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Genetics

Functional domains of the yeast regulatory protein GAL4

(eukaryotic gene regulation/galactose system regulation)

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ABSTRACT **In the yeast *Saccharomyces cerevisiae* regulation of the galactose/melibiose regulon rests on a dosage-dependent functional interplay between the positive regulator of transcription, the GAL4 protein, and the negative regulator, GAL80 protein. We have used this interplay to select *in vitro* generated fusions between the yeast *ADH1* promoter and *GAL4* coding sequence that overproduce GAL4 protein, allowing the identification of GAL4 protein in crude extracts from yeast. One type of these constructions produces a GAL4 protein that lacks its normal NH_2 terminus. This protein is unable to complement a *gal4* lesion but still retains a domain that functionally antagonizes the negative regulatory protein. One defect in this truncated protein is its inability to be concentrated in the nucleus. However, the nuclear localization defect is complemented by full-length GAL4 protein. The truncated protein also appears to effect changes in the relative transcriptional levels of the structural genes. These observations imply that GAL4 protein consists of several domains, including ones for nuclear localization, interaction with the negative regulatory protein, and, possibly, separable transcriptional activation domains for the structural genes.**

The galactose/melibiose metabolism system of the yeast *Saccharomyces cerevisiae* is a paradigm for studying eukaryotic gene regulation. The transcription of four structural genes, whose expression is required to cleave external melibiose (*MEL1*) and to convert galactose to glucose 1-phosphate (*GAL1, GAL7, GAL10*), is induced by galactose and severely repressed by the presence of glucose. Control of transcription is largely mediated through the products of positive (*GAL4*) and negative (*GAL80*) regulatory genes. The *GAL4* gene is defined by recessive mutations (*gal4*) that make the system uninducible and semidominant $GAL4^c$ alleles that allow constitutive transcription of the structural genes. There are also two types of alleles that characterize the *GAL80* gene—recessive *gal80* lesions that permit constitutive expression and dominant $GAL80^s$ alleles that suppress expression of the *GAL/MEL* structural genes under all growth conditions. The *gal4* mutations are epistatic to the *gal80* and $GAL80^s$ alleles (1, 2).

GAL4-responsive sequences have been delineated 5′ to *GAL* structural genes (3–6), and recently GAL4 protein has been shown to specifically protect bases in these sequences *in vivo* (7, 8). Since GAL4 RNA (9) and protein activity (10, 11) are produced constitutively, it follows that GAL80 protein blocks GAL4 protein activity through a posttranslational interaction rather than by repressing transcription of the *GAL4* gene. Dosage studies with cloned *GAL4* (2) and *GAL80* genes (12) have implied that GAL80 protein may also interact with control regions at the structural genes. In keeping with the above observations, two working models for GAL4–GAL80-mediated regulation have been proposed—one in which a GAL4–GAL80 protein complex interacts with control regions (2) and one in which GAL4 and GAL80 proteins independently compete for sites in the control sequences (12). Several other fungal regulatory systems appear to rest on a similar balance between positive and negative regulatory proteins (1, 13, 14).

Toward the aim of determining the details of GAL4–GAL80 regulation of the galactose/melibiose system, we have first attempted to identify GAL4 protein *in vivo* in yeast. This has necessitated the creation of overproducers of GAL4 protein since GAL4 mRNA (and presumably protein) is made in very low amounts under normal conditions [≈0.001% of poly(A)-enriched RNA (ref. 8; this work)]. We describe here the construction of yeast strains that overproduce GAL4-encoded protein using a selection scheme that relies on the fact that regulation of the *GAL/MEL* structural genes rests on a balance between positive and negative regulatory proteins (2, 12).

A related aim is to define the various functions of GAL4 protein and to demarcate the sequences of *GAL4* gene that encode these functional domains. While generating the overproducing GAL4 constructions, we made one that produced an NH_2-terminal truncated protein. This protein is not only unable to nuclear-localize (15), but, as we show here, its phenotypic effects indicate that the protein still retains the domain that functionally antagonizes the GAL80 protein activity. We also present evidence that suggests that the truncated protein is "helped" into the nucleus by wild-type GAL4 protein and that there are two regions of GAL4 protein for activation of transcription of the structural genes.

