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The DNA Binding and Activation Domains of Gal4p Are Sufficient for Conveying Its Regulatory Signals

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The transcriptional activation function of the *Saccharomyces cerevisiae* activator Gal4p is known to rely on a DNA binding activity at its amino terminus and an activation domain at its carboxy terminus. Although both domains are required for activation, truncated forms of Gal4p containing only these domains activate poorly in vivo. Also, mutations in an internal conserved region of Gal4p inactivate the protein, suggesting that this internal region has some function critical to the activity of Gal4p. We have addressed the question of what is the minimal form of Gal4 protein that can perform all of its known functions. A form with an internal deletion of the internal conserved domain of Gal4p is transcriptionally inactive, allowing selection for suppressors. All suppressors isolated were intragenic alterations that had further amino acid deletions (mini*GAL4*s). Characterization of the most active miniGal4 proteins demonstrated that they possess all of the known functions of full-length Gal4p, including glucose repression, galactose induction, response to deletions of *gal11* or *gal6*, and interactions with other proteins such as Gal80p, Sug1p, and TATA binding protein. Analysis of the transcriptional activities, protein levels, and DNA binding abilities of these miniGal4ps and a series of defined internal mutants compared to those of the full-length Gal4p indicates that the DNA binding and activation domains are necessary and sufficient qualitatively for all of these known functions of Gal4p. Our observations imply that the internal region of Gal4 protein may serve as a spacer to augment transcription and/or may be involved in intramolecular or Gal4p-Gal4p interactions.

Transcriptional activators have modular but complex domain structures. In addition to DNA binding domains and activation domains, some activators have other regulatory domains, such as the ligand binding domains of the steroid hormone receptors (69). The *Saccharomyces cerevisiae* activator Gal4p is a modular protein of 881 amino acids (aa) (21). The first 100 aa at the amino (N) terminus of Gal4p are sufficient for its DNA binding, dimerization, and nuclear localization (Fig. 1) (8, 9, 26, 57). The last 113 aa at the carboxy (C) terminus have been mapped as the activation domain (Fig. 1) (36). The core activation function can be further constrained to a 34-aa stretch at the very end of the C terminus (33, 36) that has been shown to interact with basal transcription factors, most strongly with the TATA binding protein (TBP) (39). Due to the difficulty of obtaining full-length Gal4p in an active form, Gal4 fusion proteins are commonly used as activators in in vitro assays or as controls for measuring the potencies of other activation domains. These fusion proteins generally consist of the Gal4p DNA binding domain fused to its own activation domain or to other strong activation domains, such as the activation domain of the viral activator VP16. However, truncated Gal4ps are weaker activators than full-length Gal4p, even when they are overexpressed (40).

Although it is rarely included in activator fusion constructs, the internal region of Gal4p between the DNA binding domain and activation domain accounts for about two-thirds of the total mass of Gal4p. Its function is not well understood, but several observations imply that this region may play a role in Gal4p function. First, a group of fungal transcriptional regulators have homology within part of their internal regions (10, 37, 54). For example, there is a 61% sequence similarity and 41% identity between aa 232 to 398 of Gal4p and aa 378 to 540 of Lac9p (the Gal4p homolog of the milk yeast *Kluyveromyces lactis*) (54). Another member of this group of proteins, Ppr1p, is a positive regulator of an unrelated metabolic pathway (pyrimidine synthesis). Between aa 272 to 412 of Gal4p and aa 385 to 529 of Ppr1p, there is 51% sequence similarity and 24% identity. Second, missense mutations in this internal region have been found to inactivate Gal4p (22). In a genetic selection for mutations that inactivated Gal4p, 4 of 41 point mutations isolated were localized within or very close to the internal homology domain (22). In addition, a deletion analysis by Stone and Sadowski (60) implied that there are multiple inhibitory domains within the internal region of Gal4p that convey repression on glucose-grown cells (see Fig. 6).

