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Carbon Source Dependence of Transposable Element-Associated Gene Activation in *Saccharomyces cerevisiae*

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Seven *cis*-dominant mutations leading to the overproduction of the glucose-repressible alcohol dehydrogenase isozyme ADHII (structural gene, *ADH2*) in *Saccharomyces cerevisiae* have previously been shown to be due to insertion of a transposable element, Ty, in the 5′ regulatory region of the *ADH2* gene. We showed that although mating-competent cells (**a**, α, **a/a**, or α/α cells) overproduced both ADHII enzyme and *ADH2* mRNA, mating-incompetent cells (**a/α** or ste$^-$ cells) produced much less ADHII enzyme and *ADH2* mRNA. This mating type effect on *ADH2* expression was greatest in the presence of a normally derepressing carbon source, glycerol, and much less apparent in the presence of a repressing carbon source, glucose. In addition, Ty insertion led to an aberrant carbon source response in mating-incompetent cells—the normally glucose-repressible ADHII becomes glycerol repressible. The mating type effect and aberrant carbon source response in mating-incompetent cells was specific for Ty-associated mutations in the 5′ flanking region of the *ADH2* gene in that a non-Ty mutation in the same region did not show these effects. Finally, Ty1 RNA levels also showed **a/α** suppression, which was apparent only during growth on a nonfermentable carbon source such as glycerol. This suggests that Ty-mediated gene expression is subject to regulation by both mating competence and carbon catabolites.

Wiame and co-workers isolated and studied overproducing mutants of *Saccharomyces cerevisiae* that were affected in arginine catabolism and urea utilization (7, 8, 14). The *cargA*$^+$*O*^{h}, *cargB*$^+$*O*^{h}, and *durO*^{h} mutations led to 40- to 550-fold overproduction of arginase, ornithine transaminase, and the urea carboxylase-allophanate hydrolase enzyme complex, respectively, in haploid (**a** or α) and mating type-homozygous diploid (**a/a** or α/α) cells. However, the overproduction induced by these mutations was much diminished in mating type-heterozygous (**a/α**) diploid cells. This unexpected suppression of constitutive overproduction in *MAT* heterozygous strains has been called the mating type effect or **a/α** suppression.

Subsequently, a similar mating type-regulated mutation, *CYC7-H2*, leading to 20-fold overproduction of iso-2-cytochrome *c*, was isolated by Rothstein and Sherman (22). Cloning studies showed that the *CYC7-H2* mutation was due to the insertion of a moderately repetitive transposable element, Ty1, in the 5′ region of the gene coding for iso-2-cytochrome *c* (10, 11). The *cargA*$^+$*O*^{h} mutant strain was also shown to contain the insertion of a Ty element in the *cargA* region (13). Errede and co-workers (10) showed that the overproducing phenotype was independent of sporulation functions, but some positive correlation with mating competence was demonstrated. Thus, haploids (**a** or α) and diploids homozygous at their *MAT* loci (**a/a** or α/α) were able to mate and showed Ty-mediated overproduction. However, diploids heterozygous at their *MAT* loci (**a/α**) and haploids containing some sterile mutations (*ste*) could not mate and showed significantly decreased overproduction. Errede and co-workers proposed the acronym ROAM (regulated overproducing alleles responding to mating signals) for the class of mutations represented by *cargA*$^+$*O*^{h}, *cargB*$^+$*O*^{h}, *durO*^{h}, and *CYC7-H2*. In addition, these workers suggested that Ty elements may resemble regulatory regions adjacent to some mating functions and that Ty elements throughout the genome are able to respond to mating-specific signals present only in mating-competent cells.

The present work deals with ADHII, an alcohol dehydrogenase isozyme of *S. cerevisiae*. This isozyme is glucose repressible and hence is thought to function in the utilization of ethanol. Its structural gene has been designated *ADH2* (formerly *ADR2*, see Table 1 for other changes in nomenclature). Nine *cis*-dominant mutations leading to overproduction of ADHII have been localized to the 5′ noncoding region of the *ADH2* gene (22a, 27). Two of these *ADH2*^{c} mutations, *ADH2-4*^{c} and *ADH2-5*^{c}, contain a polyadenylate tract 5′ to the ADHII coding sequences which has been elongated from the 20 adenines found in the wild-type strain (23) to 55 and 54 adenines, respectively (22a). The remaining seven *ADH2*^{c} mutations, *ADH2-1*^{c}, *-2*^{c}, *-3*^{c}, *-6*^{c}, *-7*^{c}, *-8*^{c}, and *-9*^{c}, contain the insertion of a transposable element, either Ty1 or Ty2, at different positions in the 5′ region of the *ADH2* gene (27). We observed a novel carbon source dependence of **a/α** suppression in the presence of each of the different Ty-associated *ADH2*^{c} mutations, but not in strains containing a non-Ty *ADH2*^{c} mutation.

MATERIALS AND METHODS

Strains. Since the nomenclature in the ADH system has become unnecessarily cumbersome, we are attempting to simplify the gene designations as shown in Table 1. In addition, the former *ADR3* locus, which is a *cis*-linked regulatory region of the ADHII-coding gene, will be designated as part of the *ADH2* gene along with the structural sequences (e.g., the former *ADR3-6*^{c} mutation which contains the insertion of a Ty1 element in the 5′ regulatory region of the ADHII-coding gene will be called the *ADH2-6*^{c} mutation). The *ADR1*, *ADR4*, *ccr1*, *ccr2*, *ccr3*, and *ccr80* loci will maintain their previous gene designations.

