Overproduction of the GAL1 or GAL3 Protein Causes Galactose-Independent Activation of the GAL4 Protein: Evidence for a New Model of Induction for the Yeast *GAL/MEL* Regulon

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The transcriptional activation function of the *Saccharomyces cerevisiae* GAL4 protein is modulated by the GAL80 and GAL3 proteins. In the absence of galactose, GAL80 inhibits the function of GAL4, presumably by direct binding to the GAL4 protein. The presence of galactose triggers the relief of the GAL80 block. The key to this relief is the GAL3 protein. How GAL3 and galactose activate GAL4 is not understood, but the long-standing notion has been that a galactose derivative formed by catalytic activity of GAL3 is the inducer that interacts with GAL80 or the GAL80-GAL4 complex. Here we report that overproduction of the GAL3 protein causes constitutive expression of *GAL/MEL* genes in the absence of exogenous galactose. Overproduction of the GAL1 protein (galactokinase) also causes constitutivity, consistent with the observations that GAL1 is strikingly similar in amino acid sequence to GAL3 and has GAL3-like induction activity. Cells lacking the GAL10-encoded UDP-galactose-UDP-glucose epimerase retained the constitutivity response to overproduction of GAL3, making it unlikely that constitutivity is due to endogenously produced galactose. A galactose-independent mechanism of constitutivity is further indicated by the inducing properties of two newly created galactokinaseless alleles of GAL1. On the basis of these data, we propose a new model for galactose-induced activation of the GAL4 protein. This model invokes galactose-activation of the GAL3 and GAL1 proteins which in turn elicit an alteration of the GAL80-GAL4 complex to activate GAL4. This model is consistent with all the known features of the system and has important implications for manipulating GAL4-dependent transcriptional activation *in vitro*.

The GAL4 protein of the yeast *Saccharomyces cerevisiae* is a DNA-binding transcriptional activator of galactose pathway genes. It is part of a complex molecular switch that responds to carbon sources (16). In the absence of galactose, the transcription activation function of the GAL4 protein is blocked by its association with the GAL80 protein (8, 18, 21, 22, 26). The presence of galactose triggers a rapid relief of the GAL80 block, resulting in GAL4-mediated galactose pathway gene activation (16). The key to this rapid activation of GAL4 activity is the GAL3 protein (31, 33). Cells lacking the GAL3 protein and its counterpart activity in the GAL1 protein cannot activate GAL4-mediated gene transcription in response to galactose (2, 3, 7, 24, 31). The long-standing model of GAL3 function holds that GAL3 catalyzes the conversion of galactose to an inducer molecule (2, 3, 7, 16, 24, 31, 32). On the basis of a variety of data, the likely target for the presumed inducer is thought to be the GAL80 protein (21, 25, 27, 35).

Recent efforts have been directed toward identifying the presumed galactose-derived inducer molecule, with the aim of elucidating the mechanism of the transcriptional switch. However, attempts by several investigators to identify a galactose-derived or galactose-dependent low-molecular-weight inducer have been unsuccessful (8, 21, 35). The lack of evidence for such a GAL3-dependent galactose derivative led us to consider an alternative mechanism of induction. In this article, we present data that lead us to propose that GAL3 does not catalyze the conversion of galactose to an

inducer. Rather, our data support a new model of induction for this system that invokes galactose-dependent conversion of GAL3 to a form that is capable of relieving the GAL80 inhibition of GAL4 activity.