As the transcriptional activator for the expression of galactose catabolism (*GAL*) genes (21), the activity of Gal4p is controlled by multiple levels of regulation. *GAL* gene expression is highly modulated by carbon sources. In the presence of glucose, Mig1p binds to the *GAL4* promoter and represses the transcription of the *GAL4* gene, thereby leading to lower expression of the Gal4p-regulated genes (17, 44). In a noninducing carbon source (glycerol-lactic acid or raffinose), the activity of Gal4p is repressed by Gal80 protein (67). This repression is conveyed by Gal80p binding to the 34-aa activation domain at the C terminus of Gal4p (25, 34–36). Upon the addition of galactose, Gal4p initiates transcription of the *GAL* genes within minutes. The key factor for this rapid induction is Gal3p (58, 68), a catalytically deficient Gal1p (galactokinase) homolog (2, 6). During the induction, Gal3p is thought to interact with Gal80p and alter the interaction between Gal80p and the C terminus of Gal4p such that the activation domain is accessible

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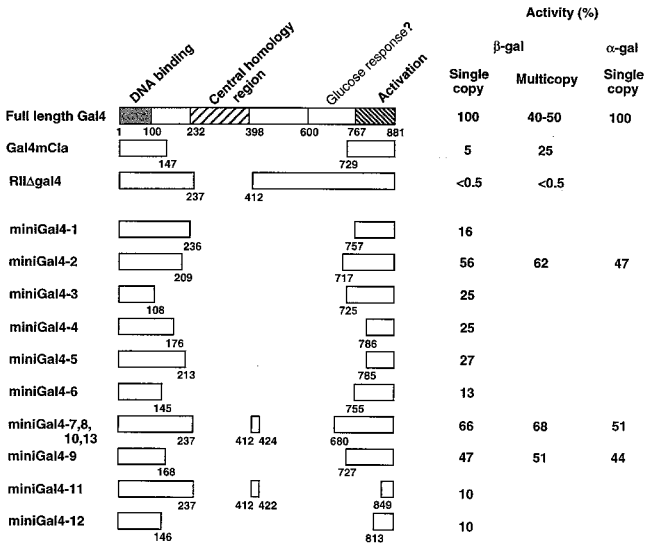

FIG. 1. Schematic representations and activities of the full-length Gal4p, Gal4pmCla, RIIΔgal4p, and the miniGal4ps identified in the genetic selection. All Gal4ps were under the control of the *GAL4* promoter. Single-copy Gal4ps were expressed from the yeast centromeric plasmid pSB32. Multicopy Gal4ps were expressed from the 2μm plasmid YEp351. A chromosomal integrated β-galactosidase (β-gal) gene driven by the *GAL1/10* promoter was used as the reporter gene. Activities of Gal4ps were measured by β-galactosidase assay.

(32, 62). The internal region of Gal4p may play a role in this alteration.

The activity of Gal4p is also influenced by gene products which may not be directly involved in galactose catabolism, such as Gal11p, Sug1p, and Gal6p. Although it was initially identified in a selection for genes involved in galactose metabolism (46), Gal11p has been shown to be a component of the general transcription apparatus (28). Deletion or mutation of *gal11* reduces the expression of all of the *GAL* genes (45, 61). Sug1p was identified via a genetic selection for extragenic suppressors of defects in the activation domain of Gal4p (63). Recent evidence indicates that Sug1p is a component of the regulatory subunit of the 26S proteasome (51, 64). The newly discovered nucleic acid binding protease Gal6p is the yeast homolog of the mammalian bleomycin hydrolase (72). Its transcript and protein levels are regulated by Gal4p, while a deletion of *GAL6* leads to higher expression of the *GAL* genes (76). Unlike the interaction between Gal4p and Gal80p, the effects of these three proteins on Gal4p are not defined and could be mediated through the internal portion of Gal4p.

Here we report the characterization of a series of internally truncated *GAL4* mutants (mini*GAL4*s) identified from a genetic selection. Some of them are nearly fully active at physiological levels although more than half of the wild-type protein is missing. Additional defined internal deletion mutants were also constructed. Their in vivo and in vitro functions were tested and compared to those of the miniGal4ps and the full-length Gal4p. Our findings suggest that the N-terminal DNA binding domain and C-terminal activation domain contain all of the known regulatory response functions of Gal4p. The large internal region may serve as a spacer to quantitatively influence Gal4p's transcriptional potency, possibly through intramolecular or Gal4p-Gal4p interactions.