MATERIALS AND METHODS

Strains and Plasmids. Yeast strains described in Table 1 are closely related to the standard strain SJ21 (**a** *ade1 gal4-2 ura3-52 leu2-3,112 MEL1*) and have been described (2). SJ21R is a spontaneous *GAL4* revertant of SJ21 and was used as a wild-type standard. Δ21R is strain SJ21R with a 1-kilobase (kb) *Xho* I–*Sal* I deletion of *GAL4* chromosomal sequence and contains no measurable GAL4-hybridizable mRNA. The protease-deficient strain used was 5d (α *pep4 ura3-52 leu2-3,112 gal4-2*), which is closely related to SJ21. Plasmids were maintained and propagated in the *Escherichia coli* strains RR1 and DH1. Media and growth conditions have been described (2).

The *CEN3* and *ars1* sequences in plasmid YCpLF+4 are from plasmid pYe(CEN3)41 (16). The *ADH1* promoter sequence is from plasmid AAH5 provided by G. Ammerer (17).

Nucleic Acid and Protein Analysis. DNA sequencing was done essentially by the method of Maxam and Gilbert. The S1 nuclease transcriptional start-site was determined by incubating 50 μg of total RNA with DNA 5′ end-labeled at the *Xho*

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Abbreviations: kb, kilobase(s); bp, base pair(s).

I site of p41-1 (Fig. 3*b*). Incubations were overnight at 50°C and S1 nuclease treatment was at 45°C for 30 min following the protocol of Maniatis *et al.* (18). RNA transfer blot analysis was done by the method of Thomas (19) using vacuum-distilled glyoxal. RNA transfer blot and Southern hybridizations were done using nitrocellulose and nick-translated DNA fragments labeled with a kit from Bethesda Research Laboratories. The plasmid used to probe for GAL10 mRNA was Sc4911 provided by T. St. John and for MEL1 the plasmid was pMP555 (20). Protein for immunoblots was prepared by glass-bead vortex spinning of yeast cells in a 5 mM Na_2HPO_4/1 mM $MgCl_2$, pH 6.5, buffer and pelleting the crude extract. The pellet was resuspended in 1% $NaDodSO_4$ buffer for loading on $NaDodSO_4$ gel—most of the GAL4 protein is found in pellets of crude extracts. Proteins were transferred by the method of Towbin *et al.* (21) and the nitrocellulose was treated as described by Batteiger *et al.* (22). Antibody to GAL4 protein was produced in rabbits. The antigen was a partially purified GAL4 protein isolated from *E. coli* harboring a λP_L fusion to the *GAL4* coding sequence. The overproduced GAL4 polypeptide was from sequences between the *Xho* I and *Sal* I restriction sites in *GAL4* and, thus, was missing the first 74 and last 434 amino acids of the 881 amino acid protein (unpublished results). α-Galactosidase plate and soluble extract assays were done as described (2).

RESULTS

Selection of Overproducers of GAL4 mRNA. We have shown that increased levels of *GAL4* gene have pronounced effects on the expression of the structural genes in the galactose/melibiose regulon (2). We devised a selection scheme that used this dosage effect to select for enhanced *GAL4* gene expression on plasmids that fused the *GAL4* coding sequence to the strong yeast promoter of *ADH1* (17). The basis of this strategy is that the *GAL80*^{s} allele represses expression of the *GAL1, -7, -10*, and *MEL1* genes so that a GAL4 GAL80^s strain will not grow on galactose medium (23) or express *MEL1*-encoded α-galactosidase activity. Multiple copies of the *GAL4* gene will begin to overcome this repression (2). We reasoned that if a series of fusions were made on a CEN vector between the *ADH1* promoter and *GAL4* coding sequence, we could select successful fusions by their ability to confer growth on galactose in a GAL80^s GAL4 strain and that the relative strength of expression could be determined by the level of α-galactosidase expression since increased GAL4 dosage increases α-galactosidase expression (2). For this purpose the chimeric plasmid depicted in Fig. 1 (YCpLF+4) was constructed. The essential elements of the plasmid are (*i*) the *CEN3* fragment, which maintains copy number at about one per cell (24), (*ii*) the *LEU2* gene for selection of yeast transformants independent of the Gal phenotype, (*iii*) the *ADH1* promoter, and (*iv*) the intact *GAL4* gene. The constructions left a unique *Bam*HI site equidistant (≈450 bp) from the 3′ end of the *ADH1* promoter (marked by the *Eco*RI restriction site) and the translational start of the *GAL4* gene. This plasmid complements a Gal4 strain but does not allow a GAL80^s GAL4 strain to grow on galactose medium or express α-galactosidase activity.

