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Analysis of the Galactose Signal Transduction Pathway in *Saccharomyces cerevisiae*: Interaction between Gal3p and Gal80p

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The *GAL3* gene plays a critical role in galactose induction of the *GAL* genes that encode galactose-metabolizing enzymes in *Saccharomyces cerevisiae*. Defects in *GAL3* result in a long delay in *GAL* gene induction, and overproduction of Gal3p causes constitutive expression of *GAL*. Here we demonstrate that concomitant overproduction of the negative regulator, Gal80p, and Gal3p suppresses this constitutive *GAL* expression. This interplay between Gal80p and Gal3p is direct, as tagged Gal3p coimmunoprecipitated with Gal80p. The amount of coprecipitated Gal80p increased when *GAL80* yeast cells were grown in the presence of galactose. When both *GAL80* and *GAL3* were overexpressed, the amount of coprecipitated Gal80p was not affected by galactose. Tagged *gal3* mutant proteins bound to purified Gal80p, but only poorly in comparison with the wild type, suggesting that formation of the Gal80p-Gal3p complex depends on the normal function of Gal3p. Gal3p appeared larger in Western blots (immunoblots) than predicted by the published nucleic acid sequence. Reexamination of the DNA sequence of *GAL3* revealed several mistakes, including an extension at the 3′ end of another predicted 97 amino acids.

When galactose is added to a logarithmically growing culture of the yeast *Saccharomyces cerevisiae*, a set of genes (collectively called *GAL* genes hereafter) that encode galactose-metabolizing enzymes start to be transcribed within a few minutes (26, 27). This rapid induction has long been known to involve *GAL3*-encoded protein Gal3p (9, 10, 32), in addition to Gal4p (transcription activator) and Gal80p, the antagonist of Gal4p (see reference 13 for a review). Recessive mutations of *GAL3* cause a long delay, more than 48 h, in the induction of *GAL* expression (17, 29, 30). Gal3p was once believed to be required for conversion of galactose to an unknown inducer (5, 30). This idea has been excluded by the results of Bhat et al. (2–4), who showed that overexpression of Gal3p causes expression of *GAL* genes in the absence of galactose. This finding indicates that metabolism of galactose is not required for the functioning of Gal3p. Here we demonstrate that concomitant overexpression of Gal80p and Gal3p suppresses the constitutive expression of *GAL* genes. This finding strongly suggests that balanced synthesis of Gal3p and Gal80p is important for the induction of *GAL* genes and predicts that Gal3p directly interacts with Gal80p in the cell under the inducing conditions. In fact, influenza virus hemagglutinin (HA) epitope-tagged Gal3p (HA-Gal3p), expressed in *GAL80* wild-type *S. cerevisiae*, forms a complex with Gal80p which can be coprecipitated with an anti-HA antibody. In addition, we isolated HA-Gal3p missense mutants that produce apparently full-length proteins. These mutant proteins form complexes with purified Gal80p less efficiently than does the wild type, suggesting that the ability to bind Gal80p is closely correlated with Gal3p function. Models for the role of complex formation between Gal3p and Gal80p in galactose induction are discussed.

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MATERIALS AND METHODS

Strains and plasmids. *S. cerevisiae* NFG1 (*MAT***a** *gal3*::*HIS3 gal80*::*LEU2 ade his3 leu2 trp1 ura3*) and NFG5 (*MAT***a** *gal3*::*HIS3 ade his3 leu2 trp1 ura3*) are derivatives of MT8-1 (*MAT***a** *ade his3 leu2 trp1 ura3* [28]). The *GAL3* gene was disrupted by using a 9.6-kb fragment derived from pNN42 (gift of Ron Davis; 25). Another clone of *GAL3* was isolated in our laboratory from a YEp24-based library (gift of David Botstein; 6) as a plasmid capable of complementing galactose nonfermentation in a *gal3* null yeast strain (NFG5), which is referred to as pSK124. Plasmid pHAGAL3, which overproduces HA-tagged Gal3p, was constructed as follows. A 2.7-kb *Eco*RI fragment of pSK124 containing the *GAL3* open reading frame (ORF) was inserted into pBluescript II at the *Eco*RI site. A 0.6-kb fragment containing a 5′ part of the *GAL3* ORF and its flanking region was excised from the resultant plasmid with *Pst*I and *Ssp*I and replaced with a chemically synthesized oligonucleotide with a sequence corresponding exactly to that of the excised region of the ORF but bearing *Pst*I and *Ssp*I linkers at the 5′ and 3′ ends, respectively. A 2.1-kb fragment which had the *Pst*I site immediately upstream of the *GAL3* ORF was then excised from the resultant plasmid with *Pst*I and *Hin*dIII and inserted into pVT102UHA at the corresponding sites. Plasmid pVT102UHA was constructed by inserting a chemically synthesized oligonucleotide with *Bam*HI and *Xho*I linkers at the 5′ and 3′ ends into pVT102U such that a copy of the HA epitope with the initiation codon at the N terminus would be encoded. Two plasmids capable of overproducing Gal80p (pAHGAL80 and pFJGAL80) were constructed as follows. A 1.5-kb fragment encoding Gal80p from pTF8005 (19) was inserted into pVT102L (31) downstream of the *ADH1* promoter to give rise to pAHGAL80. A 1.7-kb *Sph*I fragment which encompasses the *GAL80* ORF and the *ADH1* promoter was then excised from pAHGAL80 and inserted into pTV3 (22) to yield pFJGAL80. Yeast transformation was performed by the method described by Dohmen et al. (8).