MATERIALS AND METHODS

Strains, media, and genetic methods. The wild-type *S. cerevisiae* strain used was SJ21R (*MATa ade2 ura3-52 leu2-3,112 MEL1*) (17). The disruption of the chromosomal *GAL3* gene in SJ21R to produce 21R-3D was carried out essentially as described previously (31). Strain 21R-10D (2) was derived from SJ21R by creating a chromosomal deletion-disruption of the *GAL10* gene by using pWJ220 (provided by R. Rothstein). The 21R-10D deletion-disruption has been confirmed by Southern analysis (2). This deletion removed all but the first 40 N-terminal and final 113 C-terminal amino acids encoded by *GAL10* (a 1.5-kb internal *EcoRI*-to-*SalI* fragment was replaced by an *EcoRI*-to-*SalI* fragment containing the yeast *LEU2* gene) and has been shown to have no detectable residual UDP-galactose-UDP-glucose epimerase (1a). Strain YM147 (*MATa gal1Δ-152 ura3-52 trp1-259*) was provided by Mark Johnston. Strain TTD6-2C (*MATa gal3 gal1 gal7 MEL1 trp1 ura3-52 leu2-3,112 ade1*) has been described elsewhere (31). *Escherichia coli* HB101 (6) was used to propagate plasmids. *E. coli* and yeast transformations were done as described previously (10, 15). Yeast transformants were grown in synthetic complete medium lacking uracil (13) and containing appropriate carbon sources. Carbon sources were sterilized separately and added to the medium to a final concentration of 2% (wt/vol)

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galactose or 3% (vol/vol) glycerol plus 2% (wt/vol) potassium lactate (pH 5.7).

Plasmids. Plasmid pJK-1 consists of a 6.4-kb *Sau3A* yeast genomic fragment containing the entire *GAL1* gene (which encodes galactokinase) inserted at the unique *Bam*HI site of YEp24. YEp24 (5) is a 2 μ m replicon-based plasmid that carries the yeast *URA3* gene. YCp50 (20) is a CEN/ARS-based plasmid that carries the yeast *URA3* gene. pT1-3B is YEp24 bearing the wild-type *GAL3* gene (31). pT5 is a derivative of pT1-3B in which the yeast *LEU2* gene has been inserted at the *Bgl*II site internal to the *GAL3* coding region (31).

In vitro mutagenesis and screening. pJK-1 carries a fully functional *GAL1* gene specifying both galactokinase activity and a GAL3-like induction activity (2, 3). pJK-1 was mutagenized in vitro by using hydroxylamine as described previously (29) and transformed into *E. coli*. A pool of mutagenized plasmid DNA was prepared from approximately 10⁶ *E. coli* transformants. The mutagenized plasmid pool was used to transform yeast strain YM147 (*gal1Δ*) to uracil prototrophy. Approximately 20,000 uracil prototrophs were subsequently replica plated onto galactose plates lacking uracil to screen for cells carrying plasmids no longer capable of providing *GAL1*-specified galactokinase activity. Six transformants unable to grow on galactose plates were identified. Of these, only three retained the ability to produce full-length galactokinase protein, as determined by immunoblot analysis (data not shown). Plasmids were isolated from these three transformants and were amplified in *E. coli*. These plasmids were transformed into 21R-3D (*gal3* disruption strain) to identify plasmids that retained *gal3*-complementing activity (2, 3) as judged by scoring for rapid growth on galactose plates lacking uracil. Because of the *gal3*-associated induction lag, growth of the *gal3* disruption strain (21R-3D) on galactose is retarded by 2 to 3 days compared with growth of its wild-type parental strain, SJ21R. One of the three plasmids (pJK-1-3) had lost both *gal3*- and *gal1*-complementing activities. The other two plasmids retained GAL3-like activity, but upon further analysis, only one of them (pJK-1-2-1) had completely lost galactokinase activity. Plasmids pJK-1-2-1 and pJK-1-3 were used for further analysis.

Construction of *CYC1::GAL1* and *CYC1::gal1* fusion plasmids. The *GAL1* coding regions from pJK-1, pJK-1-2-1, and pJK-1-3 were individually put under the control of the yeast *CYC1* promoter in a CEN vector as follows. A 672-bp fragment carrying the *CYC1* promoter was amplified from YCpR2 (28) by polymerase chain reaction (PCR). CYC01 (5'TACCGGATCGATGGCTCGAGAACAATAGGG3') and CYC02 (5'CGCCGGATCCGTGTGTATTTGTGTTTGCCG C3') are the upstream and downstream primers, respectively, used for the amplification of the *CYC1* promoter fragment. These primers have a *Cla*I (CYC01) or a *Bam*HI (CYC02) site at the 5' ends. The wild-type and mutated *GAL1* coding regions from pJK-1, pJK-1-2-1, and pJK-1-3, carried on 1,600-bp fragments, were separately amplified by PCR. BGAL01 (5'CCGAGGATCCCTATACCTTAACGTC AAGGAG3') and BGAL02 (5'GGTCAGTCGACGAGAA GTCGTTCTGAACAAAG3') are the upstream and downstream primers, respectively, used for the amplification of the *GAL1* coding region. These primers have a *Bam*HI (BGAL01) or a *Sall*I (BGAL02) site at the 5' ends. They were synthesized on a Milligene DNA synthesizer in the core facility at the Milton S. Hershey Medical Center, Pennsylvania State University. The PCR-amplified fragment containing the *CYC1* promoter was digested with *Cla*I and