The plasmid YCpLF+4 was cut at the *Bam*HI site and then treated with exonuclease BAL-31. The digestion of the ends was monitored to produce a mean of end points near the *Eco*RI site on the *ADH1* promoter side but somewhat beyond the translational start of the *GAL4* gene on the other side. *Bam*HI linkers were attached and the ends religated, producing a family of plasmids with various fusions of the *ADH1* promoter to the *GAL4* gene. An array of such plasmids recovered from *E. coli* transformants was used to transform a GAL80^s GAL4 leu2 strain to Leu$^+$ phenotype and Gal$^+$

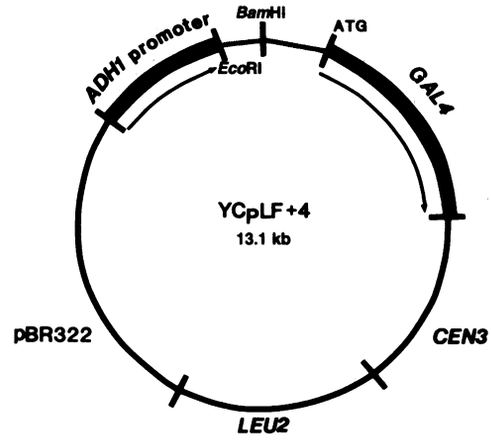

FIG. 1. Organization of the YCpLF+4 plasmid. The *Bam*HI to *GAL4* ATG sequence is genomic DNA upstream of *GAL4* gene. The DNA from the *Bam*HI site to the *Eco*RI site is 2-μm DNA used as "filler." The *Bam*HI to *Eco*RI and *Bam*HI to ATG distances are about 450 base pairs (bp). This plasmid maintains copy number at about one per cell, but because part of the *CEN3*-associated *ars* sequence has been deleted, it is fairly unstable under nonselective conditions (i.e., 5–10% loss per generation).

isolates were selected from these. The Leu$^+$ Gal$^+$ transformants were streaked onto a medium containing glucose, and the relative constitutive expression of α-galactosidase (*MEL1*) was determined in a plate overlay assay. We reasoned that the higher the level of *GAL4* expression, the higher would be the level of *MEL1* expression.

Several transformants that had high α-galactosidase expression in the overlay assays were chosen for further characterization (9 of 130 transformants showed constitutive α-galactosidase activity on glucose medium as tested by plate assay). GAL4 mRNA was overproduced in these isolates, as is apparent in the example shown in Fig. 2. The level of GAL4 mRNA overproduction ranged from 100 to 300 times normal levels. That this overproduction of GAL4 mRNA was due to *GAL4* transcription controlled by the *ADH1* promoter and not loss of copy number control of the CEN plasmid was demonstrated in two ways. (*i*) In crosses between transformants and a GAL80^s leu2 strain, most of the meiotic products segregated 2:2 for the Gal$^+$, constitutive phenotype, as would be expected for a CEN vector and not for a high copy number plasmid. The Leu$^+$ and Gal$^+$ phenotypes cosegregated, indicating that the Gal$^+$, constitutive phenotype was not due to a chromosomal *gal80* or *GAL4*^{c} mutation (unpublished data). (*ii*) The transcription of *GAL4* was regulated in a

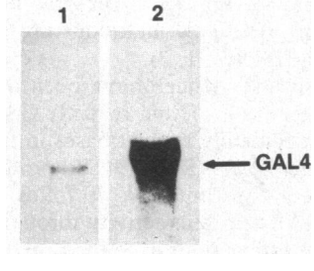

FIG. 2. Overproduction of GAL4 mRNA. RNA was run on a 1% agarose gel and blotted to nitrocellulose. The blot was probed with a nick-translated (4×10^8 cpm/μg) fragment of GAL4 comprising 2.5 kb of the coding sequence. Lane 1, 13 μg of poly(A)-enriched mRNA from a wild-type strain, SJ21R, grown on glycerol/lactic acid medium; lane 2, 40 μg of total RNA from strain Δ21R bearing p41-1 plasmid grown on glucose medium (without leucine). p41-1 is a plasmid derived from YCpLF+4, which fuses *ADH1* to *GAL4* coding sequence to produce a Gal$^+$ phenotype in a GAL80^s GAL4 strain.