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TABLE 1. Revised ADH nomenclature

ADH isozyme	Former enzyme designation	Former gene designation	New enzyme designation	New gene designation
Fermentative ADH	ADHI	*ADC1*	ADHI	*ADH1*
Oxidative ADH	ADHII	*ADR2*	ADHII	*ADH2*
Mitochondrial ADH	mADH	*ADM*	ADHIII	*ADH3*

All yeast strains used in this study were defective in fermentative alcohol dehydrogenase (ADHI) and mitochondrial alcohol dehydrogenase (ADHIII) activities due to *adh1-11* and *adh3* mutations, respectively. Strains 43-2b (α *his4 ura1 ADR1 ADH2-F*) and 11-13c (**a** *ade2-119 trp2 ADR1 ADH2-S*) and their derivatives were used as wild-type strains. *ADH2^c* mutant strains used were those described previously (3, 4) and progeny from their matings with the wild-type strains. **a/a** and α/α strains were derived from **a**/α strains by UV irradiation and isolation of mating-competent colonies.

All ste$^-$ mutant strains showed temperature-sensitive mating such that mating occurred at room temperature but not at 34°C. The *ste4-3*, *ste5-4*, *ste7-1*, and *ste11-1* mutant strains had originally been derived from strain 381G (**a** *SUP4-3 cry1 his4-580 trp1 ade2-1 tyr1 lys2*) (12).

Media. Basic yeast medium (YM) containing 1% (wt/vol) yeast extract, 2% (wt/vol) Bacto-Peptone (Difco Laboratories), 0.002% (wt/vol) adenine, and 0.002% (wt/vol) uracil was supplemented with 10% glucose (YM-glucose) for glucose-repressing conditions. Cells were maintained in continuous exponential growth for approximately 20 h at 30°C by two successive transfers into fresh media. Derepressed cells were obtained by growth in YM supplemented with 3% glycerol (YM-glycerol). Cells were grown for approximately 30 h at 30°C with two successive transfers into fresh media.

Alcohol dehydrogenase activity assay. Alcohol dehydrogenase activity at 25.0°C was determined spectrophotometrically by following the kinetics of NAD^+ reduction at 340 nm in the presence of ethanol as previously described (6).

S1 mapping of *ADH2* mRNA levels. S1 mapping was carried out as described by Nasmyth et al. (19). The 330-nucleotide single-stranded DNA probe was complementary to the 5′ end of *ADH2* mRNA. It included 89 nucleotides of pBR322 sequences from the *Sal*I site to the *Sph*I site and extended from an *Sph*I site in the 5′ noncoding region of the *ADH2* gene to an *Eco*RV site 67 nucleotides within the ADHII-coding sequences. The 5′ end of the probe, which was within the *ADH2* structural sequences, was labeled with ^{32}P by a phosphate exchange-kinase reaction (2). The labeled probe was hybridized in DNA excess to 10 μg of total yeast RNA isolated by the method of Schultz (24). The hybrids were treated with 400 U of *Aspergillus oryzae* S1 nuclease (Sigma Chemical Co.) to remove unhybridized DNA sequences followed by NaOH treatment to hydrolyze RNA sequences. The sample was denatured in 90% formamide and run for 3 h on an 8% polyacrylamide sequencing gel (18). The protected ^{32}P-labeled DNA fragments were visualized by autoradiography. Control experiments demonstrated that the amount of protected DNA probe was proportional to the amount of added yeast RNA for a given RNA preparation.

Northern blot hybridization analysis of Ty1 RNA. Northern blot hybridization analysis was carried out as described by Thomas (26). Total yeast RNA (15 μg) prepared by the method of Schultz (24) was electrophoresed through a 1.1% agarose gel and transferred to nitrocellulose. The probe, plasmid pBR322 · 2^{c}TyPst, was constructed by J. Osterman and contained a 0.7-kilobase (kb) *Pst*I-*Pst*I fragment isolated from the cloned *ADH2-2^c* Ty element. The plasmid was nick translated with [^{32}P]dTTP and hybridized to the immobilized RNA. The Ty elements of *ADH2-2^c*, *-6^c*, *-7^c*, and *-8^c* differ from that of *ADH2-3^c* in two major areas of the internal ε section (27). Due to its origin in the 2-kb substitution region, the probe shows marked specificity for the Ty1 (J. Osterman, personal communication) of which the *ADH2-2^c* and *ADH2-6^c* Ty elements are members (26a). RNAs containing sequences homologous to the probe were visualized by autoradiography of the hybridized Northern blot.

RESULTS

a/α suppression in Ty-associated *ADH2^c* mutant strains. a/α suppression of ADHII specific activity was studied in the presence of each of seven Ty-associated *ADH2^c* mutations, *ADH2-1^c*, *-2^c*, *-3^c*, *-6^c*, *-7^c*, *-8^c*, and *-9^c*. An a/α diploid strain and its two parental haploid strains for each *ADH2* allele were grown in continuous log phase in either glucose- or glycerol-containing medium to ensure complete repression or derepression, respectively. The resulting ADHII specific activities are shown in Table 2. Values in Table 2 indicate the level of a/α suppression, defined as the ratio of ADHII specific activity in mating-competent haploid strains to that in mating-incompetent a/α strains of the relevant genotype. All seven sets of Ty-associated *ADH2^c* mutant strains showed a/α suppression with respect to the mating-competent haploid strains. a/α suppression was greatest in the presence of the derepressing carbon source, glycerol, and ranged from about a 10-fold effect with the *ADH2-1^c* mutation to a 70-fold effect with the *ADH2-6^c* mutation. Suppression was much lower under glucose repression, ranging from 1.5- to 2.4-fold a/α suppression, but it was still greater than that of the two sets of control strains. The control wild-type *ADH2* strains showed strong repression of ADHII specific activity in the presence of glucose and derepression of about 160-fold in activity in the presence of glycerol. No a/α suppression was observed. The second set of control strains contained the *ADH2-4^c* allele, which is not due to Ty insertion in the *ADH2* region. The data in Table 2 demonstrate that the control *ADH2-4^c* mutant strains showed no a/α suppression. These strains had partially constitutive levels of ADHII specific activity in the presence of glucose and derepression of about 20-fold in activity in the presence of glycerol.

