The Mechanism of Inducer Formation in *gal3* Mutants of the Yeast Galactose System Is Independent of Normal Galactose Metabolism and Mitochondrial Respiratory Function

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ABSTRACT

Saccharomyces cerevisiae cells defective in *GAL3* function exhibit either one of two phenotypes. The *gal3* mutation in an otherwise normal cell causes a 2–5-day delay in the galactose triggered induction of *GAL/MEL* gene transcription. This long term adaptation (LTA) phenotype has been ascribed to inefficient inducer formation. The *gal3* mutation causes a noninducible phenotype for *GAL/MEL* transcription if cells are defective in Leloir pathway function, in glycolysis or in respiratory function. It was recently shown that multiple copies of the intact *GAL1* gene partially suppress the LTA phenotype of *gal3* cells. Here we report that constitutively expressed GAL1 restored *gal3* mutants to the rapidly inducible phenotype characteristic of wild-type cells and conferred rapid inducibility to *gal3 gal10*, *gal3 gal7* or *gal3 rho*$^-$ strains that are normally noninducible. As shown by immunoblot analysis, the *GAL1*-mediated induction exhibits phosphorylation of the GAL4 protein, suggesting a mechanism similar to *GAL3*-mediated induction. Altogether our results indicate that the deciding factor in the inducibility of the *GAL/MEL* genes in *gal3* strains is the Gal3p-like activity of Gal1p. Based on the above we conclude that inducer formation does not require normal metabolism of galactose nor does it require mitochondrial respiratory function. These conclusions vitiate previous explanations for *gal3* associated long-term adaptation and noninducible phenotypes.

TRANSCRIPTIONAL induction of the *Saccharomyces cerevisiae* galactose and melibiose pathway genes occurs within a few minutes of exposure to galactose (ADAMS 1972). Gal3p plays an essential role in providing this rapid response (JOHNSTON 1987). It has been postulated that, in the presence of galactose, Gal3p catalyzes the formation of an inducer or coinducer molecule (TSUYUMU and ADAMS 1974; BROACH 1979). However, the activity of Gal3p remains unknown.

The elusiveness of the Gal3p function and the identity of the inducer is in part due to the complexity of phenotypes caused by *gal3* mutations. *gal3* mutants exhibit one of two phenotypes depending upon the state of other cellular functions. In respiratory competent *gal3* cells, transcriptional induction of galactose pathway genes takes two to five days, a phenotype called long term adaptation (LTA) (WINGE and ROBERTS 1948). In contrast, induction does not occur in respiratory incompetent *gal3* cells (DOUGLAS and PELROY 1963). The noninducible phenotype is also observed in *gal3* cells defective in the *GAL1*, *GAL7*, *GAL10* (BROACH 1979), *GAL5* or *PGI1* (BHAT, OH and HOPPER 1990) genes. On the basis of the noninducibility of cells of genotype *gal3 gal1*, *gal3 gal7* and *gal3 gal10*, it was postulated that the *GAL3*-derived inducer is an intermediate of normal galactose metabolism (BROACH 1979).

New leads concerning inducer formation have come from the recent discovery that the Gal3p is strikingly similar in sequence to the Gal1p (BAJWA, TORCHIA and HOPPER 1988). This finding suggested that Gal1p and Gal3p may share some function. The *GAL1* gene was subsequently found to partially suppress the LTA phenotype of respiratory competent *gal3* cells when it is present in high copy number (BHAT, OH and HOPPER 1990).

We wished to define the basis for the multiple-copy *GAL1* effect and to determine whether the effect is dependent or independent of galactose metabolism and mitochondrial function(s). Results presented here indicate, contrary to previous interpretations (DOUGLAS and PELROY 1963; TSUYUMU and ADAMS 1973; BROACH 1979), that neither normal galactose metabolism nor mitochondrial respiratory function(s) are required for induction in *gal3* cells, provided that sufficient *GAL1* expression is present at the time of galactose addition. In light of this, together with previous work and the striking sequence similarity of the *GAL3* and *GAL1* genes, we suggest that the Gal1p and Gal3p operate through a similar mechanism with respect to induction.