Media and yeast growth conditions. Basal medium containing 0.67% yeast nitrogen base without amino acids (Difco), 0.5% Casamino Acids, and 20 μg of adenine sulfate per ml was supplemented with appropriate nutrients and carbon sources; ESD or ESGlyLac contained 2% glucose or 1% each glycerol and sodium lactate. EBGal agar contained 2% Polypeptone (BBL Microbiology Systems), 1% yeast extract, 2% galactose, 0.002% ethidium bromide, and 2% agar. To prepare induced and uninduced cells, yeast cells were grown in ESD overnight to the logarithmic phase, washed once with sterile water, and inoculated into ESGlyLac. The culture was grown to an optical density at 600 nm of

1.0 and then divided into two equal parts. One was induced with galactose to a 2% final concentration, while the other was untreated (uninduced cells). Both cultures were grown for an additional 6 h.

Preparation of whole-cell extract. Yeast cells were collected by centrifugation at 1,500 × *g* for 5 min in the cold. Extraction buffer (10 mM Tris-HCl [pH 8.0], 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride) and glass beads (0.5-mm diameter) were added to the cell pellet at two and one times the volume, respectively. The mixture was vigorously agitated on a Voltex-type mixer for 1 min and chilled on ice. This cycle of agitation and cooling was repeated three times. The sample was then centrifuged at 15,000 rpm for 15 min in a microcentrifuge, and the resulting supernatant was collected by decantation to yield a whole-cell extract.

Immunoprecipitation. Into 0.5 to 1 mg of protein of a whole-cell extract, 1 μl of anti-HA monoclonal antibody (Babco) was added. The reaction mixture was allowed to stand for 2 h at 4°C, and then 20 μl of a 50% suspension of protein A-Sepharose 4B (Pharmacia) in extraction buffer (see above) was added. The mixture was gently rocked overnight at 4°C and centrifuged at 5,000 rpm in a Tomy MRX150 microcentrifuge for 1 min. After the supernatant was decanted, the pellet was washed three times with an equal volume of washing buffer (phosphate-buffered normal saline–0.05% Tween 20), and bound proteins were extracted by boiling with 10 μl of sample buffer (83 mM Tris-HCl [pH 6.8], 20% glycerol, 4.6% sodium dodecyl sulfate, 0.004% bromophenol blue). An aliquot of the supernatant sample was subjected to immunoblot analysis to detect HA-tagged Gal3p or Gal80p with an anti-HA monoclonal antibody or rabbit anti-Gal80 peptide antiserum (18), respectively. The antigen-antibody complex was detected by using the Super Signal CL-HRP Substrate System (Pierce) in accordance with the supplier's protocol. The presence of background bands on the Western blots (immunoblots) was due mostly to immunoglobulin G, which varied from experiment to experiment because of changes in exposure time for detection.

In the analysis of HA-Gal3p mutants, NFG1 harboring pHAGAL3 or its derivatives which bear missense mutations in the *GAL3* ORF (see below) was grown in the presence of 2% galactose. Whole-cell extracts were prepared from these strains as described above and mixed with protein A-Sepharose beads which had been conjugated to an anti-HA monoclonal antibody (10 μl of beads was mixed with 2 μl of the antibody). After 3 to 4 h of incubation at 4°C, 1 μg of purified Gal80p (33) was added to each sample and the incubation was continued for another couple of hours. The following procedures were performed as described above except that anti-Gal3p rabbit serum was used to detect HA-Gal3p or its mutants.

UDPGal-4 epimerase assay. A UDPGal-4 epimerase assay was performed by the two-step method described by Fukasawa et al. (11).

Isolation of *GAL3* missense mutants. pHAGAL3 plasmid DNA was treated with hydroxylamine as described by Silhavy et al. (24). The mutagenized plasmid was introduced into NFG5, and Ura^+ transformants were screened for poor growth on EBGal agar. Yeast cells harboring plasmids carrying missense mutations in *GAL3* were screened for the presence of normal-size HA-Gal3p by immunoblot analysis.

Nucleotide sequencing. The nucleotide sequence was determined by using a Taq-Dyedeoxy Terminator Cycle Sequencing kit and an automatic sequencer (Applied Biosystems).