The ADHII specific activities exhibited by the Ty-associated *ADH2^c* haploid strains in Table 2 showed variable levels of derepression, no derepression, or even repression of ADHII specific activity as the carbon source was shifted from glucose (normally repressing) to glycerol (normally derepressing). In each a/α diploid strain containing Ty-associated *ADH2^c* mutations, but not in the control wild-type *ADH2* or the non-Ty-associated *ADH2-4^c* mutant strains, an anomalous response to carbon source was seen. Growth on the normally derepressing carbon source, glycerol, yielded lower ADHII specific activity than did growth in the normally repressing YM-glucose.

To determine whether a/α suppression of ADHII specific activity was due to diploidy or *MAT* heterozygosity, four *MAT* homozygous strains were selected after UV-induced recombination of an a/α diploid strain homozygous for either *ADH2-4^c*, *ADH2-2^c*, or *ADH2-6^c* mutations. Thus, diploid strains with a given *ADH2^c* allele were isogenic except at their *MAT* loci. The ADHII specific activities of these

TABLE 2. a/α Suppression of ADHII activity with seven Ty-associated *ADH2*^{c} mutations[a]

Genotype		Growth in glucose		Growth in glycerol	
ADH2	Mating type	ADHII sp act (mU/mg of protein)[b]	a/α Suppression[c]	ADHII sp act (mU/mg of protein)[b]	a/α Suppression[c]
+	a or α	8 (8)	1.0×	1,200 (990–1,400)	0.9×
+/+	a/α	8		1,300	
4^c	a or α	140 (140)	1.2×	2,700 (2,100–3,300)	0.8×
$4^c/4^c$	a/α	120		3,300	
1^c	a or α	580 (450–700)	2.4×	830 (750–920)	14×
$1^c/1^c$	a/α	240		61	
2^c	a or α	680 (680)	2.0×	590 (530–650)	16×
$2^c/2^c$	a/α	340		38	
3^c	a or α	790 (770–820)	2.3×	200 (130–270)	18×
$3^c/3^c$	a/α	350		11	
6^c	a or α	570 (470–680)	2.1×	1,100 (1,100–1,200)	65×
$6^c/6^c$	a/α	270		17	
7^c	a or α	410 (390–430)	1.7×	480 (330–630)	34×
$7^c/7^c$	a/α	240		14	
8^c	a or α	230 (230–240)	1.5×	110 (70–150)	37×
$8^c/8^c$	a/α	150		3	
9^c	a or α	510 (500–510)	2.1×	290 (290–300)	41×
$9^c/9^c$	a/α	240		7	

[a] Cells were grown at 30°C in either YM-10% glucose, which normally represses ADHII specific activities, or in YM-3% glycerol, which normally derepresses ADHII specific activities. These strains were maintained in continuous exponential growth for approximately 20 h (YM-10% glucose) or 30 h (YM-3% glycerol) by two successive transfers into fresh media. ADHII specific activity was assayed at 25°C as previously described (6). Data were obtained by testing one or two strains of the relevant genotype with one or two trials. The mean ADHII specific activity is shown above.

[b] The values in parentheses indicate the range of values obtained with different strains.

[c] The ratio of ADHII specific activities in mating-competent strains to those in mating-incompetent a/α strains.

diploid strains as well as those of the original haploid parental strains are presented in Table 3. Diploidy in the absence of *MAT* heterozygosity did not affect the ADHII specific activities of these strains. However, as reported in Table 2, *MAT* heterozygosity did lead to decreased ADHII specific activities in strains containing the Ty-associated *ADH2*^{c} mutations *ADH2-2*^{c} and *ADH2-6*^{c}. Again, this a/α suppression appeared greatest upon growth on the derepressing carbon source, glycerol, with a 20-fold effect in *ADH2-2*^{c} mutant strains and a 70-fold effect in *ADH2-6*^{c} mutant strains. Growth of these two sets of strains in the presence of glucose yielded a greatly reduced effect of *MAT* heterozygosity. As expected for the control *ADH2-4*^{c} mutant strains (see Table 2), ADHII specific activities were quite similar in **a**, α, **a/a**, α/α, and a/α strains, irrespective of the state of the *MAT* alleles.

***ste*-induced suppression of ADHII activity in Ty-associated *ADH2*^{c} mutant strains.** *ste* mutations lead to a loss of mating ability in haploid strains much as does the a/α diploid state (12). Errede and co-workers (10, 11) observed that some *ste* mutations also mimic the a/α state by leading to decreased activity of four Ty-associated genes, *CYC7-H2*, *cargA*$^+$*O*^{h}, *cargB*$^+$*O*^{h}, and *durO*^{h}.

