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Regulation of Basal and Induced Levels of the *MEL1* Transcript in *Saccharomyces cerevisiae*

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The *MEL1* gene in *Saccharomyces cerevisiae* is required for the production of α-galactosidase and for the catabolism of melibiose. Production of α-galactosidose is induced by galactose or melibiose and repressed by glucose. Inducibility is controlled by the positive and negative regulatory proteins GAL4 and GAL80, respectively. We have cloned the *MEL1* gene to study its transcriptional expression and regulation. Evidence is presented that the *MEL1* gene encodes α-galactosidase and that *mel*^{0} is a naturally occurring allele which lacks the α-galactosidase-coding sequences. RNAs prepared from wild-type cells and from cells carrying either the noninducible *gal4-2* or *GAL80*^{S}*-100* allele grown on three different carbon sources were examined by Northern hybridization analyses. In wild-type cells under noninducing conditions, such as growth on glycerol-lactic acid, the *MEL1* transcript was detected at a basal level which was 1 to 2% of the fully induced level. The basal level of expression was diminished in cells carrying the *gal4-2* mutant allele but not in cells carrying the *GAL80*^{S}*-100* allele. The basal and induced RNA levels are repressed by glucose. Size determinations of the *MEL1* transcripts detected in glycerol-lactic acid- and galactose-grown cells provided no evidence for two distinct transcripts.

Production of α-galactosidase in the yeast *Saccharomyces cerevisiae* depends on the presence of the *MEL1* gene (5, 24). *S. cerevisiae* normally lacks this gene, but *S. cerevisiae* strains containing *MEL1* have been derived by interspecies matings with *S. carlsbergensis* strains which carry this locus (16, 24, 46). Sporulation and dissection of *mel*^{0} *S. cerevisiae* × *MEL1 S. carlsbergensis* diploids result in only 2:2 segregation for the ability to produce α-galactosidase and to ferment melibiose. In α-galactosidase-producing cells of either species, expression of α-galactosidase is under carbon source regulation. It is induced by either galactose or melibiose and is repressed by glucose (24, 33). Enzymatic activity is detected internally, membrane associated, and fully excreted into the growth medium (5, 16, 25, 27). The external form is known to be glycosylated (28).

The galactose or melibiose inducibility of α-galactosidase enzyme activity in *S. cerevisiae* is regulated by the functional interplay of the products of two major regulatory genes, *GAL4* and *GAL80* (24; O. M. Kew, Ph.D. thesis, University of Washington, Seattle, 1976). *GAL4* functions as a positive regulator, and *GAL80* functions as a negative regulator. The GAL4 and GAL80 proteins also regulate the coordinate induction of galactokinase, epimerase, uridyl transferase, and galactose permease encoded by the *GAL1*, *GAL10*, *GAL7*, and *GAL2* genes, respectively (10, 13, 14, 32, 38, 40). In contrast to the galactose-metabolizing enzymes, there is a significant level of basal α-galactosidase activity under noninducing conditions such as growth on glycerol, lactic acid, or ethanol (5, 24). Mutations in *GAL4* and *GAL80*, which result in noninducibility (*gal4* or *GAL80*^{S}) or constitutivity (*GAL4*^{c} or *gal80*) for α-galactosidase and the galactose pathway enzymes, have been described (15, 24, 31; O. M. Kew, Ph.D. thesis).

Although the transcriptional expression and regulation of the galactose-metabolizing enzymes have been studied (43), no information is available concerning either the transcriptional expression or regulation of the α-galactosidase gene. We have cloned *MEL1* from *S. cerevisiae* by complementation of a *mel*^{0} strain, and we have provided evidence that it is the structural gene for α-galactosidase. Its transcriptional expression and regulation were studied under noninducing (basal), glucose-repressing, and inducing conditions. The data reported here demonstrate that (i) expression during growth on three different carbon sources is regulated via transcription, (ii) by agarose gel analyses there is no difference in size for the transcripts detected under noninducing or inducing conditions, (iii) both basal and induced levels of expression are dependent on a functional GAL4 protein, and (iv) the basal level of expression is not affected by the *GAL80*^{S}*-100* allele, which prevents the induced level of expression.

MATERIALS AND METHODS

Strains and plasmids. The bacterial strain used for all transformation and cloning procedures was *Escherichia coli* K-12 RR1 (F$^-$ *pro leu thi* Str^r *hsdR hsdM endoI*) and was obtained from K. Tatchell. The yeast strains used in these studies are described in Table 1. Plasmid YEp24 (3) was obtained from D. Botstein. Plasmid YEp13 (4) was obtained from K. Nasmyth. Plasmid Sc4911 (43), which contains DNA sequences internal to the *GAL10* coding sequences in a pBR322 vector, was obtained from T. St. John. Plasmids pMP550 and pMP575 were constructed in this laboratory as described below. Restriction fragments used in construction of pMP575 or as a hybridization probe were isolated from agarose gels by electrophoresis.