RESULTS AND DISCUSSION

Nucleotide sequence of *GAL3*. Preliminary Western blots with tagged Gal3p indicated a discrepancy between its apparent size and that deduced from the nucleotide sequence reported by Bajwa et al. (1). We then prepared rabbit antiserum against a chemically synthesized peptide from residues Thr-212 to Val-224 of Gal3p based on the nucleotide sequence. The antiserum reacted with a protein specifically produced in *GAL3 S. cerevisiae* but not in *gal3* null *S. cerevisiae* in an immunoblot analysis (Fig. 1). The protein was detected only when the yeast was grown in the presence of galactose (Gal3p is induced by galactose [1]) or carried a high-copy plasmid bearing *GAL3*. These results indicate that this protein is Gal3p, the molecular size of which is 57 kDa rather than 49 kDa (4).

Therefore, we determined the nucleotide sequences of *GAL3* alleles from two independent sources, plasmids pNN42 and pSK124. Although the sequences of the two clones coincided with each other, there were significant differences from the sequence previously reported by Bajwa et al. (1), not only in the coding region but also in the 3′-flanking region. In accordance with the Western blot in Fig. 1, our sequence

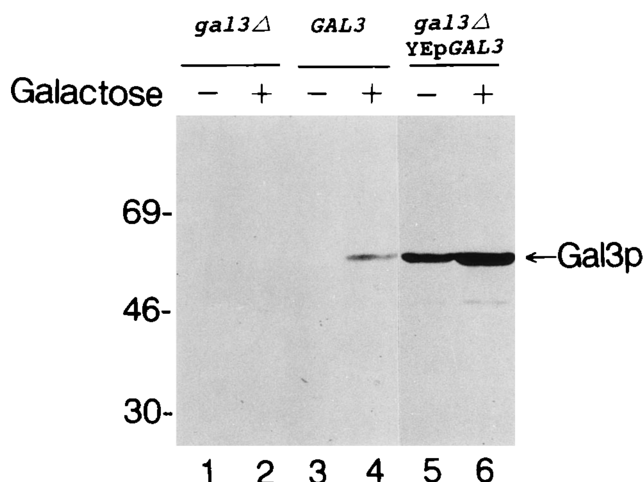

FIG. 1. Western blot analysis of Gal3p. Cells of yeast strains NFG5 (*gal3*Δ; lanes 1 and 2), MT8-1 (*GAL3*; lanes 3 and 4), and NFG5 harboring pSK124 (*gal3*Δ/YEpGAL3; lanes 5 and 6) were grown to the log phase in ESGlyLac medium containing appropriate nutrients in the presence (+) or absence (−) of 2% galactose. Whole-cell extracts prepared from these cells were subjected to electrophoresis on a 0.1% sodium dodecyl sulfate–7% polyacrylamide gel. Each lane contained 80 μg of protein, and separated proteins were blotted to a reinforced nitrocellulose membrane. Gal3p was probed with rabbit anti-Gal3p peptide serum. Molecular mass standard sizes are indicated to the left in kilodaltons.

predicted that the molecular size of Gal3p would be 57 kDa rather than 49 kDa (4).

At the final step of the preparation of this report, we noticed that the nucleotide sequence of *GAL3* had been submitted to EMBL-GenBank-DDBJ under accession number Z48008. The sequence was determined in the chromosome IV project at the Sanger Centre, Hinxton, Cambridge, England, and coincided perfectly with ours except for nucleotide position 109 (A in the database but C in our sequence). The difference causes an alteration in the amino acid from K (AAA) to Q (CAA) but not in the reading frame.

Similarity between Gal3p and Gal1p. When the ORF of *GAL3* determined by ourselves was compared to that of *GAL1* recently determined by Smits et al. (24a), a striking similarity was observed throughout the whole sequence (Fig. 2). The similarity is much more remarkable than that described by Bajwa et al. (1), strongly suggesting that the two genes evolved from a common origin. This is due not only to the correction of the *GAL3* sequence but also to that of the *GAL1* sequence (see reference 24a). That the two genes are homologous to each other explains, as first pointed out by Bhat et al. (4), why Gal1p is capable of partially complementing *gal3* mutations (2, 4). *Saccharomyces* Gal3p presumably has no galactokinase activity (4). By contrast, *Kluyveromyces* Gal1p (galactokinase) appears to have dual functions, the regulatory functions in induction and phosphorylation of galactose (16). It remains to be determined which domain is responsible for each function.