MATERIALS AND METHODS

Strains, media, yeast transformation, and β-galactosidase assay. *S. cerevisiae* YJ0Z is *MAT*a Δ*gal4* Δ*gal80 ura3-52 leu2-3,112 his3 ade1 trp1 MEL1* and has a integrated *lacZ* reporter gene controlled by the *GAL1* promoter (32). YJ0Z80 is YJ0Z with a copy of *GAL80* integrated into the chromosome at the *URA3* locus (33). YJ0Z80Δgal3 results from replacing the *Nsi*I-*Eco*RV fragment of chromosomal *GAL3* with the *HIS3* gene in YJ0Z80. YJ0Z80Δgal1Δgal3 results from replacing the entire coding region and the following 60-bp 3′ untranslated region of chromosomal *GAL1* with the *TRP1* gene in YJ0Z80Δgal3.

Growth media were as described previously (56). Glucose liquid cultures contained 2% glucose. Uninduced liquid cultures contained 3% glycerol and 2% lactic acid (Gly-Lac). Two percent galactose was added to uninducing media for galactose induction. The lithium acetate method was used for yeast transformations (20).

α-Galactosidase and β-galactosidase activities were assayed as described previously with whole-cell extracts prepared by the glass bead method (24). All assays were done with at least three independent transformants with standard deviations of less than 20%. For illustration purposes, the activities of Gal4p derivatives are presented as percentages of the activity of full-length Gal4p in the same background. The β-galactosidase activity of pSB32-Gal4 is 2,000 to 2,500 U in strain YJ0Z and 1,000 to 1,500 U in strain YJ0Z80 when fully induced by galactose. The α-galactosidase activity of pSB32-Gal4 is 530 to 570 U in strain YJ0Z.

Plasmids and sequencing. Full-length *GAL4* was cloned as a *Bam*HI-*Hin*dIII fragment including its native promoter into the *LEU2* CEN4 plasmid pSB32 as described previously (33). pSB32-RIIΔgal4 was made by replacing the *Xba*I-*Sal*I fragment of *GAL4* with a double-stranded annealed oligonucleotide having an *Nco*I or *Sma*I site. pSB32-Gal4mCla was constructed by K. Leuther and contained the first 147 and 152 aa of Gal4p (31).

The DNA sequences encoding aa 571 to 881, 672 to 881, 718 to 881, 789 to 881, and 842 to 881 were PCR amplified as *Sal*I-*Hin*dIII fragments by using full-length *GAL4* as a template. These fragments were used to exchange the *Sal*I-*Hin*dIII fragment in pSB32-GAL4 or pSB32-RIIΔgal4 to result in IntΔ plasmids designated as IntΔ1 to -10.

Multicopy *GAL4* and derivatives were cloned by inserting the *Bam*HI-*Hin*dIII fragment from the respective pSB32 clones into the *Bam*HI-*Hin*dIII sites of the 2μm plasmid YEp351.

S10 tagging of Gal4p was done by inserting a double-stranded, annealed oligonucleotide encoding the 11-aa S10 (from phage T7) epitope (Novagen) into the *Nco*I site at the start ATG of *GAL4*. S10-tagged mini*GAL4*s were constructed by exchanging the *Xho*I-*Hin*dIII fragments between mini*GAL4*s and tagged full-length *GAL4*.

The Δgal3 plasmid was made by replacing the *Nsi*I-*Eco*RV fragment of a pUC-GAL3 plasmid with the *Nsi*I-*Sma*I fragment of *HIS3*. The *Ssp*I-*Xho*I fragment of the Δgal3 plasmid was used to disrupt *GAL3*. The construction of a Δgal1 plasmid utilized the pYES2.0 plasmid (Invitrogen), which contains the *GAL1* promoter. First, a 500-bp 3′ untranslated region of *GAL1* was obtained by PCR and cloned as a *Bam*HI-*Xho*I fragment into pYES2.0, and then a *Kpn*I-*Bam*HI fragment of *TRP1* was cloned into the polylinker of pYES. The *Spe*I-*Xho*I fragment of the Δgal1 plasmid was used to disrupt *GAL1*.

gal4D in pSB32 was described previously (63) and consists of Gal4p truncated at aa 853. mini*gal4D*s in pSB32 were constructed by exchanging the *Pvu*II-*Hin*dIII fragments between mini*GAL4*s and *gal4D*. YEp24-mini*gal4D*s contain the *Bam*HI-*Eco*RV fragment of the pSB32-mini*gal4D*s.

Plasmids for in vitro translations of Sug1p and TBP were described previously (39, 65). For *Escherichia coli* expression, miniGal4ps were cloned into pKM263 (40) for expression as His6-glutathione transferase (His6-GST) fusion proteins from the T7 RNA polymerase promoter, with a TEV protease (Gibco BRL) cleavage site between GST and miniGal4ps.