*Bam*HI, and the fragments containing the *GAL1* coding region were digested with *Bam*HI and *Sall*I. The fragments were then gel isolated and ligated to the larger vector fragment isolated from YCp50 after digestion with *Sall*I and *Cla*I. The ligation mixture was transformed into *E. coli*. Constructs bearing the *GAL1* coding region in the proper orientation with respect to the *CYC1* promoter were identified by restriction analysis. The resulting YCp50 (CEN-based) plasmids bearing the *GAL1* coding region from pJK-1, pJK-1-2-1, and pJK-1-3 were named pYCJBGAL1, pYCJBgal1-2-1, and pYCJBgal1-3, respectively. These plasmids are referred to as single-copy alleles. To transfer the *CYC1::GAL1* fusion constructs from pYCJBGAL1, pYCJBgal1-2-1, and pYCJBgal1-3 to the multicopy vector YEp24, each plasmid was linearized by digestion with *Cla*I, and the ends were rendered flush with the Klenow fragment of DNA polymerase. The linearized plasmids were then digested with *Sall*I, and the *CYC1::GAL1* fragments were gel isolated. These fragments were ligated to the larger vector fragment obtained by digesting YEp24 with *Pvu*II and *Sall*I. The resulting plasmids were named pYEJBGAL1, pYEJBgal1-2-1, and pYEJBgal1-3. These plasmids are referred to as multicopy alleles. The conditions used for the PCRs were those specified by Perkin Elmer. Twenty-eight cycles of denaturation (94°C, 1 min), annealing (45°C, 2 min), and elongation (72°C, 3 min) were carried out in a thermal cycler apparatus (MJ Research, Inc.).

Nucleic acid and protein methods. Plasmid isolations were carried out according to published procedures (4, 12). pJK-1 (wild-type *GAL1*), pYCJBgal1-2-1, and pYCJBgal1-3 were sequenced by the dideoxy method (30) with appropriate primers and the double-stranded sequencing technique (29) as originally described by Chen and Seeburg (9). Total cell extracts were prepared as previously described (3). α -Galactosidase activity was determined as described before (3). Proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (3), transferred to nitrocellulose (3), and probed with GAL7 (13) and GAL1 (14) antisera at a dilution of 1:4000. GAL3 antiserum (3) was used at a dilution of 1:500. Antigen-antibody complexes were visualized with alkaline phosphatase-conjugated anti-rabbit antibody (Promega). Determinations of protein in the cell extracts were carried out with the Bio-Rad protein reagent.

RESULTS

Overproduction of the GAL3 or GAL1 protein causes constitutive expression of the *GAL1* and *GAL7* genes. We monitored the expression of the *GAL1* and *GAL7* genes in the *gal3* disruption strain (21R-3D), which bears multiple plasmid-borne copies of the *GAL3* gene, grown in the absence of galactose. Overproduction of the GAL3 protein was confirmed by probing the blot with antiserum to the GAL3 protein (Fig. 1B). The GAL7 and GAL1 proteins were readily detected by immunoblot assays of extracts obtained from cells bearing a plasmid with the wild-type *GAL3* gene (Fig. 1) but not from cells bearing a plasmid containing a *gal3* disruption allele (Fig. 1). To determine whether excess GAL3 protein also causes constitutive expression of the *MEL1* gene, another gene under galactose induction control, the *MEL1*-specified α -galactosidase enzyme activity was assayed. The level of α -galactosidase activity in glycerol-lactate-grown cells bearing multiple copies of the wild-type *GAL3* gene was 40% of that observed in galactose-induced wild-type cells (Table 1).