To determine whether *ste*-induced suppression of ADHII specific activities in Ty-associated *ADH2*^{c} mutant strains occurs, as does a/α suppression, strains were constructed that contained the *ADH2-6*^{c} mutation and a temperature-sensitive allele of the *ste4*, *ste5*, *ste7*, or *ste11* mutations. Table 4 presents the ADHII specific activities of these *ste ADH2-6*^{c} mutant haploid strains as well as of *STE ADH2-6*^{c} mutant haploid strains after growth at the restrictive temperature, 34°C. Shown in Table 4 are measures of the

TABLE 3. a/α Suppression of ADHII activity in *ADH2-2*^{c} and *ADH2-6*^{c} mutant strains[a]

Genotype		Growth in glucose		Growth in glycerol	
ADH2	Mating type	ADHII sp act (mU/mg of protein)	a/α Suppression	ADHII sp act (mU/mg of protein)	a/α Suppression
4^c	a or α	140 (140)	1.2×	2,700 (2,100–3,300)	0.8×
$4^c/4^c$	a/a or α/α	130 (110–160)	1.1×	2,600 (1,400–3,900)	0.8×
$4^c/4^c$	a/α	120		3,300	
2^c	a or α	680 (680)	2.0×	590 (530–650)	16×
$2^c/2^c$	a/a or α/α	500 (480–530)	1.5×	930 (850–1,100)	24×
$2^c/2^c$	a/α	340		38	
6^c	a or α	570 (470–680)	2.1×	1,100 (1,100–1,200)	65×
$6^c/6^c$	a/a or α/α	390 (280–480)	1.4×	1,300 (940–1,700)	76×
$6^c/6^c$	a/α	270		17	

[a] One to four strains of the relevant genotype were tested in one or two trials. See the footnotes to Table 2 for more details.

TABLE 4. *ste*-induced suppression of ADHII activity in *ADH2-6*^{c} mutant strains[a]

Genotype	Growth in glucose		Growth in glycerol	
	ADHII sp act (mU/mg of protein) ± SD[b]	*ste*-induced suppression[c]	ADHII sp act (mU/mg of protein) ± SD[b]	*ste*-induced suppression[c]
STE4 ADH2-6^c	1,200 ± 170 (1,100–1,500)	1.5×	1,400 ± 470 (630–2,000)	3.3×
ste4 ADH2-6^c	790 ± 180 (580–970)		420 ± 110 (280–580)	
STE5 ADH2-6^c	820 ± 79 (740–920)	1.5×	1,400 ± 930 (130–2,700)	2.4×
ste5 ADH2-6^c	540 ± 100 (440–680)		580 ± 430 (94–1,200)	
STE7 ADH2-6^c	700 ± 200 (580–1,100)	1.4×	1,700 ± 1,100 (230–2,600)	5.7×
ste7 ADH2-6^c	510 ± 100 (380–630)		300 ± 280 (71–770)	
STE11 ADH2-6^c	1,000 ± 190 (820–1,300)	1.5×	3,500 ± 1,200 (1,300–4,800)	15×
ste11 ADH2-6^c	680 ± 55 (630–730)		240 ± 180 (87–580)	

[a] Cells were grown in continuous log phase at 34°C since all the *ste* mutations were temperature sensitive. Data were obtained from six segregants isogenic for the relevant mutant genes, each with one to seven trials.

[b] ADHII specific activities ± the standard deviation about the mean are given. The values in parentheses indicate the range of values obtained with the different strains.

[c] The ratio of ADHII specific activities in mating-competent *STE ADH2-6*^{c} strains to those in mating-incompetent *ste ADH2-6*^{c} mutant strains.

amount of *ste*-induced suppression, defined as the ratio of ADHII specific activity in mating-competent *STE ADH2-6*^{c} mutant strains to that in mating-incompetent *ste ADH2-6*^{c} mutant strains. *ste*-induced suppression of ADHII specific activity was most evident upon growth in YM-glycerol, ranging from a 2.4-fold effect with *ste5* to a 15-fold effect with *ste11*. The values in parentheses in Table 4 represent the range of ADHII activities seen with six segregants of the relevant genotype. The range of values was quite broad, indicating that *ste*-induced suppression is very sensitive to genetic background as noted by Errede et al. (10). Corresponding control strains containing the non-Ty-associated mutation *ADH2-4*^{c} instead of the Ty-associated *ADH2-6*^{c} allele were also tested (data not shown). With the *ADH2-4*^{c} mutant strains, *ste*-induced suppression was not evident under derepressing conditions. The ratio of ADHII specific activities in *STE ADH2-4*^{c} mutant strains to those in *ste ADH2-4*^{c} mutant strains varied from 1.0- to 1.3-fold.

To determine whether *ste*-induced suppression is less apparent under glucose repression as is **a**/α suppression, *ste ADH2-6*^{c} and *STE ADH2-6*^{c} mutant haploid segregants were grown in the presence of glucose at 34°C. As can be seen in Table 4, ADHII specific activities were suppressed by *ste* mutations to a much lower extent under glucose repression, ranging from a 1.4- to a 1.5-fold effect. The corresponding control *ADH2-4*^{c} mutant strains showed no *ste*-induced suppression (data not shown). The ratio of ADHII specific activities in *STE ADH2-4*^{c} mutant strains to those in *ste ADH2-4*^{c} mutant strains ranged from 0.8 to 1.0.

We noted that although the *STE ADH2*^{c} mutant strains showed some ADHII derepression upon switching from glucose as a carbon source to glycerol, the **a**/α-like *ste ADH2-6*^{c} mutant strains showed an anomalous response to carbon source as did the **a**/α diploid strains studied in the previous section. Some repression of ADHII specific activity occurred upon growth of most of the *ste ADH2-6*^{c} mutant strains in the normally derepressing carbon source, glycerol. No such aberrant behavior was seen with the control *ste ADH2-4*^{c} mutant strains.

Errede et al. (10, 11) have examined the effect of several *ste* mutations as well as other mutations on ROAM gene expression. Our results were in essential agreement with their results. However, although we observed a slight *ste5*-induced suppression of ADHII activity, Errede et al. observed no suppression. Also, although *ste11* induced the highest level of suppression in our hands, Errede et al. observed the greatest suppression with *ste7*. These results could illustrate the magnitude of genetic background effects and suggest that *ste*-induced suppression controls the expression of Ty-associated genes in an indirect manner.