A Gal80-VP16 fusion protein (the last 78 aa of VP16 fused to the C-terminal end of the full-length Gal80p) was expressed in yeast from the 2μm, ADH1 promoter-driven plasmid pVT102U. This construct was made by inserting an *Nco*I-blunted *Nco*I-*Hin*dIII fragment of Gal80-VP16 into the *Pvu*II-*Hin*dIII site of pVT102U.

The mini*Gal4* mutants and selected plasmid constructs were sequenced by using the fmol PCR sequencing kit (Promega).

Western analysis. For Western analysis of S10-tagged proteins, yeast strain YJ0Z80 was transformed with S10-tagged, full-length Gal4p or miniGal4ps expressed from pSB32. Cells were grown in selective noninducing media to an optical density at 600 nm (OD_{600}) of 0.6. Cells were washed and broken by the glass bead method in buffer A_{50} (25 mM Tris · Cl [pH 7.5], 50 mM NaCl, 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol) in the presence of protease inhibitors. Three volumes of 2× sodium dodecyl sulfate (SDS) sample buffer were added to the unfractionated broken-cell homogenate and stored at −80°C. A small aliquot of the samples without SDS sample buffer was denatured by adding 3 volumes of 2% SDS and boiling for 5 min. The boiled samples were centrifuged in a microcentrifuge at 13,000 × *g* for 1 min. The supernatant was further diluted with water and used to determine the protein concentrations in the samples. Between 150 and 200 μg of total protein was loaded onto a 6% (separating) Tricine-SDS polyacrylamide gel. Proteins were then transferred from the gel to polyvinylidene difluoride membranes (MSI). The membranes were blocked in 5% nonfat dry milk and incubated with anti-S10 antibody (Novagen) as the primary antibody and horseradish peroxidase-conjugated antimouse antibody (Bio-Rad) as the secondary antibody. The membranes were developed by using the ECL Renaissance (chemiluminescence) kit from Dupont.

Western analysis of overexpressed proteins followed a protocol similar to that described above except that the broken yeast cell homogenate was centrifuged at 13,000 × *g* for 15 min in a microcentrifuge and the supernatant was used for SDS gels. An antibody raised against the C-terminal last 146 aa of Gal4p was used to detect Gal4ps.

Western blots were quantitated on a Molecular Dynamics Personal Densitometer with the Imagequant software.

Expression of miniGal4ps and GST pull-down assays. The miniGal4p expression plasmids were transformed into *E. coli* BL21(DE3)(pLysS). Transformants were cultured overnight in L broth with 25 μg of ampicillin per ml and 25 μg of chloramphenicol per ml. Larger cultures in L broth were inoculated 1:50 with the overnight culture with 75 μg of ampicillin per ml and 25 μg of chloramphenicol per ml. Cultures were grown at room temperature to an OD_{600} of 1.0 to 1.2. GST fusion proteins were purified on glutathione-Sepharose 4B as described previously (70), except that the *E. coli* cells were resuspended in phosphate-buffered saline supplemented with 500 mM NaCl and 20 μM $ZnSO_4$. GST pull-down assays were performed as described previously (39).

Gel mobility shift assays. Gel mobility shift assays with crude yeast extracts were performed as described previously (39). Gal4 antibody against its DNA binding domain was as described previously (33). Antibody against the Gal4p C-terminal 146 aa was made by K. Leuther (25). These polyclonal antibodies were immunopurified with a protein A column (Pierce), and the immunoglobulin G fraction was used in the gel mobility shift assays. For comparisons of relative DNA binding abilities, Western analysis was used to monitor the amount of yeast extract so that each DNA binding reaction mixture contained the same amount of Gal4p derivatives. The amount of labeled double-stranded probe ranged from 0.1 to 4 ng in 20-μl binding reaction mixtures.

Image processing. Images on X-ray film or phosphoimager printouts were scanned by using Ofoto 1.1 software and an Apple One scanner at 8 bits/150 to 300 dpi. The images were then imported into MacDraw Pro and then into Adobe Illustrator or Adobe Photoshop for labeling. Figures were printed on a Kodak XLS 8600 PS dye sublimation printer.