We recently discovered that the *GAL1*-encoded protein

FIG. 1. Immunoblot analysis of GAL7, GAL1, and GAL3 proteins in extracts of a *gal3* disruption strain (21R-3D) containing either a plasmid (pT1-3B) bearing the wild-type *GAL3* gene (3^+) or the corresponding plasmid (pT5) bearing the *gal3* deletion (3^Δ). Extracts were prepared from cells which had been grown to mid-log phase in glycerol-lactate (– Gal) and from cells which had been grown to mid-log phase in glycerol-lactate and induced with galactose for 6 h (+ Gal). Lanes loaded with the 3^Δ extract received 10 μ g of total protein, whereas lanes loaded with the 3^+ extract received 5 μ g of total protein. The probes used were GAL7 antiserum (A), GAL3 and GAL7 antisera (B), and GAL1 antiserum (C). The migration of size markers (molecular masses in kilodaltons) is indicated on the right. The polypeptide migrating slightly faster than the GAL7 polypeptide is a nonspecific cross-reacting polypeptide whose occurrence is not correlated with *GAL* gene expression (Fig. 1A and B, 2B, 3B, and 5A and B). Proteins were separated by electrophoresis on SDS–10% polyacrylamide gels.

has GAL3-like induction activity that is not galactokinase activity (2, 3). This discovery, together with evidence that GAL3 has no detectable galactokinase activity (3), suggests that GAL1 has two distinct activities, galactokinase and GAL3-like induction activities. Similar conclusions have

TABLE 1. α -Galactosidase activity in SJ21R bearing plasmid pT5 or pT1-3B

Plasmid ^a	Carbon source	Sp act ^b	% Sp act
pT5 (<i>gal3</i> disruption)	Glycerol-lactate	<1	<1
pT5 (<i>gal3</i> disruption)	Glycerol-lactate-galactose	80	100
pT1-3B (<i>GAL3</i>)	Glycerol-lactate	32	40

^a SJ21R bearing pT5 (*gal3* disruption plasmid) was grown in the presence of glycerol-lactate. At a cell density of 10^7 /ml, galactose to a final concentration of 2% (wt/vol) was added to one half of the culture; the other half received the same volume of distilled water. Cells were harvested 6 h after the addition of water or galactose. SJ21R bearing pT1-3B (*GAL3* gene on YE24) was grown in the presence of glycerol-lactate. At a cell density of 10^7 /ml, the same volume of distilled water was added as described above, and cells were harvested after 6 h.

^b α -Galactosidase specific activity is expressed as nanomoles of *para*-nitrophenylphosphate liberated per milligram of protein per minute under the assay conditions described in Materials and Methods.

FIG. 2. Immunoblot analysis of the GAL1 and GAL7 proteins in extracts obtained from the *gal3* disruption strain 21R-3D transformed with single-copy plasmids bearing the indicated alleles of *GAL1* expressed from the *CYC1* promoter. Extracts were prepared from either cells grown to mid-log phase in glycerol-lactate (– Gal) or cells grown to mid-log phase and induced with galactose for 1 h (in the case of *GAL1* and *gal1-2-1*) or 4 h (in the case of *gal1-3* and the vector-only control) (+ Gal). The probes used were GAL1 antiserum (A) and GAL7 antiserum (B). Proteins were separated by electrophoresis on SDS–12.5% polyacrylamide gels. Molecular mass markers are as defined in the legend to Fig. 1.

been drawn from work on the *GAL1*-encoded protein of *Kluyveromyces lactis* (23). Accordingly, we expected that overproduction of the GAL1 protein also would lead to constitutive expression of *GAL* genes. Overproduction was achieved by placing *GAL1* under the control of the *CYC1* promoter on a single-copy (pYCJBGAL1) (Fig. 2A) or a multicopy (pYEJBGAL1) (Fig. 3A) plasmid in a *gal3* disruption strain (21R-3D). As expected, *GAL1* was not expressed in the strains bearing the corresponding control plasmids (vectors alone) (Fig. 2A and 3A). The *GAL7* gene was expressed constitutively in cells bearing multicopy *GAL1*

FIG. 3. Immunoblot analysis of the GAL1 and GAL7 proteins in extracts obtained from the *gal3* disruption strain 21R-3D transformed with multicopy plasmids bearing the indicated alleles of *GAL1* expressed from the *CYC1* promoter. Extracts were prepared from cells grown to mid-log phase in glycerol-lactate. The probes used were GAL1 antiserum (A) and GAL7 antiserum (B). Proteins were separated by electrophoresis on SDS–12.5% polyacrylamide gels. Molecular mass markers are as defined in the legend to Fig. 1.