manner characteristic of the *ADH1* gene. Denis *et al.* (25) have shown that ADH1 steady-state RNA levels are lower by a factor of 10 in yeast grown on a nonfermentable carbon source than on glucose medium. In contrast, wild-type GAL4 RNA levels are increased severalfold on a nonfermentable versus glucose medium (9), the reverse of the control of the *ADH1* promoter. RNA transfer blot analysis from a strain bearing the *ADH1–GAL4* fusion gene grown on glucose medium showed 10-fold more GAL4 RNA than when grown on glycerol medium—consistent with *ADH1* regulation of *GAL4* expression (data not shown).

Two Classes of *ADH1–GAL4* Fusions. Plasmids from the selected yeast strains were extracted and introduced into a GAL80 gal4 leu2 strain. Surprisingly, two types of plasmids were found—some could complement the *gal4* lesion and others could not. One type of plasmid, represented by p1A, allowed a GAL80^s GAL4 strain and a GAL80 gal4 strain to grow on galactose, whereas the other type of plasmid, represented by p41-1, allowed growth on galactose medium of the GAL80^s GAL4 strain but not the GAL80 gal4 strain. To determine if the phenotypes observed were influenced by the presence of a *GAL80^s* allele, the p1A and p41-1 plasmids were introduced into GAL80 GAL4 and GAL80^s gal4 strains. The phenotypes of these strains with and without the p1A and p41-1 plasmids are presented in Table 1. Apparently the p1A plasmid produces a GAL4 protein that has full biological activity and the p41-1 plasmid produces a GAL4 protein that relieves the repressive effects of the GAL80 or GAL80^s protein but cannot complement a *gal4* mutation. Because of this unusual phenotype, the p41-1 *ADH1–GAL4* fusion was characterized further.

Characterization of the p41-1 Plasmid and Its Products. Restriction enzyme analysis of p41-1 indicated that part of the 5′-terminal protein coding sequence of *GAL4* was missing. The sequence at the junction between the *ADH1* promoter and *GAL4* in plasmid p41-1 is presented in Fig. 3*a*. The BAL-31 exonuclease digestion had left the *ADH1* promoter intact with 31 bp of the filler DNA remaining. The digestion on the *GAL4* side of the restriction site had proceeded 142 bp into the translational coding sequence of *GAL4*, with the *Bam*HI site demarcating the boundary between the *ADH1* and *GAL4* regions of the fusion. Fig. 3*a* also shows the S1 nuclease-determined transcriptional start sites of the p41-1 GAL4 RNA produced on glucose medium. The major start site in p41-1 is five bases upstream from the normal start of the *ADH1* gene (26). Sequencing of the p1A plasmid junction site indicated that the *ADH1* promoter is fused 5 bp upstream of the first ATG in *GAL4* sequence (unpublished data).

The first ATG sequence downstream from the S1 nuclease-determined transcriptional start sites in p41-1 would be the in-frame ATG at +234 of the translational sequence of *GAL4* (Fig. 3*b*). When the remaining 76 bp of *GAL4* sequence between the *Bam*HI and *Xho* I (+217) restriction sites in p41-1 were deleted (Fig. 3*b*), the resulting plasmid still conferred the phenotype of p41-1, arguing that translation

Table 1. Phenotypes produced by p41-1 and p1A plasmids in various genetic backgrounds

Yeast strain transformed	Plasmid					
	Growth on galactose			Constitutive *MEL1* expression		
	p1A	p41-1	None	p1A	p41-1	None
GAL80^s GAL4	+	+	−	+	+	−
GAL80 gal4	+	−	−	+	−	−
GAL80 GAL4	+	+	+	+	+	−
GAL80^s gal4	+	−	−	+	−	−

Constitutive expression of *MEL1* was determined by a plate assay of the strains grown on glucose medium.

