meu1p also shares 55% homology with a region of *p*urine *n*ucleoside *p*hosphorylases, PNP. The region of PNP that is homologous to Meu1p is conserved in PNPs from humans, mouse, and *Bacillus subtilis;* no function has been ascribed to this region of PNP (Jonsson *et al.* 1991). No other significant homologies to genes or proteins of known function were identified. Further computer-aided analysis of *MEU1* failed to find any functional motifs, such as DNA-binding domains. However, three repeats of a 7-bp motif found in the promoters of the *SUC2* and *MAL* genes (Sarokin and Carlson 1985; Hohmann and Gozalbo 1988), which like *ADH2* are subject to catabolite repression, were identified in the 5′ region of *MEU1.* This 7-bp motif is not repeated elswere within a 7.8-kb DNA sequence (Saville *et al.* 1995) containing *MEU1,* suggesting it is likely functionally relevant to *MEU1* expression.

MEU1 was localized to chromosome *XII* during the initial characterization of plasmids isolated by this selection. This localization was confirmed by hybridization of *MEU1* probes to the grid of primary yeast genomic fragments in phage λ provided by Olsen (Riles *et al.* 1993). *MEU1*-specific probes hybridized to λ clone 6234 that has been localized to chromosome *XII* (Riles *et al.* 1993). Clone 6234 also contains the *PPR1* gene (Kramerer *et al.* 1984; Lilielund *et al.* 1984; Riles *et al.* 1993). These results are in agreement with the DNA sequence submitted to GenBank by Saville *et al.* (1995).

***MEU1* transcription is not glucose regulated:** Northern blot analysis of *MEU1* transcription was done to estimate the mRNA size and to determine whether transcription was glucose-regulated. RNA was extracted from repressed and derepressed cultures of MC71-18Ba. Northern blots analyzed with either a *MEU1* cDNA probe or a larger clone 74 probe showed a single mRNA species with the same M_r, providing further evidence that *MEU1* is the only transcribed region on the genomic fragment. The size of the transcript was consistent with that of the *MEU1* coding region. The presence of the *MEU1* transcript in approximately equal quantities in RNA isolated from repressed and derepressed cultures indicated that the *MEU1* transcript is not glucose-regulated (data not shown).

***MEU1* is not an essential gene:** The effect of null alleles of *MEU1* on various phenotypes was examined. Null chromosomal alleles of *MEU1* were made by one-step gene disruption in a diploid using two constructs in which most or all of the *MEU1* coding sequences were replaced with a DNA fragment containing the *LEU2* gene. These results demonstrated that *MEU1* was not essential for cell viability. Sporulation of diploids heterozygous for the deleted *MEU1* alleles always yielded four viable spores, two of which contained only the deleted allele. Growth rates of *MEU1*Δ::*LEU2* strains on plates containing glucose, ethanol, or glyc-

TABLE 4

Effect of *MEU1* deletion on expression of *ADH2* and reporter genes

	ADHII activity[a]			
	ADH2[b]		(UAS2)/*ADH2*[c]	$(UAS2)_2$/*ADH2*[d]
Strains[e]	r[f]	dr[g]	r	r
5-5A (*meu1ΔI*)	<0.5	770	20	70
5-5B (*MEU1*)	<0.5	1,500	60	90
4-10A (*meu1ΔE*)	<0.5	750	20	50
4-10B (*MEU1*)	<0.5	1,300	30	90

[a] ADHII activity in mU/mg. All values represent the mean of at least three independent transformants. In all cases the standard error of the mean was <12% of the value cited.

[b] *ADH2* represents the chromosomal *ADH2* gene of MC71-18B.

[c] (UAS2)/*ADH2* is a construct (pRSU2-3) in which the *ADH2* gene is driven from its basal promoter by a single UAS2 element.

[d] (UAS2)2/*ADH2* is analogous to pRSU2-3 except that it contains two UAS2 elements (pRSU2-10).