Media and growth conditions. Bacterial cells were grown in L broth medium at 37°C. Ampicillin (100 μg/ml) was added to cultures containing transformed cells. Chloramphenicol (100 to 200 μg/ml) was added to cultures used for large-scale isolation of plasmid DNA (17). Nontransformed yeast cells were grown on YEP medium (0.5% yeast extract, 1.0% Bacto-Peptone [Difco Laboratories, Detroit, Mich.], and 20 μg of adenine per ml), and transformed cells were grown on uracil- or leucine-deficient medium (42) to maintain the free plasmid or to select for integrants. Yeast media were supple-

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TABLE 1. Yeast strains used in this study

Strain	Genotype	Source
108-3c	α *MEL1 trp1 ura1*	D. C. Hawthorne
DBY745	α *MEL1 ade1 leu2-3,112 ura3-52*	D. Botstein
D8921R-13d	α *mel*^{0} *ade1 leu2-3,112 ura3-52*	This work
P18-1a	**a** *mel1-18 leu2-3,112 trp1 ura1*	This work
D9258-5b	**a** *gal4-2 gal10 MEL1 ade6 ade8 ura1*	This work
418-6a	**a** *GAL80*^{S}*-100 MEL1 his trp*	D. C. Hawthorne
E56-2c[a]	α *MEL1 ade1*	C. Michels

[a] This strain is *S. carlsbergensis*. All other strains are *S. cerevisiae*.

mented with 3% glycerol–2% lactic acid, 2% glucose, 2% galactose, or 2% galactose–3% glycerol–2% lactic acid. All chemicals and sugars were purchased from Sigma Chemical Co., St. Louis, Mo., and all media were purchased from Difco Laboratories, Detroit, Mich.

UV mutagenesis and genetic techniques. 108-3c cells (10^7 cells per ml) were irradiated at a distance of 46 cm for 2 min (0.8 J/mm^2 per s) with a 15-W General Electric UV germicidal lamp (General Electric Co., Schenectady, N.Y.). Cells were spread on galactose plates and incubated at 30°C in the dark. These conditions resulted in survival rates of 3 to 10%. The colonies were screened for α-galactosidase activity by overlay assay with a chromogenic substrate (see below). Those colonies which were white (negative) were subjected to genetic analysis and quantitative enzyme assays. Genetic techniques and tetrad dissections were carried out as described by Mortimer and Hawthorne (34).

Construction of a yeast genomic DNA pool. A yeast plasmid recombinant DNA pool was constructed by using genomic DNA from diploid strain MPY1041 (108-3c × 418-6a), which is homozygous at the *MEL1* locus. Pool construction was necessary since existing *S. cerevisiae* pools had been derived from strains lacking α-galactosidase activity (presumably naturally occurring *mel*^{0} strains). Total genomic high-molecular-weight DNA was isolated from spheroplasted diploid cells of strain MPY1041 by the CsCl purification method of Olson et al. (37). Size-fractionated DNA from *Sau*3A partial digests was prepared on sucrose gradients as described by Maniatis et al. (30). DNAs from the 10- to 15-kilobase (kb) fractions were ligated into the *Bam*HI site of the vector YEp24 which had been treated with bacterial alkaline phosphatase. Independent bacterial Amp^r transformants (ca. 20,000) were obtained. Of the transformants, >90% contained inserts of genomic DNA. Restriction endonucleases, T4 DNA ligase, and bacterial alkaline phosphatase were purchased from either Bethesda Research Laboratories, Gaithersburg, Md., or New England Biolabs, Beverly, Mass., and were used in accordance with the conditions recommended by the manufacturer.

Preparation of plasmid DNA. Large-scale isolations (≥1-liter cultures) of plasmid DNA were carried out by fractionating cleared bacterial cell lysates on CsCl density gradients containing ethidium bromide, as described by Guerry et al. (17). Small-scale preparations (from colonies on plates to 200-ml cultures) were carried out either by the boiling method (21) or by an alkaline lysis method (2, 29). Plasmid DNA was isolated from yeast cells by the method of Zakian and Scott (47).

Bacterial and yeast transformations. Calcium chloride-treated RR1 cells were transformed as described by Capage and Hill (6) with supercoiled plasmid DNA isolated from bacteria or DNA precipitated from ligation reactions. Bacteria used in transformations with plasmid DNA obtained from yeast cells were prepared by the method of Zakian and Scott (47). Yeast transformations were performed with either supercoiled plasmid DNA or DNA fragments (integrative transformation) by either a spheroplasting method (20) or a lithium acetate method (22).

α-Galactosidase assays. α-Galactosidase activity was determined qualitatively by an agar overlay method (R. G. Buckholz, Ph.D. thesis, University of Hawaii, Honolulu, 1981). Whole-cell homogenates prepared from mid-log yeast cultures (2×10^7 to 5×10^7 cells per ml) of steady-state noninduced (glycerol-lactic acid), catabolite-repressed (glucose), or induced (galactose ± glycerol-lactic acid) cells were used for quantitative analysis of α-galactosidase activity by the method of Kew and Douglas (24) with minor modifications. After stopping the reaction with 100 mM Na_2CO_3, we spun samples in a microfuge to pellet membrane fragments which interfere with the spectrophotometric readings. The amount of *p*-nitrophenyl-α-D-galactopyranoside (Sigma Chemical Co.) hydrolyzed was determined by reading absorbance at 400 nm and calculating the concentration of *p*-nitrophenol by using its molar extinction coefficient (18.3×10^3). The protein concentration of each whole-cell homogenate was measured by using sodium dodecyl sulfate-solubilized samples and the method of Hess et al. (19). This method was used because it allows accurate quantification of membrane proteins.

Preparation of total and poly(A)$^+$ RNA. Total RNAs were prepared from mid-log yeast cultures by repeatedly extracting cell homogenates (in 50 mM Tris–25 mM EDTA [pH 9.0]) with phenol-chloroform (1:1, vol/vol), and the RNA in the final aqueous phase was precipitated with ethanol overnight at −20°C. A polyadenylic acid-containing [poly(A)$^+$] RNA-enriched fraction was obtained by affinity chromatography of total RNA on an oligodeoxythymidylate-cellulose column with two consecutive passages as described by Bantle et al. (1). Poly(A)$^+$ and total RNA preparations were stored in 10 mM Tris–1 mM EDTA (pH 7.5) at −70°C.