MATERIALS AND METHODS

Chemicals and media: Isopropyl-α-D-thiogalactopyranoside, T4 DNA ligase, DNA polymerase (Klenow fragment),

alkaline phosphatase and the restriction enzymes used were purchased from BRL, Inc., Gaithersburg, Maryland. [α-^{32}P] dCTP was bought from Amersham Corp., Arlington Heights, Illinois. Yeast cells were grown in yeast extract-peptone (YEP) medium containing 1% (w/v) bactopeptone (Difco, Detroit, Michigan), 0.5% (w/v) yeast extract (Difco) and 25 mg of adenine per liter. Solutions of appropriate carbon sources were sterilized separately and added to the medium to obtain final concentration of 2% glucose (YEPD), 2% (w/v) galactose(YEP GAL) and/or 3% (v/v) glycerol plus 2% (v/v) potassium lactate (pH 5.7) (YEPG/L). Yeast strains carrying plasmids were maintained in synthetic complete medium lacking either uracil (Ura$^-$) or leucine (Leu$^-$) with appropriate carbon sources.

Strains and genetic methods: *Escherichia coli* strain HB101 was used to propagate the plasmids (BOYER and ROULLAND-DUSSOIX 1969). *S. cerevisiae* haploid strains 21R (**a** *ura3–52 leu2–3 leu2–112 ade1 ile MEL1*) (JOHNSTON and HOPPER 1982), 21R-3D (**a** *ura3–52 leu2–3 leu2–112 ade1 ile MEL1 gal3::LEU2*) (BHAT, OH and HOPPER 1990) and TTD6–2C (**a** *gal3 gal1 gal7 MEL1 trp1 ura3–52 leu2–3,112 ade1*) (TORCHIA and HOPPER 1986) have been described. YM147 (**a** *galΔ-152 ura3–52 trp1–289*) is a *GAL1* deletion strain obtained from MARK JOHNSTON. An α mating type derivative of 21R-3D was obtained by *HO* gene induced mating type switching (RUSSEL *et al.* 1986), as described (BHAT, OH and HOPPER 1990). Strain 21R-10D (*gal10*) was derived from 21R using one step gene disruption technique (ROTHSTEIN 1983) as described under strain construction. Strain 21R-3D.10D was obtained by crossing α 21R-3D to **a** 21R-10D and screening the isolated tetrads to identify the doubly disrupted strain. Petite derivatives of 21R and 21R-3D were obtained by growing the strains in YEPD containing 20 μg/ml of ethidium bromide (MAHLER and WILKIE 1978) and screening individual colonies for the inability to grow on YEPG/L plates. Yeast and *E.coli* transformations were done as described elsewhere (ITO *et al.* 1983; DAGERT and EHRLICH 1979). Standard genetic techniques were used (MORTIMER and HAWTHORNE 1966).

Strain and plasmid constructions: YEp24 was provided by D. BOTSTEIN (BOTSTEIN *et al.* 1979). Plasmid pJK-1 consists of vector YEp24 carrying at its *Bam*HI site a 6.4-kb *Sau*3A yeast genomic fragment encompassing the entire *GAL1* gene (J. KIRSCHMAN and J. E. HOPPER, unpublished results). A disruption of the chromosomal *GAL10* gene was done in the wild-type strain (21R) as follows. Plasmid pWJ220, used for disrupting the chromosomal *GAL10* locus, was kindly provided by ROD ROTHSTEIN. The essential elements of this plasmid are: (a) pUC 18 containing the yeast *URA3* gene at the *Hin*dIII site of the polylinker, (b) an *Ava*I-*Eco*RI fragment bearing the *GAL1*-10 divergent promoter region at the *Eco*RI site of the polylinker, (c) an *Ava*I-*Sal*I fragment containing the yeast *LEU2* gene inserted at the *Sal*I site of the polylinker, and (d) a *Sal*I fragment containing the 3′ part of the *GAL10* gene and a portion of the *GAL7* gene inserted at the *Sal*I site of the polylinker. This plasmid was linearized by digesting with *Pst*I and used to transform 21R to leucine prototrophy (LEU$^+$). LEU$^+$ transformants were then screened for uracil auxotrophy and for inability to grow on galactose as the sole carbon source. Genomic DNA isolated from colonies that were unable to grow on galactose was subjected to SOUTHERN (1975) analysis. When probed with a labeled 2.0-kb *LEU2* fragment, genomic DNA from a wild-type strain digested with *Bam*HI and *Bgl*II showed a 3.0-kb *LEU2* fragment while the *GAL10* disrupted strain showed a 4.5-kb disrupted locus and a 3.0-kb *LEU2* fragment as expected.