[e] Congenic strains resulting from the dissection of ascii derived from sporulation of MC71-18B**a**/α *MEU1/meu1Δ* strains. *MEU1* designates haploid progeny with a wild-type allele. *meu1ΔI* designates a strain carrying the *MEU1* internal disruption allele. *meu1ΔE* designates a strain containing the *MEU1* external disruption allele.

[f] Repressed growth conditions.

[g] Derepressed growth conditions.

erol at several temperatures (25°, 30°, 33°, and 37°) were not different from that of their wild-type siblings. Likewise, *Meu1Δ* strains appeared normal microscopically.

***MEU1* affects *ADH2* expression:** The effect of deleting *MEU1* on chromosomal *ADH2* expression and on the expression of UAS2-dependent reporter genes was measured in repressed and derepressed cultures. Disruption of *MEU1* reduced *ADH2* expression under derepressing growth conditions about twofold, and there was no effect on repressed ADH2 activity (Table 4). ADH activity derived from two UAS2/*ADH2* reporter genes was also decreased in the absence of *MEU1*. The relatively small effect of *meu1Δ* on *ADH2* expression and that of UAS2/*ADH2* reporter genes suggested that the cell may contain another gene capable of compensating for the loss of *MEU1*. Low-stringency Southern blots were carried out to identify DNA sequences related to *MEU1*. No sequences related to *MEU1* were identified by this method (data not shown). Furthermore, no related yeast genes were identified by search of the yeast genome sequencing project databases.

It is possible that a gene exists in yeast that can compensate for loss of *MEU1* function but is not structurally homologous to *MEU1*. A candidate for such a gene is *CAT8*, a gene required for growth on gluconeogenic carbon sources such as ethanol, glycerol, and lactate (HEDGES *et al.* 1995). Since *ADH2* encodes ADHII, the enzyme catalyzing the first step in gluconeogenesis starting with ethanol as a carbon source, *CAT8* would be a logical candidate for regulating *ADH2* expression as it regulates the expression of several other gluconeogenic genes, such as *FBP1* and *ICL1*. Cat8p is a zinc cluster protein that activates FBP1 expession through two UAS elements in the *FBP1* promoter. The *FBP1* UASs are similar in sequence to UAS2 (NIEDERACHER *et al.* 1992). In particular *FBP1*-UAS1 contains a sequence, **CGG**A-CAC**CCG,** closely related to a sequence in UAS2, **CGG**AAC**ACCG.** This sequence is particularly noteworthy because it contains a CCG inverted repeat that is the common binding motif for C6-Zn cluster proteins such as *GAL4, PPR1,* and *PUT3.*

To test the effect of a *CAT8* mutation on *ADH2* expression, a deletion/disruption allele of *CAT8* was constructed and used to inactivate *CAT8* in strain MC71-18B. As expected the strain containing a disrupted *CAT8* gene grew poorly on gluconeogenic carbon sources. However, ADH enzyme assays revealed that the mutation had no effect on *ADH2* expression. When UAS1 and UAS2 reporters were tested, the *CAT8* mutation reduced activity of both equally, about twofold. We attribute this modest effect to the decreased growth rate caused by the mutant allele, rather than to a specific effect on gene expression. Derepression of *ADH2* and the expression of a UAS2-dependent reporter gene was also tested in a *cat8D meu1Δ* strain. *ADH2* derepression was not reduced in this *cat8Δ meu1Δ* background, compared to strains containing *MEU1* or *CAT8* deletions, respectively (data not shown). Thus, there is no evidence that *MEU1* and *CAT8* act in functionally equivalent but separate pathways of *ADH2* expression.

Increased *MEU1* gene dosage activates UAS2-dependent gene expression: The relationship between *MEU1* gene dosage and UAS2-dependent reporter gene activation was tested by inserting *MEU1* into related CEN- and 2μm-based plasmids. The effect of these plasmids on UAS2-dependent reporter gene expression was then tested in a strain containing the wild-type MEU2 gene. Significantly reduced levels of reporter gene expression were observed in strains containing pCEN-*MEU1* compared to those containing p2μm-*MEU1* (Table 3). In a reporter containing a single UAS2 element, no significant increase in expression was observed in strains containing pCEN-*MEU1* compared to the reporter gene alone. In the presence of pCEN-*MEU1,* expression from a reporter containing two copies of UAS2 increased about twofold. Two copies of UAS2 in the reporter enhanced expression about fivefold compared to a single copy of UAS2 in the presence of multicopy *MEU1*. Thus, reporter gene activation is sensitive to both the level of *MEU1* expression and to UAS2 copy number in the promoter.