Northern hybridizations. Northern analyses of glyoxylated RNAs were carried out with nitrocellulose (BA85; Schleicher & Schuell, Inc., Keene, N.H.) as described by Thomas (45) with minor modifications. After baking 2 to 3 h in a vacuum oven at 80°C, the blots were placed in a pan of boiling water and then allowed to cool to room temperature before being handled. The blots were placed against the inside walls of plastic jars, the prehybridization buffer was added, and the jars were capped and placed on rollers in a 40°C warm room. The prehybridization buffer consisted of 12 ml of formamide (deionized; Fisher Scientific Co., Pittsburgh, Pa.), 6 ml of 20× SSC (3 M NaCl, 300 mM sodium citrate [pH 7.0]), 0.6 ml of 500 mM $NaPO_4$ buffer (pH 7.0), 2.4 ml of 2% sodium dodecyl sulfate (BDH, Poole, England), 2.4 ml of 100× Denhardt solution (12), and 0.6 ml of a 10-mg/ml solution of salmon sperm DNA (Sigma Chemical Co.). After 12 to 36 h of prehybridization, the blot was incubated in hybridization buffer containing denatured ^{32}P-labeled probe (5×10^6 to 10×10^6 cpm; 20 to 50 ng of DNA) for 36 h. The hybridization buffer consisted of 7 ml of formamide, 4 ml of 20× SSC, 1.6 ml of 0.5 M $NaPO_4$ buffer (pH 7.0), 160 μl of 100× Denhardt solution, and 4 ml of 50% dextran sulfate (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The probe was denatured in 1 ml of formamide at 100°C for 5 to 8 min. The blots were washed as described by Thomas (45).

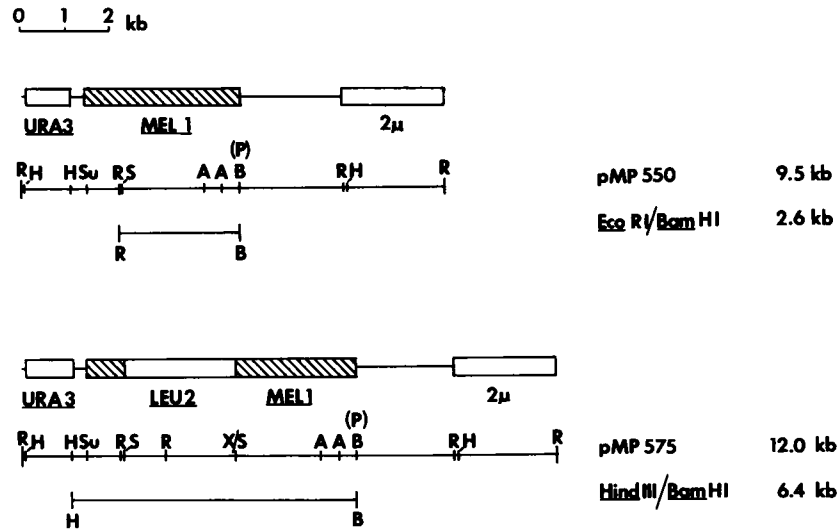

FIG. 1. Restriction maps of pMP550 and pMP575 showing the position of the insert containing *MEL1*. The *Bam*HI/*Pvu*II fragment of pBR322 was deleted during the subcloning which gave rise to pMP550. *Bam*HI linkers were ligated to the *Pvu*II-cut plasmid and subsequently digested with *Bam*HI, deleting the Tet^r gene of pBR322 and generating a *Bam*HI site. The *Eco*RI/*Bam*HI fragment (pMP550) used as the hybridization probe and the *Hin*dIII/*Bam*HI fragment (pMP575) used for integration are shown. pMP575 was generated from pMP550 by ligation of an *Xho*I/*Sal*I fragment containing *LEU2* into the unique *Sal*I site. A, *Ava*I; B, *Bam*HI; H, *Hin*dIII; P, *Pvu*II; R, *Eco*RI; S, *Sal*I; Su, *Sau*3A; X, *Xho*I.

Autoradiographic X-ray film used was Cronex 4 (Du Pont Co., Wilmington, Del.) with a Lightning-Plus screen (Du Pont Co.). Labeled-probe DNAs (200 to 500 ng) were prepared with a nick translation kit (Bethesda Research Laboratories) according to conditions recommended by the manufacturer. [α-^{32}P]dCTP (10 mCi/ml) was purchased from Amersham Corp., Arlington Heights, Ill.

Southern hybridization. Genomic DNAs were prepared from yeast spheroplasts lysed with sodium dodecyl sulfate and Tris-EDTA as described by Struhl et al. (44). Samples (100 to 200 ng) were digested to completion with restriction endonucleases and fractionated by gel electrophoresis on 0.6% agarose gels with Tris-acetate buffer. The gels were stained with ethidium bromide and exposed to long-wave UV light to visualize and mark the *Hin*dIII-digested lambda DNA standards (Bethesda Research Laboratories) and facilitate transfer of DNAs. The gels were washed for 15 min per wash, twice with 500 mM NaOH–1.5 M NaCl and twice with 200 mM sodium acetate, pH 4.0. Transfer onto nitrocellulose was accomplished with 10× SSC buffer. The blot was then treated as described for Northern hybridizations.