Analysis of *ADH2* mRNA levels in *ADH2-6*^{c} mutant strains. Previously, Williamson et al. (27) demonstrated that ADHII specific activities in both wild-type and Ty-associated *ADH2*^{c} haploid strains are correlated to *ADH2* mRNA levels as seen by S1 mapping analysis. Since we found that the *ADH2-6*^{c} mutant strains exhibited the most pronounced **a**/α suppression of our seven Ty-associated *ADH2*^{c} alleles, these *ADH2-6*^{c} mutant diploid strains were chosen for similar S1 mapping analysis of *ADH2* mRNA levels.

S1 mapping analysis was carried out with a 330-nucleotide single-stranded DNA probe which was complementary to the 5′ end of *ADH2* mRNA. The results of one such experiment are shown in Fig. 1 and indicate that the ADHII specific activity of each strain is proportional to the level of *ADH2* mRNA as measured by quantitative S1 mapping analysis. **a**/α suppression of ADHII specific activity was greatest under glycerol derepression (Table 3). Likewise, the 5′ ends of *ADH2* mRNA as mapped by S1 nuclease analysis underwent a distinct **a**/α suppression in the presence of glycerol (Fig. 1). Although bands corresponding to the 5′ end of *ADH2* mRNA were quite apparent in the **a**/**a** (glycerol) and α/α (glycerol) lanes, no such band was visible in the **a**/α (glycerol) lane. In the presence of glucose, **a**/α suppression of *ADH2* mRNA was not striking.

The results shown in Fig. 1 were quantitated by densitometric scanning of the major band of protected probe and are summarized in Table 5. As seen previously in Table 3, **a**/α suppression of ADHII specific activity was greatest under glycerol-derepressing conditions (about 90-fold). Likewise, *ADH2* mRNA also underwent **a**/α suppression since the protected probe was ≥40-fold more abundant when RNA preparations from mating-competent *MAT* homozygous strains (**a**/**a** and α/α) were compared to that from the mating-incompetent strain (**a**/α). No band is visible in the **a**/α (glycerol) lane of Fig. 1, but densitometric analysis of the autoradiogram yielded a maximum intensity of 2%. S1 mapping analysis indicated that *ADH2* mRNA shows reduced **a**/α suppression in the presence of glucose, consistent

with the reduced a/α suppression of ADHII specific activity in the presence of glucose (Table 3). S1 reactions carried out with RNA isolated from control *ADH2-4^c*/*ADH2-4^c* mutant strains showed no a/α suppression of *ADH2* mRNA (data not shown).

A more detailed examination of the data shown in Fig. 1 reveals an interesting lack of correlation between the level of protected DNA probe and enzyme activity when glucose-repressing conditions are compared to glycerol-derepressing conditions. In the presence of glucose, there was about fourfold more *ADH2* mRNA than one would expect from the glucose-repressed enzyme activities in comparison to that found with glycerol-derepressing conditions. Though increased turnover of ADHII protein in the presence of glucose may play a part, we feel that this inconsistency was probably due to the very different physiological states of yeast cells grown in the presence of the two different carbon sources. Comparison under a light microscope showed that yeast cells were visibly larger in glucose-containing media than in the presence of glycerol. More quantitatively, there was about threefold more total yeast RNA isolated and about fivefold more total protein isolated per cell under glucose-repressing conditions than under conditions of glycerol derepression for the samples subjected to S1 mapping analysis in Fig. 1. Thus, measured ADHII specific activities with total protein as a standard are probably an underestimate of ADHII activity per cell when glucose-repressed strains are studied. On a per-cell basis, the ADHII specific activities shown for glucose growth conditions in Table 2 are probably underestimates by about two- to fourfold. Thus, almost all the strains containing Ty-associated *ADH2^c* mutations, mating-competent as well as a/α strains, have lower ADHII activities when grown in the presence of the normally derepressing carbon source, glycerol, than they have in YM-glucose.

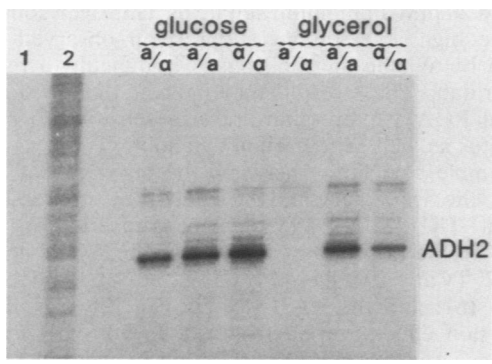

FIG. 1. S1 mapping analysis of RNA from *ADH2-6^c* mutant strains. The 330-nucleotide single-stranded DNA probe was complementary to the 5′ end of *ADH2* mRNA. The 5′ end of this probe was located 67 nucleotides internal to the ADHII-coding sequences. The ^{32}P-labeled probe was hybridized in DNA excess to 10 μg of total yeast RNA. The hybrids were treated with S1 nuclease to remove unhybridized sequences followed by NaOH treatment to hydrolyze RNA sequences. Subsequently, the sample was denatured in 90% formamide and electrophoresed for 3 h through an 8% polyacrylamide sequencing gel. The protected ^{32}P-labeled DNA fragments were visualized in the autoradiogram shown. Lane 1, a no-RNA control. Lane 2, a no-S1 nuclease control, shows several bands due to ^{32}P-induced breakdown of the probe, but almost all of the radioactivity was present at a position corresponding to the full-length probe (not shown). Lanes marked a/α, a/a, and α/α contain RNA from diploid yeast strains homozygous for the *ADH2-6^c* mutation and isogenic except at their mating type loci. Yeast strains were grown in the presence of either glucose or glycerol (Table 1). As noted by Williamson et al. (27), minor transcriptional starts in addition to the major start site may be seen.