A Plasmid containing the *GAL1* gene under the control of the *ADH1* promoter was constructed as follows. *CEN* plasmids containing the *ADH1* promoter and the *URA3* marker were derived from p1A and p41–1 (JOHNSTON *et al.* 1986). Plasmids p1A and p41–1 were digested with *Xho*I and *Hin*dIII. The 2.5-kb *Xho*I-*Hin*dIII GAL4 fragment was discarded and the larger *Xho*I-*Hin*dIII vector fragment was isolated by electrophoresis, and the ends were filled in using the Klenow fragment and dNTPs. A 1.1-kb *Hin*dIII fragment bearing the *URA3* gene isolated from YEp24 was rendered flush and then ligated to the vector fragments obtained from p1A and p41–1. The resulting plasmids, designated as p1A4ΔURA and p41–14ΔURA respectively, have a *Bam*HI site immediately 3′ to the *ADH1* promoter. These plasmids also have a *Hin*dIII site at the junction of the original *Xho*I site, and contain 234 (p1A) or 92 (p41–1) nucleotides of *GAL4* sequences between the *ADH1* promoter and the *URA3* marker.

The plasmids p1A4ΔURA and p41–14ΔURA were digested with *Bam*HI, dephosphorylated, and ligated to fragments ranging from 2.0 to 3.0 kb obtained by partial digestion of pJK-1 with *Sau*3A. Ampicillin resistant *E. coli* transformants were obtained from the ligation reactions, and the recovered pooled plasmids were transformed into yeast 21R-3D (*gal3*) to obtain plasmids containing the *GAL1* coding region ligated to the *ADH1* promoter in the proper orientation. Ura$^+$ transformants of 21R-3D were screened for fast growth on Ura$^-$ galactose plates. Using this protocol we isolated a plasmid that conferred weak suppression of the LTA phenotype of 21R-3D cells. This plasmid was designated as p41–1gal1–3. Restriction and DNA sequence analysis of p41–1gal1–3 indicated that the 3′ end of the *GAL1* gene (ST. JOHN and DAVIS 1981; YOCUM *et al.* 1984) had ligated to the 3′ end of the *ADH1* promoter sequence.

The *GAL1* gene was recovered from this plasmid on a partial *Hin*dIII fragment that was shown by DNA sequence analysis to consist of, in the 5′ to 3′ direction, 92 nucleotides of the *GAL4* coding region, 150 nucleotides of the 5′ noncoding region of the *GAL1* gene, the entire *GAL1* coding region, and approximately 2000 nucleotides of the 3′ noncoding region of *GAL1* including transcription termination signals. This fragment was ligated to *Hin*dIII digested p1A4ΔURA-1 derivative plasmid in which the original unique *Bam*HI site immediately 3′ to the *ADH1* promoter had been replaced by a *Hin*dIII site. The resulting plasmid, designated p1AGAL1, had the *GAL1* coding region in the proper orientation for expression from the *ADH1* promoter. p1AGAL1 and the corresponding vector lacking *GAL1* (p1A4ΔURA-1) are shown in Figure 1.