FIG. 4. Relative specific activity of galactokinase in the wild-type strain transformed with pT5 (□) or pT1-3B (○) with or without 0.5 mM galactose, respectively. Cultures of the wild-type strain bearing either pT1-3B (wild-type *GAL3*) or pT5 (*gal3* disruption) were inoculated at a cell density of 10^5 /ml into medium containing glycerol-lactate or glycerol-lactate with 0.5 mM galactose, respectively. After approximately 10 to 11 generations, cells were harvested at the times at which the indicated optical densities were reached, and galactokinase activity was determined. The 100% value represents the specific activity of galactokinase in the wild-type strain bearing pT5 grown to an optical density of 0.05 at 600 nm.

(pYEJBGAL1) (Fig. 3B) but not in cells bearing single-copy *GAL1* (pYCJBGAL1) (Fig. 2B).

These results suggested that constitutivity of the *GAL7* gene occurs at a high dose but not at a low dose of the *GAL1* protein. To confirm this, we determined the relative amounts of the *GAL1* protein produced from multicopy (pYEJBGAL1) and single-copy (pYCJBGAL1) plasmids. We transformed these plasmids into a strain with the *GAL1* gene deleted (YM147). The level of galactokinase specific activity in extracts from this *gal1* deletion strain bearing multicopy *GAL1* (pYEJBGAL1) was fivefold higher than in extracts from the same strain bearing single-copy *GAL1* (pYCJBGAL1) (data not shown). These results, together with the results given above, imply a galactose-independent mechanism of induction that is sensitive to the dose of the *GAL1* or *GAL3* protein. Since *gal4* deletion cells do not induce the *GAL* genes in response to an increased dose of either protein (results not shown), this mode of induction requires the *GAL4* protein.

Evidence against a galactose-dependent mechanism of constitutivity. The most interesting explanation for the constitutivity observed in response to overproduction of the *GAL3* or *GAL1* protein is that these proteins can act independently of galactose to elicit induction. However, these experiments did not rule out the possible involvement of galactose. For example, contaminating galactose might be present in the medium at a level high enough for it to act as an inducer when either *GAL3* or *GAL1* is overexpressed. If this were the case, we would expect the cells to become deinduced because of the consumption of galactose as a function of cell growth. On the contrary, we observed that galactokinase activity in cells overproducing the *GAL3* protein remained

FIG. 5. Immunoblot analysis of *GAL7*, *GAL1*, and *GAL3* proteins in extracts obtained from a wild-type *GAL10* strain (SJ21R) or the isogenic *gal10* disruption strain (21R-10D) transformed with either a *GAL3* plasmid (pT1-3B) (3^+) or a *gal3* disruption plasmid (pT5) (3^Δ) grown in glycerol-lactate to mid-log phase. The probes used were *GAL7* antiserum (A), *GAL3* and *GAL7* antisera (B), and *GAL1* antiserum (C). Proteins were separated by electrophoresis on SDS-12.5% polyacrylamide gels. Molecular mass markers are as defined in the legend to Fig. 1.

constant (Fig. 4) as a function of cell growth over the course of 10 to 11 generations to a density of 6×10^7 cells per ml. In contrast, wild-type cells bearing the control plasmid (vector only) induced with 0.5 mM galactose became deinduced as a function of cell growth (Fig. 4). These results imply that the expression of *GAL* genes observed when *GAL3* is overexpressed is not due to contaminating galactose in the medium.