Effect of overproduction of Gal80p on Gal3p-induced constitutive expression of *GAL10*. Overproduction of Gal3p leads to the constitutive expression of *GAL* genes (3), suggesting a direct physical interaction between Gal3p and the negative regulator, Gal80p (2, 3). In light of this model, we determined the effect of concomitant overproduction of Gal80p on constitutive *GAL* expression. We tagged Gal3p with the HA epitope and confirmed that HA-tagged Gal3p complements a *gal3* allele. Since both *GAL80* (18) and *GAL3* (1) have UAS_G in their promoters and are inducible by galactose (1, 23), we con-

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GAL1    1:MTKSHSEEVIVPEFNSSAKELPRPLAEKCPSIIKKFISAYDAKPDFVARSPGRVNLIGEH
GAL3    1:M------NTNVPIFSSPVRDLPRSFEQKHLAVVDAFFQTYHVQPDFIARSPGRVNLIGEH

GAL1   61:IDYCDFSVLPLAIDFDMLCAVKVLNEKNPSITLINADPKFAQRKFDLPLDGSYVTIDPSV
GAL3   55:IDYCDFSVLPLAIDVDMLCAVKILDEKNPSITLTNADPKFAQRKFDLPLDGSYMAIDPSV

GAL1  121:SDWSNYFKCGLHVAHSFLKKLAPERFASAPLAGLQVFCEGDVPTGSGLSSSAAFICAVAL
GAL3  115:SEWSNYFKCGLHVAHSYLKKIAPERFNNTPLVGAQIFCQSDIPTGGGLSS--AFTCAAAL

GAL1  181:AVVKANMGPGYHMSKQNLMRITVVAEHYVGVNNGGMDQAASVCGEEDHALYVEFKPQLKA
GAL3  173:ATIRANMGKNFDISKKDLTRITAVAEHYVGVNNGGMDQATSVYGEEDHALYVEFRPKLKA

GAL1  241:TPFKFPQLKNHEISFVIANTLVVSNKFETAPTNYNLRVVEVTTAANVLAATYGVVLLSGK
GAL3  233:TPFKFPQLKNHEISFVIANTLVKSNKFETAPTNYNLRVIEVTVAANALATRYSVALPSHK

GAL1  301:EGSSTNKGNLRDFMNVYYARYHNISTPWNGDIESGIERLTKMLVLVEESLANKKQGFSVD
GAL3  293:DNSNSERGNLRDFMDAYYARYENQAQPWNGDIGTGIERLLKMLQLVEESFSRKKSGFTVH

GAL1  361:DVAQSLNCSREEFTRDYLTTSPVRFQVLKLYQRAKHVYSESLRVLKAVKLMTTASFTADE
GAL3  353:EASTALNCSREEFTRDYLTTFPVRFQVLKLYQRAKHVYSESLRVLKALKMMTSATFHTDE

GAL1  421:DFFKQFGALMNESQASCDKLYECSCPEIDKICSIALSNGSYGSRLTGAGWGGCTVHLVPG
GAL3  413:DFFTDFGRLMNESQASCDKLYECSCIETNQICSIALANGSFGSRLTGAGWGGCTIHLVPS

GAL1  481:GPNGNIEKVKEALANEFYKVKYPKITDAELENAIIVSKPALGSCLYEL
GAL3  473:GANGNVEQVRKALIEKFYNVRYPDLTDEELKDAIIVSKPALGTCLYEQ
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FIG. 2. Homology alignment of Gal3p and Gal1p (galactokinase) deduced from the respective nucleotide sequences. The latter sequence was obtained from the database of the chromosome II project at the Sanger Centre under accession number X76078 (see also reference 24a).

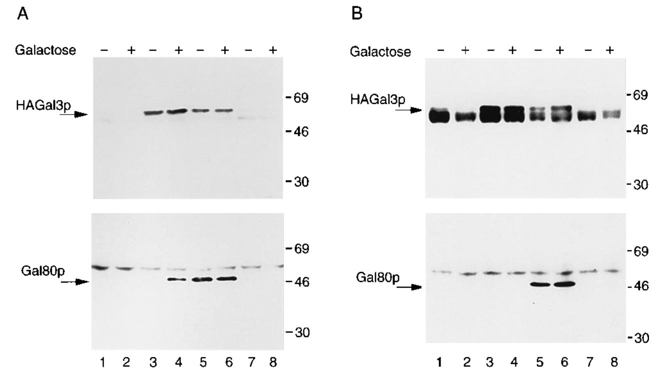

FIG. 3. Immunoprecipitation analysis of the formation of the HA-Gal3p-Gal80p complex in vivo. (A) Yeast strain NFG5 (*gal3* null *GAL80*) bearing pTV3 and pVT102UHA as vacant vectors (lanes 1 and 2), pHAGAL3 and pTV3 (lanes 3 and 4), pHAGAL3 and pFJGAL80 (lanes 5 and 6), or pFJGAL80 and pVT102UHA (7 and 8) was grown in the presence (+) or absence (−) of galactose. (B) Yeast strain NFG1 (*gal3* null *gal80* null) bearing pTV3 and pVT102UHA as vacant vectors (lanes 1 and 2), pHAGAL3 and pTV3 (lanes 3 and 4), pHAGAL3 and pFJGAL80 (lanes 5 and 6), or pFJGAL80 and pVT102UHA (lanes 7 and 8) was grown in the presence (+) or absence (−) of 2% galactose. Whole-cell extracts prepared from these cells were treated with an anti-HA monoclonal antibody. Immunoprecipitates were subjected to blot analysis as described in the legend to Fig. 1. HA-Gal3p or Gal80p was probed with an anti-HA monoclonal antibody or an anti-Gal80p peptide antibody, respectively. Molecular mass standard sizes are indicated to the right in kilodaltons.