Other techniques: Yeast cell extracts were prepared by disrupting cells with glass beads (BOSTIAN *et al.* 1980). The activity of α-galactosidase was measured as described (POST-BEITTENMILLER, HAMILTON and HOPPER 1984). The Bio-Rad reagent was used for protein determination. Western blot analysis of the Gal4p was carried out according to the procedure described previously (MYLIN, BHAT and HOPPER 1989). Plasmid preparations from *E. coli* and yeast were as described elsewhere (BIRNBOIM and DOLY 1979; DAVIS *et al.* 1980). DNA labeling was done by the random primer labeling technique using a kit from Pharmacia. Transfer of DNA and RNA to Genescreen Plus membrane was according to the manufacturers recommendations.

RESULTS

Increased basal expression of *GAL1* decreases the induction lag in a *gal3* strain: Previous experiments revealed that multiple copies of *GAL1* in *gal3* cells

FIGURE 1.—The plasmids used for the experiments. Plasmids p1A4ΔURA-1 (A) and p1AGAL1 (B) were used as the control and experimental, respectively. The *GAL1* ATG is separated from the 3′ end of the *ADH1* promoter by a 242-base pair sequence consisting of 92 base pairs of *GAL4* sequences (shaded segment) derived from the vector followed by 150 base pairs of 5′ noncoding *GAL1* sequences. Figures are not drawn to the scale.

reduced the induction lag from 60 hr to 5 hr (BHAT, OH and HOPPER 1990). Suppression of the *gal3* LTA phenotype was not observed by multiple copies of the *GAL1* promoter and controlling elements alone (BHAT, OH and HOPPER 1990). Based on those experiments and the striking sequence similarity between *GAL1* and *GAL3* we hypothesized that the Gal1p has Gal3p activity. However, high copy *GAL1* mediated induction takes 5 hr rather than the 15 min found for *GAL3* mediated induction. Since a possible explanation for the difference between the two inductions is that multiple copies of the *GAL1* promoter could titrate limiting factors required for chromosomal *MEL1* (α-galactosidase) gene induction (BAKER *et al.* 1987), we tested whether high level *GAL1* expression from a single copy gene (*GAL1* expressed from the *ADH1* promoter) would allow rapid induction.

Plasmids p1AGAL1 (*GAL1* expressed under the *ADH1* promoter) and p1A4ΔURA-1 (vector control) were transformed into a *gal3* strain (21R-3D). The ability of p1AGAL1 to express high basal level *GAL1* transcription was confirmed by Northern blot analysis (data not shown). The induction of α-galactosidase in 21R-3D transformants was monitored following galactose addition. Induction of α-galactosidase occurred almost immediately in the strain bearing the plasmid p1AGAL1. The vector control showed the long-term adaptation phenotype (Figure 2, panel A). These results indicate that it is the increased basal expression of *GAL1* in the absence of multiple copies of *GAL1* promoter elements that provides the *gal3* complementation activity.

Increased basal expression of *GAL1* reinstates induction in noninducible *gal3 gal10*, *gal3 gal7* and *gal3 rho*⁻ cells: We determined whether high constitutive *GAL1* expression is sufficient to substitute for *GAL3* in the absence of *GAL10*, or *GAL7*. The *gal10 gal3* strain (21R-3D.10D) was transformed with p1A4ΔURA-1, p1AGAL1, and pJK-1 (*GAL1* under its own promoter). The course of *MEL1* induction in these transformants is shown in Figure 2, panel B. Constitutive high expression of *GAL1* from the *ADH1* promoter causes immediate induction of α-galactosidase in the *gal10 gal3* strain. The same strain, transformed with pJK-1, exhibited a slower induction of α-galactosidase. Galactose is toxic to a *gal10* strain (MATSUMOTO, TOH-E and OSHIMA 1981); therefore the induction response could not be followed for a longer period of time. Similar results were found with respect to *GAL7* and are reported in panel C, Figure 2. The *gal3 gal1 gal7* strain (TTD6–2C) transformed with p1AGAL1 showed a rapid induction, whereas if transformed with the control plasmid, p1A4ΔURA-1, showed no α-galactosidase induction. These results, together with results from the previous experiment, indicate that *GAL1*-mediated induction in *gal3* cells is not dependent on either *GAL7* or *GAL10*.