We also considered the possibility that galactose is generated within the cell to a level sufficiently high to cause induction under conditions of elevated *GAL3* or *GAL1* protein abundance. The known pathway for production of galactose within the cell is by the conversion of UDP-glucose to UDP-galactose catalyzed by the UDP-glucose-UDP-galactose-4-epimerase, the enzyme encoded by the *GAL10* gene (11). In fact, constitutivity of the *E. coli* galactose operon genes in a galactokinaseless mutant has been shown to be due to the buildup of endogenously produced galactose from UDP-galactose generated by the epimerase-catalyzed reaction (19). Predictably, constitutivity of galactose operon genes in a galactokinaseless mutant of *E. coli* is not observed if the UDP-galactose-UDP-glucose epimerase is nonfunctional (34). By analogy with *E. coli*, if UDP-galactose were a source of galactose in yeast cells, we would expect that deletion of the *GAL10* gene would abolish the constitutivity observed in response to increased *GAL3* protein dosage. To test this, we assayed the expression of *GAL1* (Fig. 5C) and *GAL7* (Fig. 5A and B) in a *gal10* disruption yeast strain (2) bearing multiple copies of the *GAL3* gene (pT1-3B). This strain does not produce detectable levels of the epimerase (unpublished data). In the absence of *GAL10* function, we still observed the *GAL3*-

TABLE 2. α -Galactosidase activity in plasmid-bearing TTD6-2C after the addition of 2% galactose

Plasmid	% Sp act at indicated time after galactose addition ^a		
	0 h	2.5 h	5 h
pYEB24 (vector)	<1	<1	<1
pYEBgal1-2-1	100 (40)	700 (280)	1,100 (440)
pYEBgal1-3	105 (42)	105 (42)	95 (38)

^a Strain TTD6-2C (*gal1 gal3 gal7*) transformed with the indicated plasmid was grown in the presence of glycerol-lactate to a cell density of 10^7 /ml. At the indicated time points after the addition of 2% galactose, the cells were chilled on ice and collected by centrifugation. α -Galactosidase activity was determined as described in Materials and Methods. α -Galactosidase activity in TTD6-2C bearing pYEBgal1-2-1 at the time of addition of galactose is taken as 100%. Specific activity (nanomoles of *para*-nitrophenylphosphate liberated per milligram of protein per minute) is given in parentheses.

dependent constitutive expression of both *GAL7* and *GAL1* (Fig. 5). The levels of constitutive expression of *GAL1* as indicated by determinations of galactokinase activity in the *GAL10* and *gal10* extracts were, respectively, 37 and 38 nmol of galactose-1-phosphate formed per mg of protein per min.

Even more compelling evidence in support of a galactose-independent mechanism of constitutivity comes from an analysis of two newly isolated missense alleles of the *GAL1* gene that exhibited no detectable galactokinase activity. One of them is a Gly→Asp substitution at residue 166 (*gal1-2-1*), and the other is a Cys→Tyr substitution at position 175 (*gal1-3*). We overexpressed both alleles by placing them under the control of the *CYC1* promoter on single-copy plasmids (pYCBgal1-2-1 and pYCBgal1-3). The *gal3* disruption strain bearing a single-copy *gal1-2-1* allele (pYCBgal1-2-1) did not express the *GAL7* gene in the absence of galactose (Fig. 2B) but did express it in the presence of galactose (Fig. 2B). Although not shown in Fig. 2, this induction had occurred by 30 min after galactose addition. In contrast, the *gal3* disruption strain bearing a single-copy *gal1-3* allele (pYCBgal1-3) did not express the *GAL7* gene even in the presence of exogenous galactose over a period of 4 h (Fig. 2B). No induction of *MEL1* was observed even after 30 h of exposure of these cells to galactose on plates (unpublished data). These results indicated that the Gal1-2-1 and Gal1-3 proteins differed in their ability to provide an induction signal in response to galactose.