structed plasmids in which the ORF of each gene was inserted downstream of the *ADH1* promoter such that both genes were expressed constitutively to high levels. Both plasmids were then introduced into a *gal3* null yeast strain, and the expression of *GAL10*, which encodes UDPGal-4 epimerase, was investigated under noninducing or inducing conditions by determining UDPGal-4 epimerase activity. As shown in Table 1, overexpression of HA-Gal3p caused constitutive synthesis of epimerase, in agreement with a similar experiment in which normal Gal3p was used (3). This constitutive level of epimerase in HA-Gal3p-overproducing yeast cells was found to be decreased significantly by concomitant overproduction of Gal80p. These results demonstrate that balanced synthesis of Gal80p and Gal3p is important for the control of *GAL* gene expression and further support the idea that the two proteins interact with each other in vivo.

Direct interaction of Gal3p with Gal80p. To directly test the prediction that Gal3p and Gal80p physically interact, extracts were made from yeast cells bearing HA-tagged *GAL3* and either chromosomal *GAL80* or plasmid-carried *ADH1*-promoted *GAL80* in the presence or absence of galactose. Figure 3A shows that immunoprecipitation of HA-Gal3p from these extracts coprecipitated Gal80p. Notably, the amount of Gal80p coprecipitation increased when a strain bearing pHAGAL3 was grown in the presence of galactose compared with that in the same yeast strain grown without galactose (lanes 3 and 4 in Fig. 3A). These experiments suggest either (i) that galactose addition causes a modification of either HA-Gal3p or Gal80p so that the modified protein tends to bind the other or (ii) that the amount of Gal80p derived from chromosomal *GAL80* was increased by galactose induction, which enhances complex formation. To determine if the latter could be true (but not to distinguish between models i and ii), we overproduced both HA-Gal3p and Gal80p by placing each gene under the control of the *ADH1* promoter. As shown by Fig. 3, lanes 5 and 6, no significant difference in the amount of Gal80p was observed between the coprecipitates of cells grown in the absence of galactose and those grown in the presence of galactose. To investigate this point further, a similar experiment was carried out by using a strain with both *GAL3* and *GAL80* deleted, and essentially similar results were obtained (Fig. 3B). The ratio of the amounts of Gal80p precipitated with HAGal3p and HAGal3p was not changed significantly by addition of galactose. The use of the strain with both *GAL3* and *GAL80* deleted in place of the strain with only *GAL3* deleted thus excluded the possibility that galactose-induced Gal80p derived from the chromosomal copy had any influence on the result shown in Fig. 3A.

We concluded that increased HA-Gal3p-Gal80p complex formation in induced cells may result solely from increased Gal80p accumulation. However, our results do not rule out the possibility that either Gal3p or Gal80p is also modified in galactose-grown cells.

Gal3p missense mutants fail to form a complex with Gal80p. To study the correlation of the Gal3p function to complex

TABLE 1. Effect of overexpression of Gal3p and/or Gal80 on expression of UDPGal-4 epimerase

Plasmids[a]	Epimerase activity[b]	
	Uninduced	Induced
pTV3 + pVT102UHA[c]	<0.1	<0.1
pHAGAL3 + pTV3	5.1	29.5
pHAGAL3 + pFJGAL80	1.1	29.7
pFJGAL80 + pVT102UHA	<0.1	<0.1
None (wild-type strain MT8-1)	<0.1	32.2[d]

[a] The yeast strain used was NFG5 (*MAT***a** *gal3*::*HIS3 ade his3 leu2 trp1 ura3*) bearing the indicated plasmids.

[b] Epimerase activity is expressed as micromoles of UDP-glucose formed per hour per milligram of protein.

[c] pVT102UHA and pTV3 were vacant vectors for pHAGAL3 and pFJGAL80, respectively.

[d] The epimerase level in parental strain MT8-1 was determined in a separate experiment for comparison.

TABLE 2. Effect of overexpression of mutant Gal3p on the expression of UDPGal-4 epimerase

Plasmid	Epimerase activity[a]	
	Uninduced	Induced
Vacant vector[b]	<0.1	<0.1
pHAGAL3	3.2	19.9
pHAGAL3m2	0.3	3.1
pHAGAL3m3	<0.1	3.6
pHAGAL3m4	<0.1	<0.1

[a] Epimerase activity is expressed as micromoles of UDP-glucose formed per hour per milligram of protein. The yeast strain used as the host was NFG5 (*MAT***a** *gal3*::*HIS3 ade his3 leu2 trp1 ura3*).

[b] The vacant vector was pVT102UHA.

formation with Gal80p, we isolated missense mutants starting from HA-*GAL3* (Table 2). Three of the mutants tested, which were capable of producing proteins with sizes similar to that of normal HA-Gal3p, bound purified Gal80p but poorly (Fig. 4), suggesting that formation of the Gal3p-Gal80p complex is closely related to normal functioning of Gal3p.