The high copy-number *MEU1* plasmid, p2μm-*MEU1,* was introduced into a yeast strain containing the *cre2-1* allele. The *cre2-1* mutation bypasses glucose repression of *ADH2* in an *ADR1*-independent manner (DENIS 1984). Overexpression of *MEU1* suppressed the *cre2-1*

TABLE 5

Activity of a bifunctional Meu1p/β-gal fusion protein

Strains[c]	β-galactosidase activity[a]		Repressed ADHII activity[b]		
	r	dr	No UAS/ADH2	UAS2/ADH2	$(UAS2)_2$/ADH2
MC71-18B	<0.5	<0.5	<5	20	60
MC71-18B + p*MEU1Z*	20	20	<5	60	190

[a] β-galactosidase activity of strains (c) in Miller Units. r denotes cultures grown under repressing conditions (glucose) while dr denoted cultures grown under derepressing conditions. All values represent the mean value of at least three independent transformants. In all cases the standard error of the mean was <10%.

[b] ADHII activity, in mU/mg, from strains (c) transformed with reporter genes No UAS/*ADH2* (pRS314-II), UAS2/*ADH2* (pRSU2-3) or $(UAS2)_2$/*ADH2* (pRSU2-10). The reporters all contain the ADH2 basal promoter and structural gene. The No UAS construct has no UAS element, UAS2/*ADH2* contains a single synthetic UAS2 element, and $(UAS2)_2$/*ADH2* contains two copies of UAS2. All values represent the mean value of at least three independent transformants. In all cases the standard error of the mean was <12%.

[c] Yeast strains used in this experiment. MC71-18B denotes strain MC71-18B into which a vector lacking the *MEU1/lacZ* fusion gene was introduced. MC71-18B + pMEU1Z contains the *MEU1/lacZ* fusion gene. These strains were cotransformed with the ADH2-containing reporter indicated (No UAS/*ADH2*, UAS2/*ADH2*, or $(UAS2)_2$/*ADH2*).

phenotype, in that *cre2-1* strains overexpressing *MEU1* lost their ability to grow on media containing At. Conversely, overexpression of *SPT6/CRE2* yielded At^r transformants in both *CRE2 MEU1* and *CRE2 meu1Δ* strains (data not shown). Thus, although MEU1 was isolated as an activator of UAS2 expression in the absence of other *ADH2* promoter elements, its ability to suppress *cre2-1* suggests that it can affect *ADH2* expression as well. This conclusion is consistent with the observation that the *MEU1* deletion allele reduced *ADH2* expression.

The relationship of *MEU1* and *SPT6/CRE2* was investigated further by crossing a *meu1Δ* allele into the *cre2-1* background. A diploid strain (*meuΔ/MEU1 CRE2/cre2-1*) was sporulated, and the progeny were scored for growth on leu^- and At-containing media. Several tetratype tetrads were identified; that is, tetrads containing spores of the following phenotypes: Leu^+At^s, Leu^+At^r, Leu^-At^s, and Leu^-At^r (data not shown). From these results we infer that *MEU1* is not required for bypass of *ADH2* glucose repression due to *cre2-1*.

A *MEU1*p/β-gal fusion protein is distributed throughout the cell and its expression is not glucose-regulated: The subcellular localization of Meu1p might provide useful information as to its site of action. As a surrogate for the wild-type protein a plasmid containing a *MEU1-lacZ* gene fusion was made in which 1100 bp of 5′ untranslated DNA and sequences encoding the amino-terminal 334 amino acids of Meu1p were fused in-frame to *lacZ*.