Since the Gal1-3 protein did not provide a galactose-responsive induction signal at a low copy number, we determined whether it would provide one when produced at a higher copy number, as was the case for wild-type *GAL1* protein. The results of this analysis were quite striking. The *gal3* disruption strain bearing a multicopy *gal1-3* allele (pYEBgal1-3) expressed the *GAL7* gene in the absence of galactose (Fig. 3B). If constitutive expression of *GAL7* in these cells were due to endogenous galactose, addition of exogenous galactose would be expected to cause a further increase in expression. To test this possibility, we determined the level of *MEL1*-encoded α -galactosidase upon addition of galactose in TTD6-2C (*gal1 gal3 gal7*) cells bearing multicopy *gal1-3* (pYEBgal1-3). Cells bearing a multicopy *gal1-3* allele (pYEBgal1-3) did not exhibit a further increase in α -galactosidase in response to galactose (Table 2). The multicopy *gal1-2-1* allele (pYEBgal1-2-1) behaved differently. Although multicopy *gal1-2-1* provided constitutive expression of *GAL7* (Fig. 3B) and *MEL1* (Table

FIG. 6. GAL3-mediated activation of the GAL4 protein in wild-type yeast cells. This model provides a parsimonious explanation for the galactose-independent activation we observe with overproduced GAL3 protein. This model states that in wild-type cells, the function of galactose is to shift the equilibrium in favor of a form of GAL3 capable of relieving the GAL80 inhibition of GAL4 activity. In the absence of galactose, this active form of GAL3 is limiting. Overproduction of the GAL3 protein results in levels of the active form sufficient to cause induction in the absence of galactose. How the GAL3 protein acts to alter the GAL4-GAL80 protein interplay is unknown. Such alteration could occur by reconfiguration or dissociation of the complex by GAL3 directly or through a GAL3-dependent intermediate. This model represents a departure from the long-standing view that GAL3 catalyzes the conversion of galactose to an inducer molecule which in turn binds to GAL80 to alter the GAL4-GAL80 interplay. Experimental evidence indicates that the GAL1 protein can readily substitute for the GAL3 protein in this scheme. UASgal denotes the DNA site to which GAL4 protein (GAL4p) binds.

2), it differed from multicopy *gal1-3* (pYEBgal1-3) in that it mediated a response to galactose (Table 2). *MEL1* expression was increased 11-fold over the base constitutive level over a period of 5 h following galactose addition (Table 2). These results support the notion that galactose is not involved in *GAL1* protein-mediated constitutivity.

DISCUSSION

The problem of how GAL3 triggers galactose-responsive GAL4 activation has been an enigma. It has been generally thought that GAL3 catalyzes the conversion of galactose to a true inducer (7). In this report, we show that the overproduction of either GAL3 or *GAL1* protein (proteins having considerable sequence homology [1]) causes induction of the *GAL/MEL* regulon genes. It is highly unlikely that this induction is due to galactose because of the following constraints. First, since constitutivity was also observed in a *gal10* disruption strain, galactose would have to be produced by the activity of a heretofore undiscovered pathway. Second, the *gal1-3* allele, when expressed from either a single-copy or multiple-copy plasmid, does not respond to exogenous galactose yet has the intrinsic ability to cause induction. To argue that this induction is due to galactose would require that all of the following assumptions be true: (i) the Gal1-3 protein saturates at very low levels of galactose; (ii) the rate of inducer formation by the higher level of the Gal1-3 protein (multicopy *gal1-3*), but not the lower level

(single-copy *gal1-3*), is sufficient to cause induction; and (iii) the inducer is unstable.

Overall, our data indicate that induction mediated by high levels of the GAL1 protein or GAL3 protein does not involve galactose. This implies a new model (Fig. 6) of how induction might work in wild-type cells. We hypothesize that the GAL3 and GAL1 proteins can each exist in two forms, active and inactive, with respect to eliciting an altered GAL4-GAL80 interplay. In uninduced wild-type cells, the level of the active form(s) is too low to elicit induction. Addition of galactose shifts the equilibrium in favor of the active form(s), leading to the relief of the GAL80 inhibition of GAL4. Induction in the absence of galactose, as observed in our experiments, can be achieved by increasing the level of either the GAL1 or GAL3 protein. In this model, galactose binds to the GAL3 protein, forming an active GAL3-galactose complex. This active complex then somehow elicits an alteration of the GAL80-GAL4 protein complex to relieve GAL4 protein from GAL80 inhibition. This second stage could occur by direct or indirect means. The simplest means would be by direct interaction of the GAL3-galactose complex with GAL80 or the GAL80-GAL4 complex. The existence of a stable GAL3-galactose complex and the ability of such a complex to bind to GAL80 or the GAL80-GAL4 complex are testable predictions of the model.

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