That it is likewise independent of mitochondrial function is shown by the data in Figure 2, panel D. There was no induction of the *GAL/MEL* genes over a period of 96 hr in the petite *rho*⁻ (21R-3D) strain. In contrast, induction did occur in 21R-3D transformed with p1AGAL1 nearly as rapidly as the wild-type strain. Induction also occurred in cells bearing pJK-1, but in this case the induction lag was 40 hr (Figure 2, panel D). Since it was necessary to grow the transformants initially in glucose (due to the *rho*⁻ phenotype), the prolonged lag may be due to residual glucose repression (ADAMS 1972) of *GAL1*. These results indicate that elevated basal *GAL1* expression is capable of providing an inducing signal independent of mitochondrial respiratory functions.

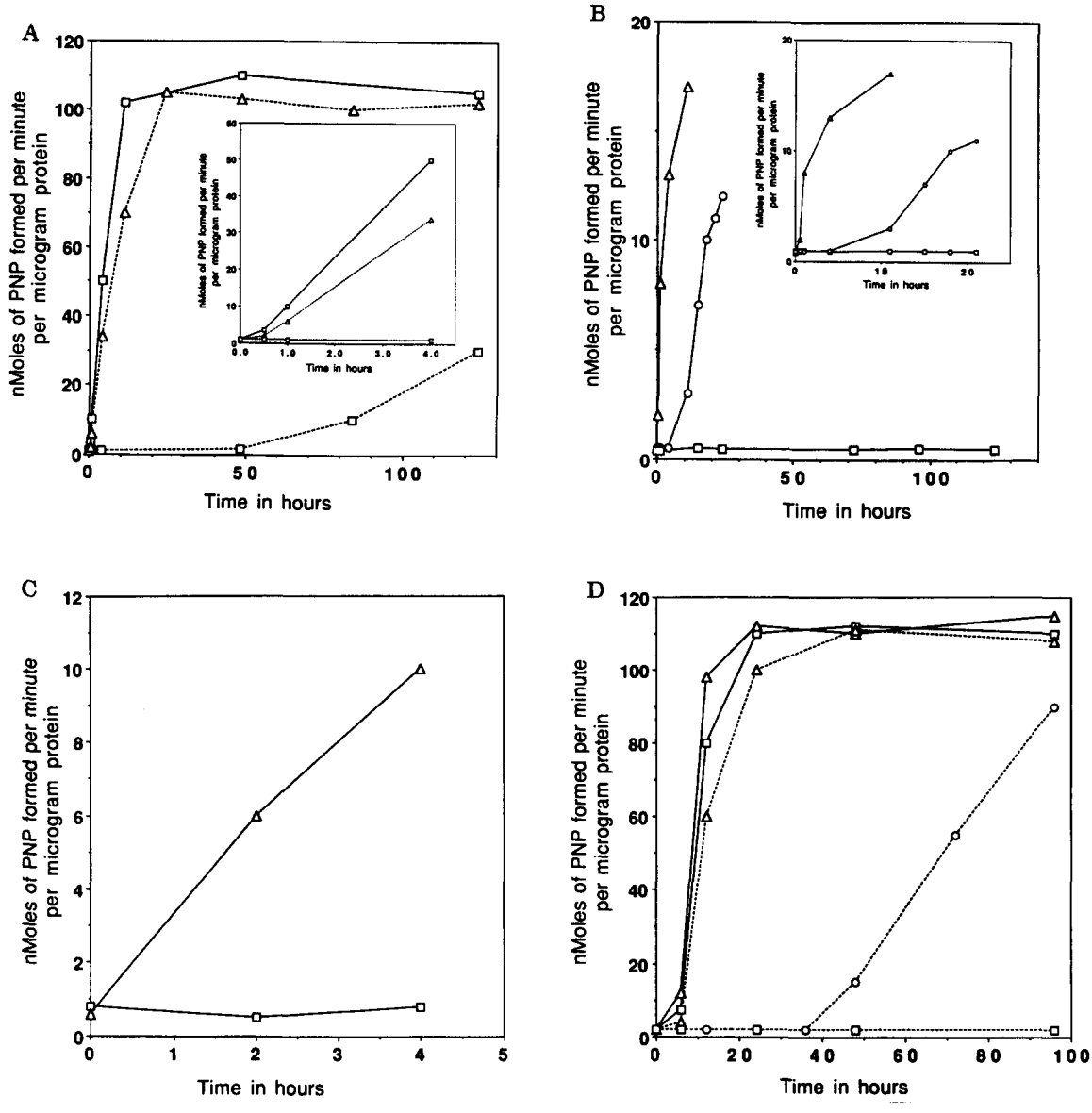

FIGURE 2.—α-Galactosidase activity in wild type (21R), *gal3* (21R-3D), *gal3 gal10* (21R-3D.10D), *gal3 gal7 gal1* (TTD6-2C) strains bearing p1A4ΔURA-1, p1AGAL1 or pJK-1. The yeast transformants were grown in Ura⁻ G/L medium. At a cell density of 2×10^7 ml, galactose was added to a final concentration of 2% (w/v) and samples were collected at indicated time points. During the experiment, cell cultures were maintained at mid-log phase by dilution into fresh prewarmed media. α-galactosidase activity was determined. Panel A, the wild-type strain transformed with p1A4ΔURA-1 (□——□), the *gal3* strain transformed with p1A4ΔURA-1 (□ - - - - - □) and p1AGAL1 (Δ - - - - - Δ). The inset shows α-galactosidase activity from 0 to 4 hours. Panel