RESULTS

Isolation and integration of *MEL1* sequences. *MEL1* DNA sequences were isolated from our recombinant DNA pool by yeast transformation with D8921R-13d (Table 1) as the recipient strain. It is wild type for all known regulatory genes required for production of α-galactosidase and the galactose-catabolizing enzymes but does not have detectable α-galactosidase activity. The *mel*^{0} designation refers to a naturally occurring null variant in this strain (see below). After initial selection on uracil-deficient medium, yeast transformants were screened for production of α-galactosidase activity by agar overlay assays on galactose plates. Those transformants which were positive in the overlay assay were used for further analyses. Of 12 plasmids isolated from three independent yeast transformants, 1 was found to confer α-galactosidase activity and allow growth on melibiose medium. The other *URA3* cotransforming plasmids, which presumably contained genomic DNA not encoding *MEL1*, were not analyzed further. The original clone which conferred the ability to produce α-galactosidase activity (defined as *MEL1*) contained a 12-kb insert of yeast DNA. It was further subcloned to obtain an α-galactosidase-positive plasmid (pMP550) containing a 3.4-kb insert of yeast DNA (Fig. 1).

During subcloning of the original plasmid, the *Bam*HI/*Pvu*II region of pBR322, which encodes the Tet^r gene, was deleted. This yielded a smaller plasmid containing a unique *Sal*I site within the insert DNA. A *Sal*I/*Xho*I fragment encoding the yeast *LEU2* gene was isolated from YEp13 and inserted into this *Sal*I site of pMP550. Results of an agar overlay analysis for α-galactosidase activity in yeast cells transformed with this plasmid (pMP575) and grown on galactose-containing medium were positive, demonstrating that the *LEU2* insertion into the *Sal*I site does not disrupt function. Therefore, since the *Sau*3A/*Sal*I fragment is too small to code for α-galactosidase, the *MEL1* protein-coding sequences must lie between the *Sal*I and the *Bam*HI sites of pMP550 (Fig. 1).

To establish the identity of the cloned DNA, integrative transformation (41) was carried out. The *Hin*dIII/*Bam*HI fragment of pMP575 (Fig. 1) was used to transform DBY745 (Table 1). *LEU2* transformants were obtained and found to have integrated the fragment DNA into the genome. This was demonstrated by the mitotic and meiotic stability of the *LEU2* phenotype, by the tight linkage of the *LEU2* phenotype with production of α-galactosidase activity, and by genomic Southern analysis. DBY745 containing the integrated cloned sequences (*LEU2*, α-galactosidase positive) is designated as IHB225. This strain was crossed to a leucine-requiring, α-galactosidase-negative strain, P18-1a (Table 1), and the resultant diploid was sporulated and dissected. Tetrads were scored for leucine prototrophy and for the ability to produce α-galactosidase on galactose plates. Of 20 tetrads dissected and analyzed, all showed tight linkage of leucine prototrophy and the ability to produce α-galacto-

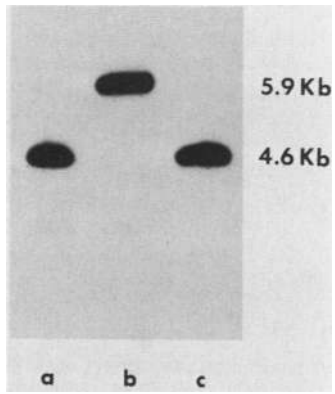

FIG. 2. Southern hybridization analysis of wild-type *S. cerevisiae*, integrated *S. cerevisiae*, and wild-type *S. carlsbergensis* strains. Genomic DNA was isolated, digested with *Eco*RI endonuclease, electrophoresed on agarose gels, transferred to nitrocellulose, and hybridized as described in the text. The hybridization probe used was [α-^{32}P]dCTP-labeled *Eco*RI/*Bam*HI fragment of pMP550. Lanes: a, DBY745 (wild-type *S. cerevisiae*); b, IHB225 (DBY745 integrated with the *Hin*dIII/*Bam*HI fragment of pMP575); c, E56-2c (wild-type *S. carlsbergensis*).

sidase (20 parental ditypes, 0 tetratypes, and 0 nonparental ditypes). In addition, segregation of *LEU2*, which is normally centromere linked (35), did not demonstrate centromere linkage in the dissected tetrads when compared with another centromere marker, *TRP1*. Results of the tetrad segregation analysis of *LEU2* and *TRP1* were 6 parental ditypes, 10 tetratypes, and 4 nonparental ditypes. These data indicate that the *LEU2* gene in the haploid strain IHB225 is now linked to the genomic *MEL1* locus rather than to the centromere as a result of the cointegration of *LEU2* and *MEL1* sequences. Since *MEL1* has not been mapped, analysis of linkage of the integrated *LEU2* marker to other markers was not possible. Demonstration of α-galactosidase activity in IHB225 by the agar overlay assay indicated that the *LEU2* insertion does not disrupt normal regulation of *MEL1* since α-galactosidase expression in the strain carrying this gene was similar to that of the wild type on all three carbon sources examined.

Comparative analysis of the DNAs from the integrated strain IHB225 and from the nontransformed parental strain DBY745 was carried out by genomic Southern hybridizations. Blots containing *Eco*RI-digested genomic DNA prepared from the two strains were hybridized with the α-^{32}P-labeled *Eco*RI/*Bam*HI fragment isolated from pMP550. The hybridization analysis revealed a single 4.6-kb *Eco*RI fragment of genomic DNA isolated from wild-type strain DBY745 (Fig. 2, lane a). *Eco*RI-digested genomic DNA prepared from the integrated strain IHB225 contained a single fragment of 5.9 kb (lane b). This is consistent with the predicted 1.3-kb increase in the *MEL1*-homologous *Eco*RI fragment if the *Hin*dIII/*Bam*HI fragment bearing the *LEU2* gene inserted into the *Sal*I site were integrated at the *MEL1* locus. Integrative transformation with linear DNA replaces the genomic sequences (41), so that in the above case with the *LEU2*-disrupted fragment, integration resulted in the disappearance of the wild-type 4.6-kb *Eco*RI fragment. Although *Eco*RI cuts within the *LEU2* sequences, a second fragment was not detected in the DNA from the integrated strain. We assume that this is due to the limited homology of this fragment with the hybridization probe. Lane c contains *Eco*RI-digested genomic DNA from a wild-type strain of *S. carlsbergensis* (E56-2c). It shows hybridization of a single 4.6-kb *Eco*RI fragment as does DBY745. This was expected since the ability to express α-galactosidase enzymatic activity was originally crossed into *S. cerevisiae from S. carlsbergensis* (16, 24, 46). Southern hybridization analysis of genomic DNAs digested simultaneously with *Xho*I and *Bam*HI detected the predicted 3.8-kb fragment for wild-type strains and the predicted 6.3-kb fragment for the integrated strain IHB225 (data not shown).