TABLE 5. ADHII activity, *ADH2* mRNA, and Ty1 RNA of *ADH2-6^c*/*ADH2-6^c* diploid strains[a]

Diploid	ADHII sp act (mU/mg of protein)	*ADH2* mRNA 5′ ends (%)	Ty1 RNA (%)
Glucose grown			
a/α	360	70	250
a/a	580	140	280
α/α	680	210	210
Glycerol grown			
a/α	12	≤2	10
a/a	1,400	100	100
α/α	870	50	90

[a] All diploid yeast strains were homozygous for the *ADH2-6^c* mutation and isogenic except at their mating type loci as described in the legend to Fig. 1. ADHII specific activities were derived from cell extracts prepared at the time of RNA isolation. *ADH2* mRNA 5′ ends were quantitated by densitometric scanning of the major band corresponding to the protected probe in the autoradiogram presented in Fig. 1. The intensity of the major band appearing with RNA prepared from the glycerol-derepressed a/a strain was assigned a value of 100%. The amount of probe protected by the other RNA samples is expressed as a percentage of this value. Ty1 RNA was quantitated by densitometric scanning of the 5.5-kb major band in the autoradiogram shown in Fig. 2. Again, a value of 100% was assigned to the band appearing with RNA prepared from the glycerol-derepressed a/a strain. A faint band was visible in the a/α (glycerol) lane of the original autoradiogram.

Northern analysis of Ty1 RNA in *ADH2-6^c* mutant strains. Elder et al. (9) have examined all of the Ty RNAs which are homologous to a complete Ty1 element. They observed a 5.7-kb RNA which hybridized to Ty1 DNA and was subject to a/α suppression. To test whether total Ty1 RNA is affected by mating competence and carbon source in the same way as the *ADH2* mRNA of Ty-associated *ADH2^c* mutant strains, we measured the level of Ty1 RNA in the RNA preparations described above by using the Northern blot hybridization technique. The probe used (see above) showed marked specificity for the Ty1 of which the *ADH2-6^c* Ty is a member. The autoradiogram resulting from this Northern hybridization is shown in Fig. 2. The major Ty1 RNA, which we observed to be about 5.5 kb, showed much more apparent a/α suppression in the presence of glycerol than in the presence of glucose. A smaller transcript of about 2.7 kb acted in a coordinate manner.

These results were quantitated by densitometric scanning of the major 5.5-kb Ty1 RNA band (Table 5). A faint band was visible in the a/α (glycerol) lane and gave a relative intensity of 10%. In the presence of glycerol, Ty1 RNAs are about 10-fold more abundant in mating-competent strains than in the mating-incompetent a/α strain. When the strains were grown in glucose-containing media, both mating-competent and mating-incompetent strains showed levels of Ty1 RNA which were similar and a/α suppression of Ty1 RNA was not evident. Thus, a/α suppression of Ty1 RNA is only apparent under glycerol-derepressing conditions.

DISCUSSION

Our results indicate that ADHII expression in the presence of Ty-associated *ADH2^c* mutations is significantly suppressed in cells unable to mate only when grown in the presence of a derepressing carbon source. *MAT* heterozygosity in diploid strains as well as *ste* mutations (*ste4*, *ste5*,

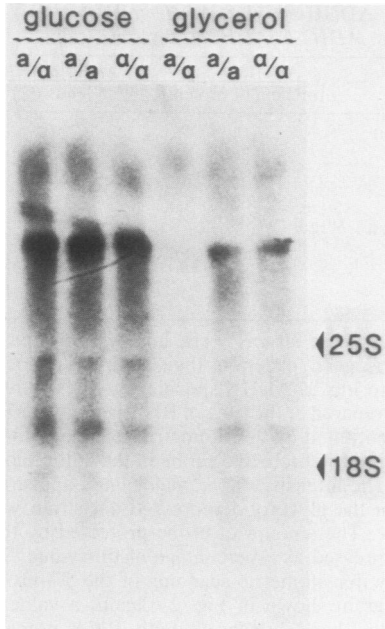

FIG. 2. Northern analysis of Ty1 RNA. Total yeast RNA (15 μg; described in the legend to Fig. 1) was run on a 1.1% agarose gel and transferred to nitrocellulose. The DNA probe has been shown to have marked specificity for the Ty1 element (J. Osterman, unpublished data) of which the *ADH2-6*^{c} Ty element is a member (26a). This probe was nick translated with [^{32}P]dTTP and hybridized to the immobilized RNA. The resulting autoradiogram is shown.

ste7, or *ste11*) in haploid strains led to reduced ADHII activity in the presence of a derepressing carbon source.

S1 mapping analysis of RNA isolated from *ADH2-6*^{c}/*ADH2-6*^{c} mutant diploid strains which are isogenic except at their *MAT* loci showed that in the presence of a derepressing carbon source **a**/α suppression of ADHII specific activity was paralleled by **a**/α suppression of *ADH2* mRNA levels. Examination of total Ty1 RNA by Northern analysis (the *ADH2-6*^{c} Ty element is a Ty1 element) showed that total Ty1 RNA also undergoes a distinct **a**/α suppression only in the presence of a derepressing carbon source. This result indicates that the particular *ADH2-6*^{c} Ty1 element inserted into the regulatory region 5′ to the *ADH2* gene also experiences **a**/α suppression of its transcription as does the *ADH2* gene. Since Ty RNAs in general are regulated by mating competence, whereas ADHII normally is not, suppression of the Ty-associated *ADH2* structural gene must be mediated by the adjacent Ty element.