Expression of the fusion protein was examined before immunolocalization by assaying levels of β-galactosidase in yeast containing p*MEU1lacZ* (Table 5). The level of β-galactosidase activity expressed under repressing or derepressing conditions did not change. Thus, like *MEU1* mRNA levels, expression of the fusion protein is not strongly glucose-regulated. The Meu1p moiety of the fusion protein was also apparently functional. Expression of UAS2-dependent reporter genes containing one or two copies of UAS2 were elevated in strains containing p*MEU1lacZ* (Table 5).

Indirect immunofluorescence using anti-serum directed against β-galactosidase was used to localize the fusion protein in yeast cells. The fusion protein was found throughout the cell in both repressing and derepressing growth conditions (Figure 4). Appropriate control experiments indicated that the lack of specific localization of Meu1p/β-gal was not an artifact of the primary or secondary antibodies used. In the same experiment, an Adr1p/β-gal fusion protein was shown to be localized to the nucleus and an ADHII/β-gal fusion protein was shown to reside throughout the cell. These results suggest that Meu1p functions in the cytoplasm or at least is not restricted to the nucleus.

DISCUSSION

UAS2 confers *ADR1*-independent, glucose-regulated expression to reporter genes (Yu *et al.* 1989). The identity of a putative UAS2-binding factor, or other proteins involved in a UAS2-dependent activation pathway, is unknown. However, genes that affect *ADH2* expression in a UAS1- or *ADR1*-independent manner, such as *CRE1, CRE2,* and *CCR4* (Denis 1984; Denis and Malvar 1990; Denis and Audino 1991), could conceivable function via UAS2.

The results presented here suggest that one class of SPT genes may function through UAS2. The SPT genes were identified by mutations that suppressed transcriptional defects caused by transposon insertions at the *HIS4* locus. One of the SPT genes, *SPT6,* is allelic to *CRE2* and to *SSN20.* A mutation in *CRE2* allows *ADR1*-independent activation of *ADH2* expression during glucose repression and suppresses the inhibitory effect of a delta insertion in the *ADH2* promoter (Denis 1984; Denis and Malvar 1990).