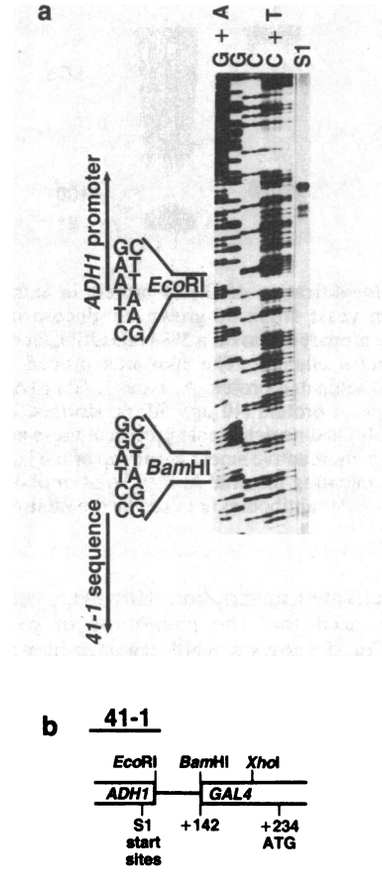

FIG. 3. (*a*) Sequence at the fusion site between *ADH1* and *GAL4* genes in p41-1 and the S1 nuclease-determined transcriptional start sites. (*b*) Organization of sequences at the junction site in p41-1.

probably does start at the AUG encoded by the +234 ATG. The p41-1 *ADH1–GAL4* fusion would then encode a truncated GAL4 protein, missing a minimum of 78 amino acids of its NH_2 terminus. This missing fragment is basic, containing 23% lysine plus arginine, and has six cysteine residues (27).

If the p41-1 plasmid encoded the protein predicted, it would have a M_r of ≈91,000 versus M_r 101,000 for full-length GAL4. Using antibody to a portion of GAL4 protein produced in *E. coli* (see *Materials and Methods*), we identified full-length GAL4 protein and the putative truncated form in protein extracts from yeast. To be able to increase the amount of GAL4 protein produced, the *ADH1–GAL4* fusions were moved from the single-copy CEN vector to a high-copy-number plasmid, p78/10 (ref. 28; D. Thomas, personal communication), and the plasmids were maintained in the protease-deficient (pep4) yeast strain 5d (29). Fig. 4 is an immunoblot demonstrating that yeast bearing the p41-1 *ADH1–GAL4* fusion do produce a protein of the predicted molecular weight. We estimate the amount of the p1A and p41-1 encoded proteins to be about 0.01–0.1% total yeast protein. These proteins were not detectable in 5d transformed with the parental plasmid (78/10) or bearing the *ADH1–GAL4* fusions on CEN plasmids (data not shown).

Phenotypic Effects of the p41-1 Plasmid. The gross phenotypic effects of the p41-1 plasmid seemed to mimic those of a *gal80* allele—i.e., the repressive effects of GAL80 protein are relieved but expression of the structural genes occurs only in the presence of an intact *GAL4* allele. The initial indications were that the truncated protein overproduced by the p41-1 plasmid was capable of titrating out the repressive effects of GAL80 protein, was unable to complement a *gal4* lesion, but did not interfere with wild-type GAL4 protein's

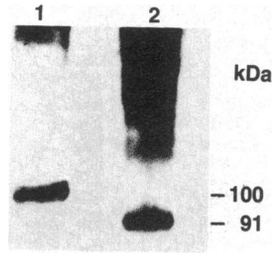

FIG. 4. Identification of GAL4 protein in extracts from yeast. Protein from yeast strain 5d grown on glucose medium (without leucine) was prepared, run on a 5% $NaDodSO_4$/acrylamide gel, and blotted to nitrocellulose. The blot was probed with anti-GAL4 antibody and iodinated protein A. Lane 1, 5d+p1A protein (40 μg); lane 2, 5d+p41-1 protein (40 μg). 5d transformed with the parental plasmid (78/10) had no detectable protein at these positions but does show the immunoreactive smear at the top of the lane. This smear is due to contaminating material in the GAL4 antibody and does not appear when other antibodies are used on the same extracts (data not shown).