***MEL1* transcriptional expression in wild-type cells.** We examined the carbon source regulation of *MEL1* transcriptional expression by using Northern hybridization analysis of poly(A)$^+$ and total RNAs prepared from 108-3c cells (Table 1) grown on glycerol-lactic acid (noninducing), glucose (repressing), or galactose (inducing) medium (Fig. 3). The *Eco*RI/*Bam*HI fragment of pMP550 was used as the hybridization probe. Owing to its low abundance and comigration with 18*s* rRNA, the basal level of the *MEL1* transcript was difficult to detect in total RNA preparations (lane d) except with very long exposures. Induced levels were readily detected in total RNA extracts (lane f). In poly(A)$^+$-enriched RNA preparations, a single-sized transcript (1.6 $\pm$ 0.05 kb) was found for cells grown on either glycerol-lactic acid (lane a) or galactose (lane c). Several exposures of the autoradiograms used for Fig. 3 were scanned utilizing an LKB 2202 ultrascan laser densitometer with a Hewlett Packard 3390A integrator to obtain quantitative assessments of the levels of the 1.6-kb *MEL1* transcript detected in RNA from glycerol-lactic acid- and galactose-grown cells. The results indicated that the induction levels are 100 to 200 times higher than the basal levels. This finding correlates with our observations and those of Buckholz and Adams (5) that the basal level of enzymatic activity is 1 to 2% of the induced level. It should also be noted that, whereas the *MEL1* transcript was readily detected in poly(A)$^+$ RNA isolated from glycerol-lactate-grown wild-type cells, the *GAL10* transcript was not detected (lane a). Neither the

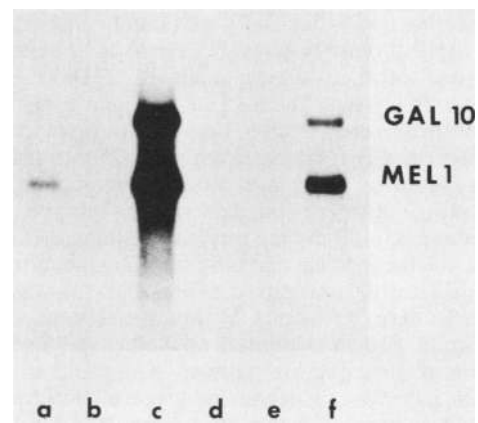

FIG. 3. Northern hybridization of poly(A)$^+$ and total RNAs prepared from wild-type (108-3c) cells grown under noninducing, catabolite-repressing, and inducing conditions. RNAs were prepared and hybridized with *GAL10* and *MEL1* sequences as described in the text. Lanes: a, 2 μg of poly(A)$^+$ RNA from glycerol-lactic acid-grown cells; b, 2 μg of poly(A)$^+$ RNA from glucose-grown cells; c, 0.4 μg of poly(A)$^+$ RNA from galactose-grown cells; d, 5 μg of total RNA from glycerol-lactic acid-grown cells; e, 5 μg of total RNA from glucose-grown cells; and f, 1 μg of total RNA from galactose-grown cells. Autoradiographic film was exposed to the blot for 25 h.

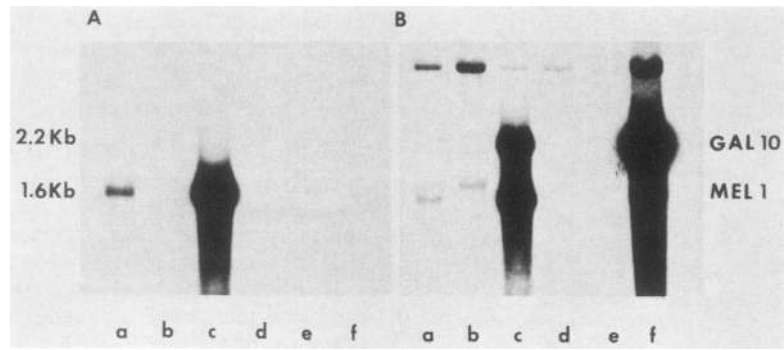

FIG. 4. Northern hybridization analysis of wild-type cells and cells carrying the *mel*^{0} allele. Poly(A)$^+$ RNAs were prepared from cells grown under noninducing, catabolite-repressing, or inducing conditions. RNAs were prepared and hybridized as described in the text. (A) RNA blot hybridized to the *MEL1*-containing *Eco*RI/*Bam*HI fragment only. (B) The same nitrocellulose blot sequentially hybridized to a *GAL10*-containing plasmid, Sc4911. The *MEL1* transcript is 1.6 kb, and the *GAL10* transcript is 2.2 kb. Lanes a through c contain RNA from wild-type cells (108-3c) grown in the following carbon sources: a, glycerol-lactic acid; b, glucose; and c, galactose. Lanes d through f contain RNA from D8921R-13d cells (*mel*^{0}) grown in the following carbon sources: d, glycerol-lactic acid; e, glucose; and f, galactose.