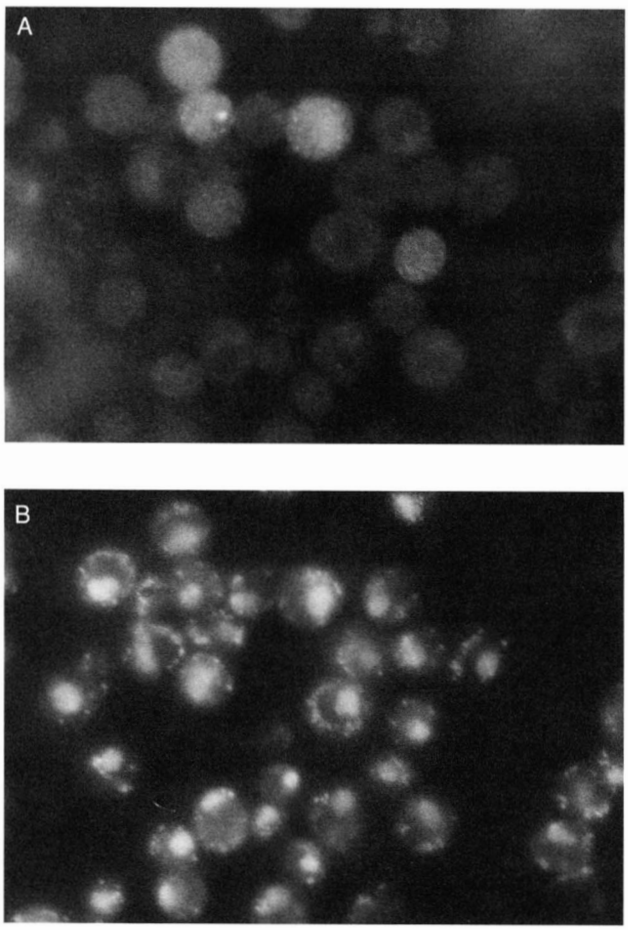

FIGURE 4.—Results of indirect immunofluorescense detection of the Meu1p/β-gal fusion protein. The top row of cells shows fluorescence from the FITC-conjugated secondary antibody. The bottom row shows fluorescence from DAPI, which was used to indicate the position of the nucleus. Column 1 shows cells containing p*MEU1lacZ* that were incubated with both the primary (mouse-α-β-gal) antibody and the secondary (FITC-conjugated goat-α-mouse) IgG. These cells were cultured under repressing conditions (no change in FITC staining was seen in cells grown under derepressing conditions, data not shown). Column 2 shows cells containing p*MEU1lacZ* that were incubated with only with the FITC-conjugated goat-α-mouse IgG. No fluorescence was seen in p*MEU1lacZ*-containing cells incubated with only the primary mouse-α-β-Gal IgG or in a strain containing p2μm-*MEU1* (no *lacZ*) cells incubated with both the primary and secondary antibodies (data not shown). Column 3 shows fluorescence from cells containing an ADHII/β-gal fusion protein known to be localized to the cytoplasm. Column 4 shows fluorescence from cells containing a Adr1p/β-gal fusion protein that was localized to the nucleus.

The spt⁻ phenotype in general can be characterized as increased transcription of inactivated genes (genes containing Ty insertions), activation in the absence of a *trans*-activator (expression of *SUC2* in a snf2/swi2 strain or *ADH2* in an *adr1*strain), and activation under normally repressing conditions (*ADH2* or SUC2 expression in the presence of glucose). Based on these phenotypes it was initially proposed that the SPT genes represented general repressors of transcription. For the "histone" class of SPT genes, which includes *SPT4, SPT5, SPT6,* and *SPT11-12* (encoding H2A and H2B), this possibility remains open (BEMIS and DENIS 1988; WINSTON and CARLSON 1992). One possibility is that these genes encode components of a complex that is the target of the swi/snf complex (WINSTON and CARLSON 1992; WINSTON 1992) that is needed to activate transcription of a large number of genes, including *ADH2.*

Altering the gene dosage of the histone class of SPT genes results in an spt⁻ phenotype (CLARK-ADAMS and WINSTON 1987, 1988; WINSTON 1992). The data presented here show that increased dosage of *SPT6* allowed a weak, strain- and *ADR1*-dependent bypass of glucose repression of *ADH2* expression, *ADR1*-independent derepression of *ADH2* expression, and increased expression of UAS2-dependent reporter genes. Overexpression of *SPT6* did not affect UAS1-dependent reporters nor UAS-independent reporters. A mutation in *CRE2/SPT6* allows *ADR1*-independent bypass of glucose repression of *ADH2* expression (DENIS 1984) and elevated expression of UAS2- but not UAS1-dependent reporter genes (data not shown). In the earlier work that showed allelism between *CRE2* and *SPT6* (DENIS and MALVAR 1990), extra copies of *SPT6* were not observed to cause increased expression of *ADH2* on glucose medium. The data in Table 2 show that the constitutive *ADH2* phenotype caused by the overexpression of *SPT6* is strain-dependent. The *ADR1*-dependence of the bypass of glucose repression by overexpression of *SPT6* suggests that the activation of *ADH2* expression by the *CRE* and *CCR4* genes, on the one hand (DENIS 1984; DENIS and MALVAR 1990), and *ADR1* on the other, may contain overlapping components. However, the *ADR1*-independent derepression caused by overexpression of *SPT6* indicates that, as with the effect of the *cre2-1* allele, *CRE/CCR4* activation can be independent of *ADR1.*