B, a *gal3 gal10* strain transformed with p1AGAL1 (Δ), pJK-1 (○) and p1A4ΔURA-1 (□). The inset shows α-galactosidase activity from 0 to 20 hr. Panel C, a *gal3 gal7* strain transformed with p1AGAL1 (Δ) and p1A4ΔURA-1 (□). Panel D, a petite derivative of the wild-type strain transformed with p1A4ΔURA-1 (□——□), p1AGAL1 (Δ——Δ), and a petite derivative of the *gal3* strain transformed with p1A4ΔURA-1 (□——□), pJK-1 (○–○) and p1AGAL1 (Δ- - - -Δ).

GAL4 protein phosphorylation occurs in *GAL1*-mediated induction: During *GAL3*-mediated induction, the GAL4 protein is phosphorylated to a form GALIII, distinguished on the basis of electrophoretic mobility (MYLIN, BHAT and HOPPER 1989). If *GAL1*-mediated induction occurs by a similar pathway as *GAL3*-mediated induction, we would expect to observe GAL4 phosphorylation. To test this hypothesis, we carried out immunoblot analysis of GAL4 protein during *GAL1* mediated induction. The results are presented in Figure 3. Strain 21R transformed with p1A4ΔURA-1 exhibits α-galactosidase induction and shows forms I and III of GAL4 protein 30 min after galactose addition. The induction of α-galactosidase in 21R-3D.10D transformed with p1AGAL1 is also observed at 30 minutes, but form III of GAL4 is not readily detectable until one hour after galactose addition. The slower appearance of GAL4III in the 21R-3D.10D cells carrying p1AGAL1 is consistent with the somewhat slower induction response observed for such cells compared to the 21R cells (see also Figure 2, panel A). A low to moderate level of GAL gene