MEL1 transcript nor the *GAL10* transcript was detected in poly(A)$^+$ RNA from glucose-grown cells (lane b). This is in accord with previous reports of no detectable α-galactosidase activity in cells grown under glucose-repressing conditions (27, 33).

Comparisons of *mel*^{0} and *mel1-18* expressions. D8921R-13d is an inducible, galactose-fermenting strain that has no detectable α-galactosidase activity. This naturally occurring deficiency (*mel*^{0}) segregates only 2:2 against α-galactosidase activity in crosses of D8921R-13d with strains containing *MEL1* (data not shown). In poly(A)$^+$ RNA prepared from D8921R-13d, there were no detectable transcripts which hybridize to the *Eco*RI/*Bam*HI *MEL1*-containing fragment (Fig. 4A, lanes d through f), even with very long exposures. However, with the labeled Sc4911 plasmid (*GAL10*) as the hybridization probe on the same Northern blot, *GAL10* transcripts were readily detected in RNA from the galactose-grown cells (Fig. 4B, lane f). When RNA preparations from galactose-grown cells containing *mel*^{0} are translated in vitro, no polypeptide is synthesized which can be immunoprecipitated by using antibody against *S. cerevisiae* α-galactosidase (unpublished data). Southern analysis of D8921R-13d revealed that this strain has no genomic sequences homologous to the probe used (Fig. 5, lane c). Attempts to integrate the *Hin*dIII/*Bam*HI fragment from pMP575 into the genome of strain D8921R-13d by homologous recombination were unsuccessful. Together these data suggest that the lack of α-galactosidase activity in the strains containing *mel*^{0} is due to a lack of α-galactosidase protein-coding sequences.

An α-galactosidase-negative strain, P18-1a, was derived from 108-3c (*MEL1*) after UV mutagenesis, as described above. Strain P18-1a exhibited no defect in the inducible expression of the galactose pathway genes and no defect in growth on galactose, glucose, or glycerol-lactic acid. The *mel1-18* allele segregated only 2:2 against *MEL1* in 18 tetrads scored for α-galactosidase activity. P18-1a, like wild-type strains, produces a 1.6-kb transcript homologous to the *Eco*RI/*Bam*HI hybridization probe under basal (glycerol-lactic acid) or inducing (galactose) conditions and not under glucose-repressing conditions (unpublished data). In contrast to galactose-grown cells containing a *mel*^{0} allele, galactose-grown strains containing a *mel1* allele have a translatable mRNA species which produces a polypeptide recognized by anti-α-galactosidase immunoglobulin G (R. G. Buckholz, personal communication). The lack of α-galactosidase activity in *mel1*-containing strains is thus due to a defective polypeptide.

Strain P18-1a did not revert to the α-galactosidase-positive phenotype (5×10^8 cells) on melibiose plates. To ascertain whether the α-galactosidase-negative phenotype of P18-1a resulted from a gross chromosomal rearrangement or a deletion, we carried out Southern analysis of genomic DNA. This analysis demonstrated that the strain has a 4.6-kb *Eco*RI genomic DNA fragment homologous to the cloned sequences (Fig. 5, lane b) as does the wild-type, parental strain 108-3c (Fig. 5, lane a). Southern hybridization of DNAs digested simultaneously with *Xho*I and *Bam*HI demonstrated the presence of genomic fragments of equivalent size (3.8 kb) in 108-3c and the P18-1a mutant strain (data not shown). From these experiments, we did not detect an alteration in the size of the hybridizing fragments (within 50 base pairs) for ca. 1 kb left of the *Eco*RI site and more than 2.6 kb right of the *Bam*HI site in the cloned sequences (Fig. 1). Strain 108-3c, in addition to being the parental strain from which strain P18-1a was derived, is one of the haploids used in the construction of the recombinant DNA pool used to clone the *MEL1* gene.

***MEL1* transcript levels in noninducible cells.** The galactose-inducible appearance of α-galactosidase activity is known to be under the control of the *GAL4* gene (24; O. M. Kew, Ph.D. thesis). To determine whether the production of the *MEL1* 1.6-kb transcript is regulated by *GAL4* and *GAL80*, Northern analyses of poly(A)$^+$ RNA prepared from wild-type cells and noninducible cells bearing either the *gal4-2* allele or the *GAL80*^{S}*-100* allele grown on three different carbon sources were carried out (Fig. 6). Both basal and induced levels of the *MEL1* transcript were diminished in strain D9258-5b (Table 1) which carries the *gal4-2* allele (lanes d and f). Hence, a functional GAL4 protein is required for normal basal expression as well as induced expression of the *MEL1* transcript. However, in 418-6a (Table 1), a strain carrying the *GAL80*^{S}*-100* allele, the basal expression (lane g) was not diminished, whereas the induced expression was completely blocked (lane i). The level of the 1.6-kb *MEL1* transcript was similar for strain 418-6a cells (*GAL80*^{S}*-100*) grown on glycerol-lactic acid (lane g) or on galactose-glycerol-lactic acid (lane i) and is comparable to the level detected in strain 108-3c cells (*GAL80*) grown on glycerol-lactic acid (lane a). The 2.2-kb *GAL10* transcript was not detected in RNA prepared from this *GAL80*^{S}*-100* strain grown on either glycerol-lactic acid or galactose-glycerol-

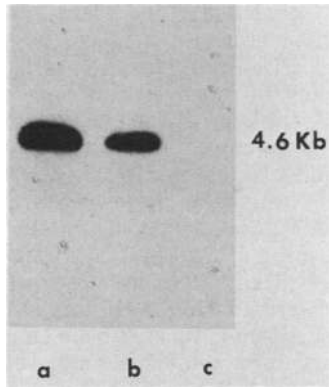

FIG. 5. Southern hybridization analysis of wild-type cells and cells containing either the *mel1-18* or the *mel*^{0} allele. Genomic DNA was prepared and hybridized as described in the legend of Fig. 2. Lanes: a, 108-3c (wild-type); b, P18-1a (*mel1-18*); and c, D8921R-13d (*mel*^{0}).

lactic acid (Fig. 7, lanes g and i). For all three of the genotypes examined, glucose-grown cells had no detectable 1.6-kb *MEL1* or 2.2-kb *GAL10* transcripts (Fig. 7, lanes b, e, and h).