Possible models for the role of Gal3p in galactose induction. Direct interaction of Gal3p and Gal80p was first suggested by Bhat and Hopper on the basis of genetic experiments (2, 3). Our findings support the models depicted in Fig. 5, which are variations of those proposed by them. In wild-type *S. cerevisiae* in the absence of galactose, Gal80p binds to Gal4p and the complex is poised at the *GAL* genes (7, 12, 14, 20, 21). A straightforward extension of the Bhat and Hopper model assumes that addition of galactose causes modification of either Gal3p or Gal80p, which increases binding of these two proteins. However, our results suggest that if this is the case the effect is modest, as we detected no increase in Gal80p-Gal3p immunoprecipitation in the presence of galactose (Fig. 3). Related to this point, in a preliminary experiment we found no evidence that Gal3p or Gal80p is phosphorylated irrespective of the noninducing or inducing conditions of the cell. Alternatively, galactose addition could result in a change of the molar ratio of Gal3p over Gal80p for an unknown reason (see below), and this would favor formation of the Gal3p-Gal80p complex. This complex formation would lead to either dissociation (14, 20) or allosteric alteration (15) of the Gal80p-Gal4p complex, which in turn activates Gal4p. We were unable to detect Gal4p in immunoprecipitates of Gal3p and Gal80p from cells grown under inducing or noninducing conditions by Western blot analysis (data not shown). This result suggests that the Gal80p that binds to Gal3p is free of Gal4p and favor model B in Fig. 5 but does not rule out model A, since the hypothetical Gal3p-Gal80p-Gal4p complex, if it exists, may release Gal3p, leaving the Gal4p-Gal80p complex intact during the course of crude-extract preparation.

If induction does arise from an increased concentration of Gal3p, perhaps in the nucleus, how can such a situation be generated? One might imagine that Gal3p is bound to an unknown structure in the cytoplasm in the absence of galactose. Once galactose is added to the medium, Gal3p is released from the structure so that it enters the nucleus to interact with

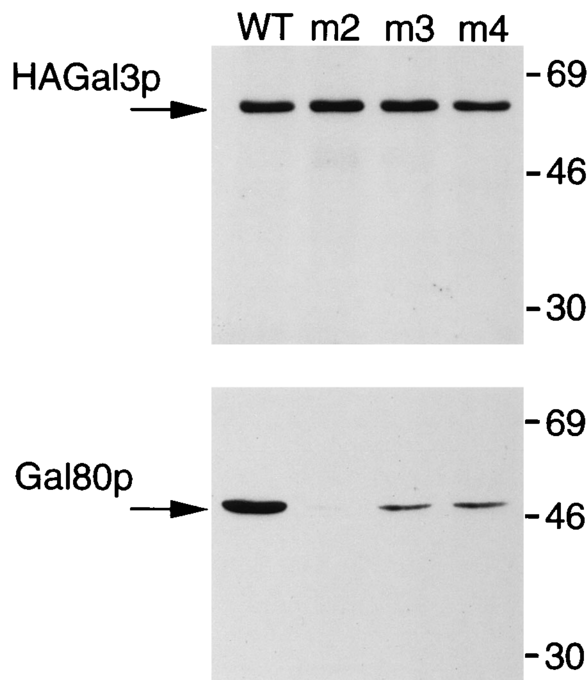

FIG. 4. Immunoprecipitation analysis of the formation of the HA-Gal3p-Gal80p complex with mutant Gal3p proteins. WT and m2 to m4 at the top stand for wild-type HAGAL3 and HAGAL3m2 to HAGAL3m4, respectively. Cells of yeast strain NFG1 bearing pHAGAL3, pHAGAL3m2, pHAGAL3m3, or pHAGAL3m4 were grown in the presence of 2% galactose. Each whole-cell extract (0.5 mg) prepared from these cells was mixed with protein A-Sepharose conjugated to an anti-HA monoclonal antibody. After 3 to 4 h of incubation at 4°C, purified Ga80p (1 μg) was added to the sample and the incubation was continued for another couple of hours. Immune complexes were subjected to Western analysis by using anti-Gal3p or anti-Gal80p serum. Molecular mass standard sizes are indicated to the right in kilodaltons.