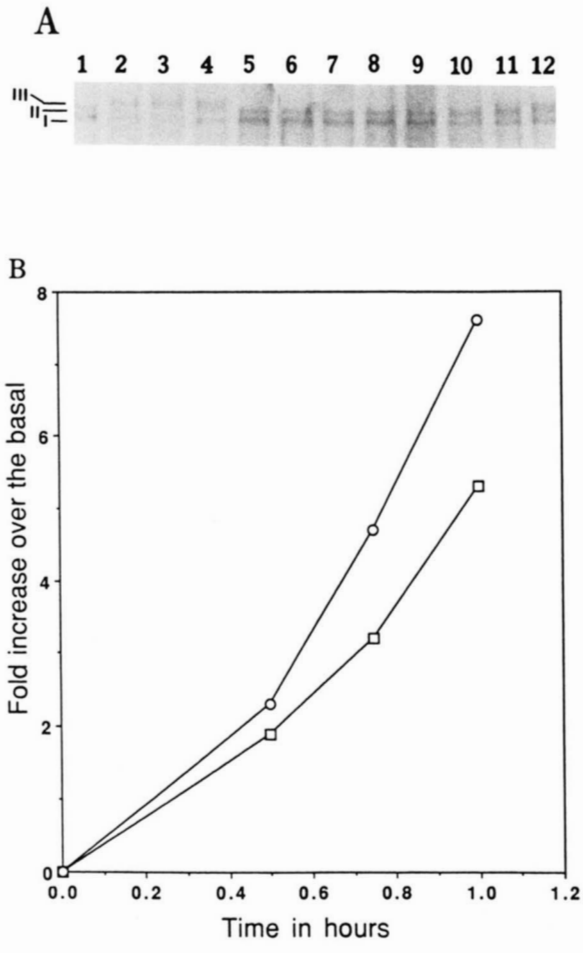

FIGURE 3.—Analysis of the induction kinetics of α-galactosidase and phosphorylation of GAL4 in a wild type strain (21R) transformed with p1A4ΔURA-1 and in a *gal3 gal10* strain (21R-3D.10D) transformed with p1AGAL1 or p1A4ΔURA-1. The above transformants were grown in Ura⁻ G/L medium. When the cell density reached 2×10^7/ml, galactose was added to a final concentration of 2%. Samples were taken at 0, 30, 45 and 60 min, and were quickly chilled in ice cold distilled water and centrifuged immediately. The resuspended cell pellets were processed for α-galactosidase activity determination and for GAL4 electrophoretic analysis by western blot. A, The electrophoretic mobility species of GAL4. Lanes 1 to 4 represent 0-, 30-, 45- and 60-min samples of 21R transformed with p1A4ΔURA-1. Lanes 5 to 8 represent 0-, 30-, 45- and 60-min samples from 21R-3D.10D transformed with p1A4ΔURA-1. Lanes 9 to 12 represent 0-, 30-, 45- and 60-min samples from 21R-3D.10D transformed with p1AGAL1. B, The fold increase of α-galactosidase over the basal. 21R transformed with p1A4ΔURA-1(○), 21R-3D.10D transformed with p1AGAL1(□). Zero time enzyme activity is taken as the baseline.

transcription occurs in the absence of detectable GAL4III (MYLIN, JOHNSTON, and HOPPER 1990). These results demonstrate that during both *GAL1*- and *GAL3*-mediated induction phosphorylation of GAL4 to form III occurs.

DISCUSSION

Our results provide new information concerning the production of the induction signal in the *GAL/MEL* regulon. We show that elevated basal *GAL1* expression is able to completely substitute for the absence of *GAL3*. These results point to a direct role for the Gal1p in the induction of *gal3* cells and necessitate a reinterpretation of past work. First, the *GAL7* and *GAL10* specified reactions of galactose catabolism are not an intrinsic part of the inducer forming mechanism, nor are the *GAL10* and *GAL7* activities required for the maintenance of the induced state. These conclusions are contrary to previous notions (BROACH 1979; NOGI 1986; TORCHIA and HOPPER 1986). Second, mitochondrial respiratory function(s) is (are) not directly part of the inducer forming mechanism. Previous workers concluded that the initiation of induction in *gal3* cells required mitochondrial respiratory competency (TSUYUMU and ADAMS 1973).