The sensitivity of mating type regulation to carbon source such that **a**/α suppression and *ste*-induced suppression effects were much greater when cells were grown in the presence of the derepressing carbon source, glycerol, than in the presence of the repressing carbon source, glucose, was unexpected. In *ADH2-6*^{c}/*ADH2-6*^{c} mutant diploid strains, ADHII specific activity and *ADH2* mRNA levels were suppressed in an **a**/α strain relative to **a**/**a** and α/α strains by 90-fold and ≥40-fold, respectively, in the presence of glycerol, but only 2- and 3-fold, respectively, in YM-glucose. *ste* mutations in *ADH2-6*^{c} mutant haploid strains showed 2.4- to 15-fold suppression of ADHII specific activity under glycerol derepression, but only 1.4- to 1.5-fold suppression in YM-glucose.

Two observations indicated that this sensitivity of mating type regulation to carbon source is not simply a result of the general carbon catabolite sensitivity of wild-type *ADH2* expression. First, the suppression seen in mating-incompetent strains was most pronounced in the presence of glycerol. Since mating-competent strains showed variable derepression, no derepression, or even repression as the carbon source was altered from glucose to glycerol, the anomalous response to carbon source exhibited by mating-incompetent strains accounts for the increased **a**/α suppression of ADHII in the presence of glycerol. Mating-incompetent strains with Ty-associated *ADH2*^{c} mutations consistently showed higher ADHII specific activities and *ADH2* mRNA levels in the presence of glucose, normally a repressing carbon source, than in the presence of glycerol, normally a derepressing carbon source. This unusual behavior suggests that Ty insertion into the *ADH2* locus endowed *ADH2* with a new carbon source sensitivity. Second, **a**/α suppression of the RNAs of Ty1 and, by extension, of RNA transcribed from the Ty1 element adjacent to the *ADH2* gene in the *ADH2-6*^{c} mutation were also sensitive to carbon source. As with *ADH2* expression, Ty1 RNAs were more strongly **a**/α suppressed in the presence of glycerol (10-fold) than in YM-glucose (no **a**/α suppression). This Ty1 RNA behavior supports the possibility that the carbon source sensitivity of mating type regulation originates from normal Ty sensitivity to the carbon source and not from normal *ADH2* carbon catabolite control.

In the iso-2-cytochrome *c* system, Errede and co-workers (10, 11) observed **a**/α suppression of *CYC7-H2* mutant strains during growth in the presence of derepressing carbon sources, much as we did with our Ty-associated *ADH2*^{c} mutant strains grown in YM-3% glycerol. However, the studies on the *cargA*$^+$*O*^{h}, *cargB*$^+$*O*^{h}, and *durO*^{h} mutant strains used media containing 3% glucose as a carbon source and 10 to 20 mM ammonium sulfate as a nitrogen source (10, 11). The high level of **a**/α suppression observed in this medium by Wiame and colleagues is difficult to reconcile with our data. These results may indicate that Ty transcription and ROAM expression are also sensitive to nitrogen catabolites as well as to carbon catabolites.

No simple correlation between the proximity of Ty elements to the *ADH2* gene and the level of **a**/α suppression was apparent. The *ADH2-2*^{c} Ty element is inserted at position −210 5′ to the translation start site of the *ADH2* gene, the *ADH2-7*^{c} Ty at −199, the *ADH2-3*^{c} Ty at −169, the *ADH2-6*^{c} Ty at −161, and the *ADH2-8*^{c} Ty at −125 (27). The **a**/α suppression effects varied from 20- to 70-fold with these mutations. Also, no direct correlation between the type of Ty element and the level of **a**/α suppression was seen. The Ty1 elements found in the *ADH2-2*^{c} and *ADH2-8*^{c} mutant strains appeared identical by restriction mapping analysis and sequencing of direct repeat δ regions (27), whereas the level of **a**/α suppression in these strains was different, i.e., about 20- and 40-fold, respectively. Finally, high ADHII activity was not necessarily correlated with increased **a**/α suppression. In the presence of glycerol, *ADH2-8*^{c} mutant haploids exhibited only 110 mU of ADHII activity per mg of protein, and *ADH2-1*^{c} mutant haploids exhibited 830 mU per mg of protein, but *ADH2-8*^{c} mutant strains showed about 40-fold **a**/α suppression and *ADH2-1*^{c} mutant strains only about 10-fold **a**/α suppression.

Elder et al. observed that when cells were in stationary phase, Ty1 and Ty2 RNAs were **a**/α suppressed about 20-fold in mating-incompetent **a**/α strains versus mating-competent strains (9). The degree of **a**/α suppression of total Ty RNA decreased to fivefold during exponential growth. Since

all of their studies were conducted with cells grown in 2% glucose-containing media, it seems likely that the exponential-phase cells were glucose repressed. The stationary-phase cells had probably utilized most of the glucose in the media and were in a state similar to that experienced by our cells while maintained in exponential growth in the presence of a derepressing carbon source such as glycerol. Our observations differed in only two respects from those of Elder and co-workers (9). First, we observed no a/α suppression of Ty1 RNAs during glucose repression, whereas Elder et al. saw fivefold a/α suppression of total Ty RNA. Second, our mating-competent strains exhibited about 2.6-fold more Ty1 RNA during glucose repression than during glycerol derepression, whereas Elder et al. detected no change in Ty RNA level between their exponential-phase and stationary-phase cells. These differences may be due to the different probes used since our probe was fairly specific for the Ty1 subclass of elements, whereas Elder and colleagues used a complete Ty1 element DNA probe homologous to both Ty1 and Ty2 RNAs. Alternatively, these differences may have resulted from differences in media conditions, since we used 10% glucose and Elder and colleagues used 2% glucose. We favor the second explanation, since earlier studies (unpublished data) with *ADH2-6*^{c}/*ADH2* heterozygous diploid strains indicated that a/α suppression of Ty-induced ADHII activity was about twofold higher when cells were grown in YM-3% glucose (3.8-fold a/α suppression) than in YM-8% glucose (1.6-fold a/α suppression). If Ty RNAs show changes in level similar to those of ADHII activity with changing glucose concentrations, then the differences in suppression observed by our laboratory and by Elder and colleagues may simply be a function of glucose concentration.