ability to activate transcription. However, with further analysis it appeared that the phenotype of p41-1 was more complex. Fig. 5 shows a RNA transfer blot analysis of the transcription of *GAL10* and *MEL1* genes in various related strains grown on a nonfermentable carbon source (i.e., noninducing, nonrepressing). In the wild-type strain there is a low-level basal expression of MEL1 RNA but not of GAL10 RNA (lane 5). This basal expression of *MEL1* is *GAL4* dependent since in a strain bearing a disruption of *GAL4* gene there is no detectable expression of MEL1 RNA (lane 4) nor is there expression in the gal80 gal4$^{\Delta}$ strain (lane 2). Apparently, then, even in the absence of GAL80-mediated repression, the GAL4 protein is required for expression of RNA of the *GAL10* or *MEL1* genes. In a gal80 GAL4 strain there is high-level expression of GAL10 and MEL1 RNAs (lane 3) and, as expected, there is also a high-level expression in a GAL80 GAL4 strain bearing the p41-1 plasmid (lane 1). If one compares lanes 1 and 3 in Fig. 5 it is clear that the relative levels of MEL1 and GAL10 RNA in each lane are reversed. In wild-type (2, 20) and gal80 strains (ref. 2; Fig. 5, lane 3) *MEL1* expression is higher than that of *GAL10*. The overproduction of the p41-1-encoded protein appears to reverse the relative level of expression (lane 1, Fig. 5).

Initially, RNA transfer blot analysis from a strain containing the p41-1 plasmid and a disruption of the chromosomal *GAL4* gene showed no detectable *GAL10* or *MEL1* transcripts. However, it was noticed that this strain grew slightly faster on glycerol/galactose medium than medium containing only glycerol, implying that there may have been a low-level expression of the *GAL1*, *-7*, and *-10* genes. A more sensitive RNA transfer blot analysis did detect a low level of *GAL10* transcript but not of *MEL1*. The steady-state level of GAL10 RNA was substantially lower than the basal *MEL1* expression in a wild-type strain (data not shown). The NH_2-terminal truncation of GAL4 protein, then, largely, but not completely, abolished GAL4 protein's ability to activate transcription and changed the relative steady-state transcript levels of the *GAL10* and *MEL1* genes, both in the absence and presence of a wild-type chromosomal copy of *GAL4* gene.

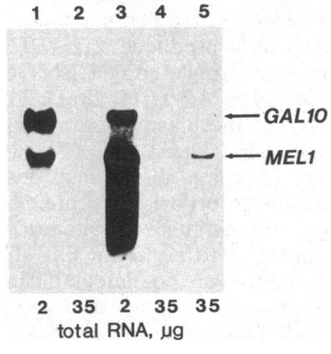

FIG. 5. RNA transfer blot demonstrating that p41-1-encoded protein changes the relative transcription of *MEL1* and *GAL10* genes. RNA was extracted from yeast strains grown on glycerol/lactic acid medium, separated on a 0.8% agarose gel, and blotted to nitrocellulose. Probes were nick-translated internal fragments of *GAL10* and *MEL1* genes labeled to the same specific activity (2×10^8 cpm/μg). Lane 1, SJ21R+p41-1; lane 2, gal4$^{\Delta}$ gal80 strain; lane 3, GAL4 gal80 strain; lane 4, gal4$^{\Delta}$ GAL80 strain (Δ21R); lane 5, wild-type strain, SJ21R.

Though the p41-1-encoded protein activates very low-level transcription by itself, in the presence of the normal low level, wild-type *GAL4* expression the overexpressed p41-1 protein permits constitutive expression of *MEL1* (Table 2) and the *GAL* structural genes (data not shown). There is circumstantial genetic evidence (2, 30) that GAL4 protein forms multimers, as might be expected of a DNA-binding protein. If this is the case, the association between wild-type and p41-1 protein apparently does not lessen the activity of the complex, as seen in Table 2. In the presence of wild-type *GAL4* expression, the p41-1 protein leads to as high a level of constitutive *MEL1* expression as does overexpressed full-length GAL4 produced by p1A plasmid. It seems that the p41-1 protein is fully complemented by wild-type levels of expression of GAL4 protein. The relative levels of α-galactosidase activity on glucose medium increases approximately proportionately to the GAL4 RNA levels. As also evident in Table 2, the p41-1 protein conditions higher than wild-type induced levels of α-galactosidase activity.

DISCUSSION

The galactose system, as well as several other systems in fungi, employs a balance between positive and negative regulatory proteins to control expression, and we have used this aspect of the regulation of the galactose/melibiose regulon of *S. cerevisiae* to select fusions between the strong yeast promoter *ADH1* and the *GAL4* coding sequence that overproduce GAL4 protein in yeast. By moving these selected fusions to high-copy-number plasmids we have greatly overproduced GAL4 protein and have identified it in crude protein extracts of yeast by immunoblots.