RESULTS

Identification of miniGal4ps. The aim of this work is to identify the minimal portion of Gal4 protein that can qualitatively function essentially as wild-type Gal4p at physiological levels. When we initiated these studies, we had found basically two types of Gal4p deletion variants. One type consists of the region of the DNA binding domain fused to that of the activation module, for example, Gal4pmCla. Gal4pmCla has only 5% of wild-type activity when expressed at physiological levels and only 25% when highly overexpressed. On the other hand, RIIΔgal4p, which has a deletion between aa 238 and 411 (the internal conserved region) (54), is transcriptionally inactive even when overexpressed (Fig. 1), despite having intact DNA binding and activation domains. This protein is expressed and stably accumulated in cells (data not shown). To determine the minimal size and amino acid sequence required for Gal4p to be a strong activator, we performed a genetic selection by capitalizing on the inactive RIIΔgal4p. A *gal4*-disrupted yeast strain, YJ0Z, was transformed with a single-copy plasmid (pSB32) expressing RIIΔgal4p under the control of the *GAL4* promoter. As RIIΔgal4p is inactive, variants capable of growing on galactose medium (Gal^+) were selected. Thirteen isolates from 10^9 cells plated were analyzed further. The Gal^+ phenotype of all of the isolates was shown to be plasmid dependent by isolating each plasmid and reintroducing it into the original Δ*gal4* strain. Sequencing of the plasmids identified nine different mutations in which *RIIΔgal4* had undergone further deletions (Fig. 1). These mutants were named mini*GAL*4s. Most interestingly, miniGal4p-2, -7, and -9, although expressed from single-copy plasmids and under the control of the *GAL4* promoter, produced high levels of transcriptional activity. With β-galactosidase as the reporter under the control of *GAL1/10* promoter, miniGal4p-2, -7, and -9 gave 56, 66, and 47% of the wild-type Gal4p activity, respectively (Fig. 1, β-gal). The *GAL1/10* promoter has four Gal4p binding sites and is greatly influenced by the cooperative occupancy of Gal4p. This makes this promoter particularly sensitive to GAL4 protein levels below certain thresholds. While under nonrepressing conditions Gal4p fully occupies the *GAL1/10* promoter, the fivefold decrease in the Gal4p level under repressing conditions leads to about a 200-fold decrease in the expression of *GAL1* (17). However, the *MEL1* gene only has a single Gal4p binding site. It would be expected, then, to respond linearly to the Gal4p level, and we have demonstrated that it does so to two- to threefold overexpression of Gal4p (24). Therefore, to eliminate the nonlinear dosage effect, we tested these miniGal4ps for their ability to stimulate *MEL1* gene expression. We found that miniGal4p-2, -7, and -9, had 47, 51, and 44% of the wild-type activity, respectively, when the natural *MEL1* gene was used as the reporter gene (Fig. 1, α-gal). We concluded from these results that miniGal4ps occupy the *GAL1/10* and *MEL1* promoters to a level similar to that of wild-type Gal4p. We then tested whether these miniGal4ps had other characteristics of full-length Gal4p.

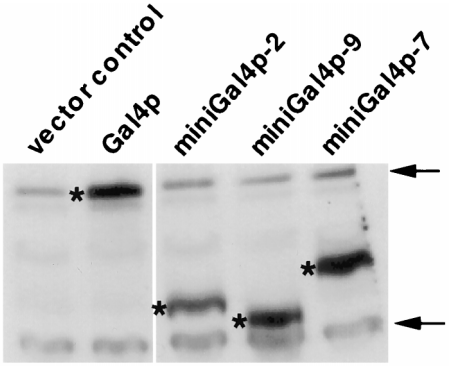

FIG. 2. Similar physiological expression levels of full-length Gal4p and miniGal4ps. The Gal4ps were tagged with the peptide epitope S10 at their N termini and visualized with an anti-S10 antibody. The proteins were expressed from the yeast centromeric plasmid pSB32 under the control of the *GAL4* promoter in the yeast strain YJ0Z80. Crude yeast extracts were used for this Western blot. Asterisks indicate the bands of Gal4ps according to their predicted sizes. Arrows point to nonspecific bands of proteins cross-reacting with the antibody, which served as approximate loading controls.