UAS2 is an activating sequence whereas the proposed Spt complex functions to repress gene expression. Thus, if UAS2 is a target for the SPT complex, it must be an indirect one, otherwise UAS2 deletion from the promoter would enhance expression. One general class of possibilities is that the putative *trans*-activator that binds UAS2 is especially sensitive to repression mediated by this complex. Alterations in the proposed Spt complex caused by mutation or overexpression might allow a more open chromatin conformation and better binding by the putative UAS2 factor. Adr1p, and hence UAS1, might not be influenced by these effects if its binding were independent of the Spt complex, as suggested by the normal regulation of UAS1-dependent reporter gene expression in the presence of multi-copy *SPT6.* It is also possible that UAS2 is not a classical UAS element. For example, it may not be the binding site for a transactivating protein. Instead, it could affect nucleosome positioning or some other aspect of chromatin function that facillitated transcription. UAS2 might be an especially sensi-

tive monitor of the function of SPT genes if it directly influenced chromatin structure.

It is also possible that *SPT6* is not involved in a UAS2-dependent activation pathway. For example, overexpression of *SPT6* or mutations in *SPT6* could enhance basal transcription. Mutations in the *MOT1* gene appear to enhance basal transcription by allowing binding of TBP to promoters in the absence of an activator (DAVIS *et al.* 1994; POON *et al.* 1994). In principle, *SPT6* could act the same way at the *ADH2* promoter. However, if this were the case, the UAS-less *ADH2* reporter and UAS1/*ADH2* should have been responsive to extra copies of *SPT6* and they were not. Thus, the phenotype created by extra copies of *SPT6* appears to be UAS-dependent and selective for a particular UAS sequence.

A second multicopy activator of UAS2-dependent transcription was studied in detail. A previously unknown gene named *MEU1* for *M*ulticopy *E*nhancer of *U*AS2 was found to activate UAS2-containing reporter genes in a manner similar to *SPT6* when overexpressed. The *MEU1* ORF was necessary for this effect. Unlike *SPT6,* overexpression of *MEU1* did not alter expression of the chromosomal copy of *ADH2.* Also, unlike mutations in *SPT6/CRE2,* mutations in *MEU1* did not cause constitutive expression of *ADH2.* Overexpression of *MEU1* suppressed loss of glucose repression at *ADH2* due to the *cre2-1* allele, suggestive of some form of interaction, most likely indirect, between these pathways. However, *a cre2-1 meu1*Δ strain remained At^r, suggesting that *MEU1* does not act downstream of *CRE2* or *CCR4* (DENIS 1984).

A null allele of *MEU1* reduced *ADH2* derepression about twofold. Also, the *MEU1* null alleles did not dramatically reduce expression of UAS2-dependent reporter gene expression. This result suggested that other genes within the cell could compensate for the loss of *MEU1.* No structural homologues of *MEU1* were identified by low-stringency hybridization. However, this experiment does not rule out the existance of a functional homologue of *MEU1*p that lacks similarity to *MEU1* at the nucleic acid level.

CAT8 seemed a likely candidate for an activator of *ADH2* expression acting through UAS2. As with other targets of *CAT8, ADH2* encodes a gluconeogenic enzyme. More specifically, UAS2 shares sequence similarity with a *FBP1* UAS element through which *CAT8* exerts its activating influence (NIEDERACHER *et al.* 1992). However, disruption of *CAT8* had no effect on *ADH2* expression, and the mutation had a modest nonspecific effect on reporter gene activity. Thus, if *CAT8* plays a role in *ADH2* gene expression, it must be a minor one, or there is a functional homologue of *CAT8* that masks its role at the *ADH2* locus in the strain lacking *CAT8* activity.

The *meu1*Δ strains did not grow significantly slower than their wild-type siblings when tested on several carbon sources (glucose, galactose, sucrose, raffinose, glycerol, or ethanol) or at different temperatures (35–25°), and the deletion strains had no new amino acid auxotrophies. Neither the regulation or localization of a Meu1/β-galactosidase fusion protein, nor the deduced sequence of the conceptual translation product provided any clues as to the function of the *MEU1* gene.

Neither *SPT6* nor *MEU1* appear to encode a DNA binding protein that acts through UAS2. If such a protein exists, it might be encoded in the DNA sequence of one of the other UAS2-dependent activators that were identified in the screen depicted in Figure 2.

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