The most interesting point of the work we report is the phenotype of the NH_2-terminal truncated GAL4 protein and its implications toward the roles of GAL4 in regulation. The p41-1-encoded GAL4 protein is probably missing 78 amino acids of the NH_2 terminus. Overproduction of this protein relieved the repressive effects of GAL80 or GAL80^s proteins in the presence of wild-type *GAL4* expression. Even when overproduced, however, the truncated protein could not complement a gal4$^{\Delta}$ strain for growth on galactose and only activated very low-level transcription of *GAL10*. In addition,

Table 2. Effects of GAL4 plasmids on α-galactosidase activity

Plasmid	Relative GAL4 RNA levels on glucose	Medium	
		Glucose	Galactose
None (wild-type)	1	0	100
YEp24+GAL4	10	0.2	290
p41-1	300	2.0	360
p1A	200	1.8	160

All activities were determined in strain SJ21R, with or without plasmids. SJ21R is wild-type with respect to GAL4. Values are percent wild-type induced levels.

a GAL4 strain overproducing the truncated protein constitutively expressed the *GAL10* and *MEL1* genes, but the ratio of *MEL1* to *GAL10* transcripts was reversed compared to the constitutive expression in a GAL4 gal80 strain and to the induced expression in wild-type strains.

Silver *et al.* (15) have shown that when full-length or COOH-terminal deletions of GAL4 protein are fused to *E. coli* β-galactosidase protein, β-galactosidase activity will be localized to the nucleus. However, a fusion of GAL4 protein missing the first 74 amino acids to β-galactosidase could not enter the nucleus. Therefore, the p41-1-encoded protein described here should be defective in nuclear localization and this would explain its inability to complement a $gal4^{\Delta}$ strain. However, the constitutive expression of the *GAL/MEL* structural genes conditioned by p41-1 in a GAL4 $GAL80^{s}$ strain implies that the truncated protein still retains the GAL80-interactive region. Conceivably, the p41-1-encoded protein could be interacting with the GAL80 protein only outside the nucleus. However, the overproduction of the p41-1 protein reverses the relative expression of the *GAL10* and *MEL1* genes, suggesting that the truncated protein may get into the nucleus. Possibly the truncated protein is transported in association with the wild-type protein. Curiously, though, the overexpressed p41-1 protein conditioned as much expression of the structural genes as was conditioned by the overexpressed full-length GAL4 protein (Table 2), suggesting that transport of the p41-1 protein into the nucleus may not be stoichiometric.

Recently it has been demonstrated that GAL4 is a DNA-binding protein with specificity for DNA sequences 5′ to two of the *GAL* structural genes (7, 8). We have found that GAL4 protein produced in *E. coli* also has a high-affinity, nonspecific DNA-binding capability that requires the sequences deleted in the p41-1 construction (unpublished data). As yet, the specific amino acid sequences responsible for GAL4 protein's activation of transcription have not been identified. Laughon and Gesteland (27) noted a sequence starting at amino acid 84 in full-length GAL4 that has homology to the α–turn–α sequences in prokaryotic regulatory proteins. This sequence would only be 5 amino acids from the NH_2 terminus in the p41-1-encoded protein. If it were normally involved in activation of transcription one might expect its new proximity to the NH_2 terminus to perturb the function of this domain. This may bear on the observation that the p41-1 construction conditions a change in the relative transcript levels of MEL1 versus GAL10. The implication that GAL4 protein interacts differently with control regions for *GAL10* and *MEL1* genes suggests that there may be separable regions in GAL4 protein for the activation of transcription of these genes.

Laughon and Gesteland (27) noted that there were two transcripts from the *GAL4* gene, one starting just upstream of the ATG presumed to initiate full-length, fully active GAL4 protein and another transcript, comprising about 30% of the total initiations, that starts about 30 bases downstream of this ATG. In this shorter transcript the first in-frame ATG would be the same one initiating the p41-1-encoded protein. Therefore, the shorter transcripts noted *in vivo* would encode a biologically active protein—one that is (*i*) nuclear-excluded but may be helped into the nucleus by full-length GAL4 protein, (*ii*) interactive with the negative regulatory protein, and (*iii*) capable of activating transcription. The biological rationale, if any, for encoding two proteins from *GAL4* sequences remains to be found.

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