It should be noted that additional faint bands are seen in the Northern blots both with *MEL1* (Fig. 6) and with *GAL10* (Fig. 3) hybridization probes. Those transcripts which hybridize to the *MEL1* probe could not be detected in the *mel*^{0} strain, even on very long exposures, and therefore are most likely derived from those DNA sequences which are

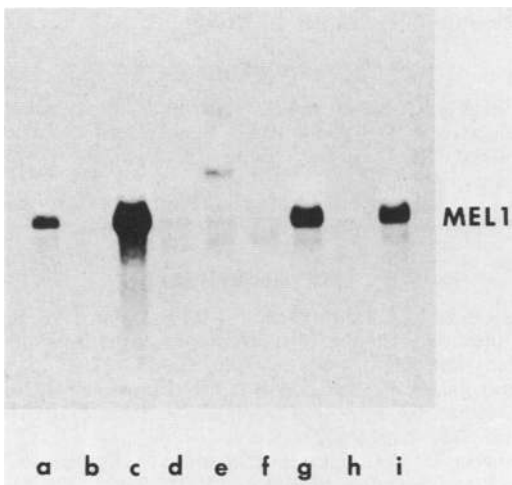

FIG. 6. Northern hybridization of poly(A)$^+$ RNA from wild-type and noninducible (*gal4-2* and *GAL80*^{S}*-100*) strains. RNA was prepared and hybridized as described in the text with only the *Eco*RI/*Bam*HI fragment containing *MEL1* for the hybridization probe. Lanes a through c contain poly(A)$^+$ RNA from strain 108-3c cells (wild-type) grown in the following carbon sources: a, glycerol-lactic acid; b, glucose; and c, galactose. Lanes d through f contain poly(A)$^+$ RNA from strain D9258-5b cells (*gal4-2*) grown in the following carbon sources: d, glycerol-lactic acid; e, glucose; and f, galactose-glycerol-lactic acid. Lanes g through i contain poly(A)$^+$ RNA from strain 418-6a cells (*GAL80*^{S}*-100*) grown in the following carbon sources: g, glycerol-lactic acid; h, glucose; and i, galactose-glycerol-lactic acid. Autoradiographic film was exposed to the blot for 116 h.

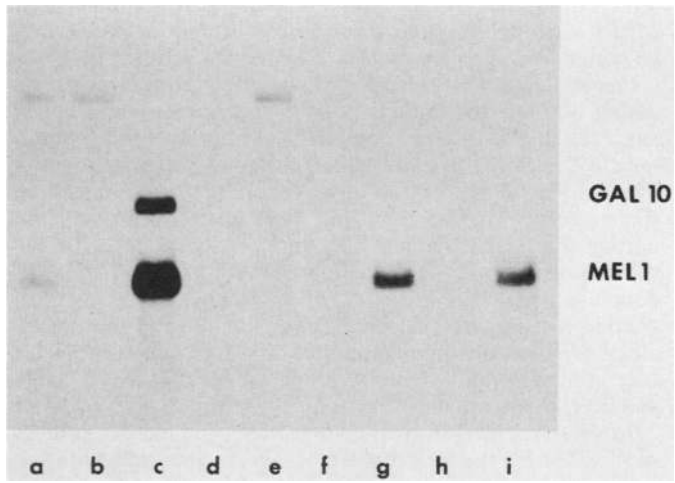

FIG. 7. Northern hybridization of poly(A)$^+$ RNA from wild-type and noninducible (*gal4-2* and *GAL80*^{S}*-100*) strains. The same blot used in Fig. 6 was rehybridized with labeled *GAL10* sequences without prior washing to remove labeled *MEL1* sequences. Lanes are as described in the legend to Fig. 6. Autoradiographic film was exposed to the blot for 46 h.

absent in D8921R-13d. The additional faint bands observed when sequences internal to *GAL10* were used as a hybridization probe against RNA prepared from wild-type cells are absent in RNA prepared from a strain deleted for *GAL1*, *GAL10*, and *GAL7* (43). Although we do not know their origin, the additional *MEL1*-hybridizing transcripts do not appear to be regulated by a carbon source or by the allelic state of the regulatory genes as is the 1.6-kb *MEL1* transcript (Fig. 6).

DISCUSSION

Our rationale for concluding that the cloned *MEL1* gene is the structural gene for α-galactosidase comes from genetic, enzymatic, RNA, and transformational analyses of strains containing either the *mel*^{0} or *mel1-18* allele, as well as genetic segregation analysis of the integrated cloned sequences. Southern analysis of the genomic DNAs prepared from P18-1a (*mel1-18*) and D8921R-13d (*mel*^{0}) cells indicates that P18-1a contains sequences homologous to cloned *MEL1* (mutant allele) and that D8921R-13d lacks these sequences (null allele). Comparable mutant and null alleles have been described by Carlson and Botstein (8) for the *SUC2* gene which encodes invertase. RNA preparations from galactose-grown cells carrying a *mel*^{0} allele do not have an in vitro-translatable α-galactosidase mRNA, whereas RNA preparations from galactose-grown cells carrying a *mel1* mutant allele produce the α-galactosidase polypeptide (R. G. Buckholtz, personal communication). In addition, Northern analysis of RNA prepared from strain P18-1a cells shows a 1.6-kb transcript which is homologous to the *Eco*RI/*Bam*HI fragment containing the *MEL1* gene (unpublished data). The level of the 1.6-kb transcript varies with the carbon source in a manner similar to the transcript variation observed in wild-type cells (108-3c). Both *mel1-18* and *mel*^{0} alleles segregate 2:2 against *MEL1*, do not complement each other, confer no known defects other than the lack of α-galactosidase activity, and are not pleiotropic in affecting the other target genes which encode the galactose-metabolizing enzymes. Furthermore, cells carrying either of these two alleles can be transformed to α-galactosidase positive with the plasmid