In light of our results, the data altogether now support the view that respiratory competence as well as galactose pathway function are only indirectly required for induction of *gal3* cells. We suggest that, in *gal3* cells, the initial low level of the Gal1p is insufficient to produce enough induction signal for detectable induction. Accordingly, detectable induction eventually occurs (LTA) in these cells due to an autocatalytic (BROACH 1979) increase in the level of the Gal1p rather than an increase in the level of Leloir pathway intermediates. The noninducibility of *gal3 gal1* cells can, therefore, be considered a consequence of the absence of both inducer-forming proteins. In the case of the noninducibility of *gal3 gal7*, *gal3 gal10*, *gal3 gal5*, *gal3 pgi1*, and *gal3 rho*⁻ cells, we suggest two possible explanations. The expression of the *GAL1* gene in these cells may be too low to initiate autocatalytic induction. Alternatively, a block in either galactose metabolism or respiratory competence may limit the level of an accessory factor(s) required for Gal1p mediated inducer formation. Low levels of such a factor would be compensated by an elevated Gal1p level.

Although p1AGAL1 produces a comparable amount of RNA as that of pJK-1 (data not shown), the former is more potent than the latter in reducing the induction lag in *gal3* cells. A plausible explanation for this is that multiple copies of pJK-1 could compete for limiting transcriptional factors, resulting in the observed slower induction of *MEL1* (α-galactosidase). The presence of multiple copies of *GAL7* has been shown to impair transcription of chromosomal *GAL10* (BAKER *et al.* 1987).

We presume that the inducing activity of *GAL1* is due to the Gal1p. The following considerations support this conclusion. First, the occurrence of a mutation in *GAL3* conferring temperature sensitivity suggests that the *GAL3* gene executes its function through its protein product (NOGI 1986). Second, a deletion in the *GAL1* coding region encompassing either the

N-terminal 29 amino acids or the C-terminal 65 amino acids, eliminates the capacity of p1AGAL1 to mimic *GAL3* (data not shown). Third, the sequence similarities between the Gal3p and Gal1p (BAJWA, TORCHIA and HOPPER 1988) and our observation that induction by either protein is associated with phosphorylation of Gal4p to form III, suggest that the inducer and its mechanism of action are the same for the two proteins.

What might be the nature of the inducing signal? Hitherto only catalytic models for induction signal formation have been considered (TSUYUMU and ADAMS 1974; BROACH 1979; LUE *et al.* 1987). Catalytic models envision a galactose derivative acting as an inducer or co-inducer. The target of a small molecular weight inducer molecule is most likely Gal80p (NOGI and FUKASAWA 1989). Gal80p inhibits the function of the DNA-binding transcriptional activator, Gal4p (JOHNSTON 1987). Gal80p and Gal4p have been shown to form a complex *in vitro* (LUE *et al.* 1987; PARTHUN and JAEHNING 1990). Presumably, binding of an inducer or a coinducer to Gal80p would relieve Gal80p inhibition of Gal4p. In accordance with a catalytic model, either Gal3p or Gal1p would produce the inducer or coinducer molecule in the presence of galactose. However, if the inducing signal is a derivative of galactose, it cannot be galactose-1-P since high level production of *E. coli* galactokinase activity in *gal3* cells cultured in the presence of galactose does not cause rapid induction (BHAT, OH and HOPPER 1990). Moreover, the inducer cannot be a molecule derived from galactose by the activity of either the *GAL7, GAL10, GAL5* or *PGI1* encoded enzymes. Repeated attempts to identify a Gal3p-dependent galactose derivative by *in vivo* and *in vitro* assays have not been successful (P. J. BHAT and J. E. HOPPER, unpublished data). It is possible that such a derivative would be very labile and/or in low abundance, making identification difficult. On the other hand, the induction signal may not be catalytically derived. A plausible alternative mechanism of induction would be a galactose-induced conversion of Gal1p and Gal3p to forms capable of binding to Gal80p; relieving Gal4p from Gal80p inhibition. Finding that Gal1p and Gal3p bind to Gal80p in the presence of galactose would provide impetus for this notion.

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