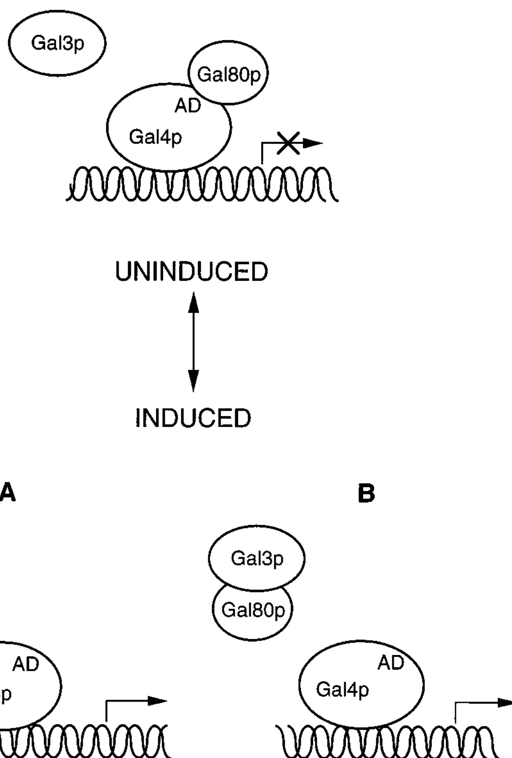

FIG. 5. Models for involvement of the Gal3p-Gal80p complex in activation of Gal4p, variations of the Bhat and Hopper model (3). In the uninduced state, Gal80p binds to Gal4p at the transcription activation domain (AD). Upon addition of galactose, Gal80p and Gal3p tend to form a complex. Complex formation leads to either allosteric change (A) or dissociation (B) of the Gal80p-Gal4p complex, resulting in exposure of the transcription activation domain.

Gal80p. It should be recalled here that *GAL3* is transcribed to a significant level in the absence of galactose (1).

These models explain, at least in part, the balanced interaction of Gal3p and Gal80p in the regulation of *GAL* gene expression. However, there are several aspects of the induction process which remain enigmatic. First, it does not explain the role of respiratory competence in induction (2). Second, in respiration-competent *gal3* mutant *S. cerevisiae*, mutations in *GAL1*, *GAL7*, or *GAL10* completely block induction (5). The failure of the combined *gal3* and *gal1* mutations to induce the *GAL* genes appears to be explained by the finding that *GAL1*-encoded galactokinase can partially complement *gal3* mutations even in *gal3 gal7*, *gal3 gal10*, or *gal3* [*rho*$^-$] mutant strains (2). *GAL1* and *GAL3* appear to be homologs, as first pointed out by Bhat and Hopper (2) and Meyer et al. (16). Indeed, our corrected sequence substantially extends the percent identity between these two genes. However, this does not explain why mutations in *GAL7* or *GAL10* would disrupt the induction pathway in *gal3* mutant *S. cerevisiae*. In addition, one should address which molecule, Gal3p, Gal80p, or Gal4p, directly receives the signal of galactose addition. It might be an unknown protein functioning upstream of Gal3p that receives the signal. For instance, another protein in the cytoplasm may inhibit Gal3p from binding to Gal80 in the absence of galactose, as mentioned above, and the inhibition might be released by addition of galactose. Much remains to be elucidated in this signal transduction pathway.