We interpret our results as indicative of both a passive and an active effect of Ty insertion on *ADH2* expression. First, Ty insertion into the *ADH2* region leads to the removal of various amounts of adjacent *ADH2* regulatory sequences to sites 6 kb further upstream from the *ADH2* structural gene. Beier and Young (1) have shown that sequences conferring glucose repressibility on the *ADH2* gene are located in a 1-kb region extending 5′ from a site 176 base pairs upstream from the translation start site. Since most of the Ty insertions occur between 125 and 210 base pairs 5′ to the translation start site, the glucose-repressing region is 5′ to the sites of Ty insertion and has been displaced by about 6 kb. A passive role for the Ty elements is also supported by the finding that deletion of the region conferring glucose repressibility on the *ADH2* gene leads to full constitutive expression of *ADH2* (1). Beier and Young proposed that production of ADHII is possible if any "neutral" DNA sequence has displaced the *ADH2* glucose repression region. However, Ty insertion may not have displaced all of the regulatory apparatus since *ADH2* expression in Ty-associated *ADH2*^{c} mutant strains is not fully constitutive and is still somewhat responsive to the *ADR1*-coded positive regulatory element (3, 4), which acts in the normal derepression of ADHII activity.

Second, the Ty elements may also play an active role. Although transcription initiation in the Ty-associated *ADH2*^{c} mutant strains occurs at the wild-type site (27), *ADH2* transcription in Ty insertion mutants of *ADH2* appears to mirror transcription of the adjacent Ty element. Such concurrent transcriptional control appears to require internal ε Ty sequences. Reciprocal recombination or imprecise excision of Ty elements from *ADH2-2*^{c}, *ADH2-3*^{c}, and *ADH2-6*^{c} sites leaving solo delta (δ) sequences yields little or no ADHII activity (5). Williamson et al. (27) have found that one such mutant produced no detectable *ADH2* mRNA and presumably no Ty transcripts from the *ADH2* region. Ciriacy (unpublished results) has also found that these Ty excision mutants do not show a/α suppression of their already low ADHII activity. Deletion mapping studies by Errede et al. (personal communication) of the *CYC7-H2* mutation as well as similar studies by Osterman (personal communication) of the *ADH2-2*^{c} mutation indicate that an internal ε Ty region near the start of Ty transcription is required for overproduction from the adjacent gene and transcription from the *ADH2*-linked Ty element. In addition, these observations indicate that activation of Ty elements and their adjacent genes is due to a positive control mechanism as originally proposed by Errede et al. (10, 11).

Concurrent transcriptional control of Ty elements and their adjacent genes appears to require that Ty transcription proceed away from the adjacent gene. *CYC7-H2*, *cargA*$^+$*O*^{h}, and the seven Ty-associated *ADH2*^{c} mutations show a similar Ty orientation such that Ty transcription is directed away from the adjacent gene, and all cause an overproduction from the adjacent gene, which is sensitive to mating type regulation. In contrast, insertion of a Ty element in either orientation into the 5′ region of the *HIS4* gene can lead to a his4$^-$ phenotype (20). In gene conversion studies of Ty elements adjacent to a *his4* gene, Roeder and Fink (21) observed a new *HIS4*$^+$ phenotype that was sensitive to mating competence in strains with Ty transcription proceeding away from the *HIS4* gene. The original Ty elements in *his4-912* and *his4-917* may be transcriptionally inactive as may be some of the internally deleted Ty elements constructed by Errede and Osterman. The *his4-912* and *his4-917* Ty elements may have changed to an active form by gene conversion. Recently, work by Pearlman, Rose, and Roeder (personal communication) has identified an internal fragment near the Ty transcription start site in the *his4-917* Ty element as the critical difference between the active and inactive Ty forms. Thus, regulatory signals may interact with an internal ε region of the Ty element leading to activation of Ty transcription and consequently to divergent adjacent transcription of the gene.

Some indications that mating and sporulation functions are linked to carbon catabolite control have been observed. The work of Liao and Thorner (15) suggests that *STE5*, a gene involved in mating regulation, affects cAMP metabolism. Derepression of several enzymes has been observed in the presence of added cAMP (16), although literature to the contrary (17) is also available. Recent work by Shuster (25) showed that some "start" mutants (*cdc28*, *cdc36*, and *cdc39* strains) which mimic the α-factor arrest seen just before conjugation were suppressed by carbon catabolite derepression. However, the relationship between mating functions and carbon catabolite control remains murky. In contrast, sporulation of yeast is inhibited by glucose and increased in the presence of the derepressing carbon source, acetate.

A positive regulatory determinant has been proposed by Errede et al. (10, 11) to be present in mating-competent strains and to lead to Ty-activated ROAM expression. Our work indicates that this positive regulatory determinant or another mating or sporulation regulatory determinant is sensitive to carbon catabolites. Positive regulatory determinant production may be activated in mating-competent strains, but a low level of production may also occur in mating-incompetent strains. In addition, positive regulatory determinant production may be further activated by glucose metabolites and decreased in the presence of derepressing carbon sources. Thus, *ADH2* mRNA was most abundant in

mating-competent strains grown in YM-glucose and least abundant in mating-incompetent strains grown in YM-glycerol.

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