pMP550. In view of these data, it is unlikely that the cloned *MEL1* sequences encode a regulatory protein or processing enzymes whose expression is required for production of α-galactosidase activity. Such a hypothetical protein would be acting at both the transcriptional and translational levels since the noncomplementing *mel*^{0} and *mel1* alleles discussed here differ at these levels of expression. Furthermore, the transcriptional expression of the structural gene encoding this protein would be regulated by *GAL4*, *GAL80*, and the carbon source in a manner which parallels what is known for the expression of α-galactosidase activity. Therefore, we conclude that the cloned *MEL1* sequences on pMP550 encode the α-galactosidase polypeptide. Use of the *MEL1* clone for hybridization, selection, and translation of galactose-induced poly(A)$^+$ RNA prepared from wild-type cells should provide molecular proof.

In wild-type cells grown on galactose, the increased level of the *MEL1* transcript that we observed correlates with the increased enzymatic activity reported by other laboratories (5, 28). This indicates that transcription is most likely a major point of control for the synthesis of α-galactosidase as it is for synthesis of the galactose-metabolizing enzymes. However, data presented here demonstrate that the *MEL1* gene, unlike the *GAL1*, *GAL10*, and *GAL7* genes, has a readily detectable basal level of expression under noninducing conditions. Although the wild-type *GAL4* function has been shown to be necessary for the expression of induced levels of the *MEL1* transcript and the wild-type *GAL80* function has been shown to be required for repression of the *MEL1* transcript in the absence of galactose (T. E. Torchia, R. W. Hamilton, C. Cano, and J. E. Hopper, unpublished data), the role(s) of *GAL4* and *GAL80* in regulating *MEL1* transcription under noninducing conditions has not been previously investigated. The results of other studies (18, 23, 26, 36, 38) have suggested that a functional interplay involving the *GAL4* and *GAL80* proteins is a central feature of regulation of this system. We report here that strain D9258-5B cells (*gal4-2*), which are noninducible, do not produce detectable levels of the 1.6-kb *MEL1* transcript when grown on any of the three carbon sources studied. This indicates that basal as well as induced levels of *MEL1* expression are dependent on a functional *GAL4* gene. In contrast, strain 418-6a cells (*GAL80*^{S}*-100*), which are also noninducible, have a basal level of *MEL1* expression when grown on glycerol-lactic acid or galactose-glycerol-lactic acid. The 2.2-kb *GAL10* transcript is not detected under any growth conditions, indicating that the basal level of the 1.6-kb *MEL1* transcript observed under these growth conditions is not due to a generally increased basal level expression of all the target structural genes. In addition, *MEL1* and *GAL10* transcripts are not detected in glucose-grown cells carrying either the *GAL80*^{S}*-100* or the *GAL80* allele, indicating that glucose repression in strain 418-6a cells is functioning similar to that in wild-type cells. These data suggest that the GAL80^S-100 and GAL80 proteins do not differ significantly in their functional interplay with the GAL4 protein or the target structural genes or both under noninducing or glucose-repressing conditions but differ only in their response to the induction signal. It should be noted here that, since steady-state cultures were used in these studies without label incorporation, the levels detected reflect an equilibrium, and judgements regarding alterations in turnover rates of these messages cannot be made.

Expression of yeast α-galactosidase provides an interesting contrast to the expression of another yeast glycohydrolase, invertase. In the case of invertase, there are two levels of expression: a constitutive, basal level and a derepressible, high level. These are attributable to two distinctly sized transcripts and arise from utilization of different transcriptional start sites of a single structural gene (9). These mRNAs encode the internal and external forms of invertase, respectively. In the case of α-galactosidase, although there are internal and external enzymatic activities (5, 27), we detect no size distinction between the transcripts found under noninducing or inducing conditions.

The internal form of invertase is a nonglycosylated polypeptide and has been shown not to be a precursor to the glycosylated external form (7, 39). The internal form of the α-galactosidase enzymes has not been characterized. However, it appears to have the same molecular weight as the external form, based on Sepharose 6B and Sephadex G-200 gel filtration chromatography (28). Unlike invertase, whose basal level of expression is intracellular only, α-galactosidase activity is found both intra- and extracellularly under noninducing conditions (5, 27). These data suggest that there may be only the glycosylated form of the enzyme and that the internal activity represents a precursor population of the external population. Sucrose and melibiose are naturally occurring substrates for invertase and α-galactosidase, respectively. These disaccharides are not permeable to the yeast cell wall and consequently must be hydrolyzed extracellularly to their component monosaccharides before they can be utilized (11). The external form of invertase is expressed when the glucose concentration is lowered (derepressing conditions). This is also the case for basal level expression of α-galactosidase. However, unlike external invertase, which is expressed at high levels in derepressing medium, galactose (or a galactose metabolite) is required for high-level expression of α-galactosidase. The basal level of external α-galactosidase presumably serves to hydrolyze melibiose to provide sufficient galactose for the subsequent induction of all the target structural genes.

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