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REFERENCES

1. **Bajwa, W., T. E. Torchia, and J. E. Hopper.** 1988. Yeast regulatory gene *GAL3*: carbon regulation; UAS_{Gal} elements in common with *GAL1*, *GAL2*, *GAL7*, *GAL10*, *GAL80*, and *MEL1*; encoded protein strikingly similar to yeast and *Escherichia coli* galactokinases. Mol. Cell. Biol. **8:**3439–3447.
2. **Bhat, P. J., and J. E. Hopper.** 1991. The mechanism of inducer formation in *gal3* mutants of the yeast galactose system is independent of normal galactose metabolism and mitochondrial respiratory function. Genetics **128:**233–239.
3. **Bhat, P. J., and J. E. Hopper.** 1992. 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. Mol. Cell. Biol. **12:**2701–2707.
4. **Bhat, P. J., D. Oh, and J. E. Hopper.** 1990. Analysis of the *GAL3* signal transduction pathway activating *GAL4* protein-dependent transcription in *Saccharomyces cerevisiae*. Genetics **125:**281–291.
5. **Broach, J. R.** 1979. Galactose regulation in *Saccharomyces cerevisiae*. The enzymes encoded by the *GAL7,10,1* cluster are co-ordinately transcribed and separately translated. J. Mol. Biol. **131:**41–53.
6. **Carlson, M., and D. Botstein.** 1982. Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28:**145–154.
7. **Chasman, D. I., and R. D. Kornberg.** 1990. GAL4 protein: purification, association with GAL80 protein, and conserved domain structure. Mol. Cell. Biol. **10:**2916–2923.
8. **Dohmen, R. J., A. W. M. Strasser, C. B. Hoener, and C. P. Hollenberg.** 1991. An efficient transformation procedure enabling long-term storage of competent cells of various yeast genera. Yeast **7:**691–692.
9. **Douglas, H. C., and D. C. Hawthorne.** 1966. Regulation of genes controlling inducibility of the galactose pathway enzymes in yeast. Genetics **54:**911–916.
10. **Douglas, H. C., and G. Pelroy.** 1963. A gene controlling inducibility of the galactose pathway enzymes in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **68:**155–156.
11. **Fukasawa, T., T. Segawa, and Y. Nogi.** 1982. Uridine diphosphoglucose-4-epimerase and galactose-1-phosphate uridylyl transferase from *Saccharomyces cerevisiae*. Methods Enzymol. **89:**584–589.
12. **Hashimoto, H., Y. Kikuchi, Y. Nogi, and T. Fukasawa.** 1983. Regulation of expression of the galactose gene cluster in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **191:**31–38.
13. **Johnston, M., and M. Carlson.** 1992. Regulation of carbon and phosphate utilization, p. 193–281. *In* E. W. Jones, J. R. Pringel, and J. R. Broach (ed.), The molecular and cellular biology of the yeast Saccharomyces. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
14. **Johnston, S. A., and J. E. Hopper.** 1982. Isolation of the yeast regulatory gene GAL4 and analysis of its dosage effects on the galactose/melibiose regulon. Proc. Natl. Acad. Sci. USA **79:**6971–6975.
15. **Leuther, K. K., and S. A. Johnston.** 1992. Nondissociation of GAL4 and GAL80 *in vivo* after galactose induction. Science **256:**1333–1335.
16. **Meyer, J., A. Walker-Jonah, and C. P. Hollenberg.** 1991. Galactokinase encoded by *GAL1* is a bifunctional protein required for induction of the *GAL* genes in *Kluyveromyces lactis* and is able to suppress the *gal3* phenotype in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**5454–5461.
17. **Nogi, Y.** 1986. *GAL3* gene product is required for maintenance of the induced state of the *GAL* cluster genes in *Saccharomyces cerevisiae*. J. Bacteriol. **165:**101–106.
18. **Nogi, Y., and T. Fukasawa.** 1984. Nucleotide sequence of the yeast regulatory gene GAL80. Nucleic Acids Res. **12:**9287–9298.
19. **Nogi, Y., and T. Fukasawa.** 1989. Functional domains of a negative regulatory protein, GAL80, of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**3009–3017.
20. **Oshima, Y.** 1982. Regulatory circuits for gene expression: the metabolism of galactose and phosphate, p. 159–180. *In* J. R. Broach, E. W. Jones, and J. N. Strathern (ed.), The molecular biology of the yeast *Saccharomyces*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
21. **Parthun, M. R., and J. A. Jaehning.** 1990. Purification and characterization of the yeast transcriptional activator GAL4. J. Biol. Chem. **265:**209–213.
22. **Rose, M. D., and J. R. Broach.** 1991. Cloning genes by complementation in yeast. Methods Enzymol. **194:**195–238.
23. **Shimada, H., and T. Fukasawa.** 1985. Controlled transcription of the yeast regulatory gene *GAL80*. Gene **39:**1–9.
24. **Silhavy, T. J., M. J. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions, p. 133–134. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.

24a. **Smits, P. H. M., M. De Hann, C. Maat, and L. A. Grivell.** 1994. The complete sequence of a 33 kb fragment on the right arm of chromosome II from *Saccharomyces cerevisiae* reveals 16 open reading frames, including ten new open reading frames, five previously identified genes and a homologue of the *SCO1* gene. Yeast **10:**75–80.

25. **Stinchcomb, D. T., C. Mann, and R. W. Davis.** 1982. Centromeric DNA from *Saccharomyces cerevisiae*. J. Mol. Biol. **158:**157–179.
26. **St. John, T. P., and R. W. Davis.** 1979. Isolation of galactose-inducible DNA sequences from *Saccharomyces cerevisiae* by differential plaque hybridization. Cell **16:**443–452.
27. **St. John, T. P., and R. W. Davis.** 1981. The organization and transcription of the galactose gene cluster of *Saccharomyces*. J. Mol. Biol. **152:**285–315.
28. **Tajima, M., Y. Nogi, and T. Fukasawa.** 1985. Primary structure of the yeast *GAL7* gene. Yeast **1:**67–77.
29. **Torchia, T. E., and J. E. Hopper.** 1986. Genetic and molecular analysis of the *GAL3* gene in the expression of the galactose/melibiose regulon of *Saccharomyces cerevisiae*. Genetics **113:**229–246.
30. **Tsuyumu, S., and B. G. Adams.** 1974. Dilution kinetic studies of yeast population, *in vivo* aggregation of galactose utilizing enzymes and positive regulator molecules. Genetics **77:**491–505.
31. **Vernet, T., D. Dignard, and D. Y. Thomas.** 1987. A family of yeast expression vectors containing the phage f1 intergenic region. Gene **52:**225–233.
32. **Winge, O., and C. Roberts.** 1948. Inheritance of enzymatic characters in yeast and the phenomenon of long-term adaptation. C. R. Trav. Lab. Carlsberg. Ser. Physiol. **24:**214–315.
33. **Yun, S.-J., Y. Hiraoka, M. Nishizawa, K. Takio, K. Titani, Y. Nogi, and T. Fukasawa.** 1991. Purification and characterization of the yeast negative regulatory protein GAL80. J. Biol. Chem. **266:**693–697.