MiniGal4ps are expressed at a level similar to that of full-length Gal4p. The occupancy of miniGal4ps could be achieved through a higher protein level, and weak activators can give higher transcriptional output when overexpressed. Therefore, it was important to establish whether the near-normal activation functions of these miniGal4ps were due to overexpression. We first confirmed that the high activity of miniGal4ps was not the result of an increase in plasmid copy numbers, since the same activities were observed when mini*GAL4-2*, *-7*, and *-9* were recloned to the vector pSB32 (data not shown). Next, we directly assayed the expression levels of miniGal4ps. The physiological level of Gal4p is extremely low, roughly 100 molecules per cell (73), and difficult to detect with currently available antibodies. Therefore, we epitope tagged miniGal4ps and full-length Gal4p with an 11-aa S10 peptide. Tagging Gal4p with the peptide did not detectably change its activities (data not shown). Using antibody against S10 in a Western analysis, we observed that miniGal4ps were expressed at comparable although slightly higher (130 to 170% of wild-type) levels relative to full-length Gal4p (Fig. 2).

MiniGal4ps are repressed by glucose. The sequences of miniGal4ps showed that they still have both the DNA binding and activation domains (at least the core 34 aa) but that they are missing all of the internal region that has been proposed to be required for the direct response of Gal4p to glucose repression (60). Therefore, we tested whether miniGal4p-2, -7, and -9 still responded to glucose. When these miniGal4ps were ex-

TABLE 1. MiniGal4ps are repressed by glucose

Gal4p[a]	Activity[b] (%) in YJ0Z with:	
	Glycerol	Glucose
Full-length	100	<0.5
MiniGal4p-2	56	<0.5
MiniGal4p-7	66	<0.5
MiniGal4p-9	47	<0.5

[a] All of the Gal4ps were expressed from the yeast centromeric plasmid pSB32 and under the control of the *GAL4* promoter.
[b] The activities of Gal4ps were determined by β-galactosidase assay.

pressed under control of the *GAL4* promoter from a single-copy plasmid, no activity was seen in glucose medium (Table 1). This suggests that, like full-length Gal4p, miniGal4ps are repressed by glucose, presumably via the interaction of Mig1p with the *GAL4* and *GAL1* promoters (17, 23, 30) (see Discussion).

Gal80p interacts with and represses miniGal4ps in noninducing medium. Gal80p-mediated repression and galactose induction are two key characteristics of the Gal4p regulation (21). To test these phenotypes, we expressed miniGal4ps in yeast strain YJ0Z80. This strain has a *gal4* disruption but contains a copy of wild-type *GAL80*. In noninducing glycerol medium, the activity of full-length Gal4p was entirely repressed, whereas miniGal4ps retained marginally detectable (1% or less) activity (Fig. 3A). The release of Gal80p repression was then tested by culturing cells expressing miniGal4ps in galactose-supplemented glycerol medium. MiniGal4ps were induced to essentially the same levels as they were in a Δ*gal80* background (Fig. 1 and 3A).

The very small residual activity for miniGal4ps in glycerol is probably due to the slightly elevated protein levels of the miniGal4ps. In wild-type cells, Gal80p is in a 5- to 10-fold molar excess over Gal4p (18). Overexpression of Gal4p can override the Gal80p repression (18, 24). Moreover, the expression of Gal80p is partially controlled by Gal4p (7). We expect a lower Gal80p level in yeast cells containing miniGal4ps, which have lower activities than the wild-type Gal4p. The observation that miniGal4ps were strongly repressed by Gal80p also confirmed our estimation that miniGal4ps were not significantly overexpressed.

MiniGal4ps are dependent on *GAL3* and *GAL1* for induction. The central player in the galactose induction pathway is Gal3p. Without Gal3p, it takes days to activate the Gal4p-dependent transcription of the *GAL* genes (58, 68). We tested whether miniGal4ps are dependent on this pathway for galactose induction. A *gal3* disruption strain (YJ0Z80Δ*gal3*) was made in the background of wild-type *GAL80*. In this strain, full-length Gal4p showed no activity 24 h after addition of galactose (Fig. 3A). Unexpectedly, miniGal4ps reached the maximum of the galactose-induced activity only slightly more slowly than they did in a wild-type *GAL3* strain (Fig. 3B).

There are two likely explanations: either miniGal4ps have altered responses to Gal3p or some basal level of Gal1p is present in the system. It is known that overexpressed Gal1p causes constitutive *GAL* gene expression and can overcome the effect of a *gal3* deletion (5). We suspected that the induction of miniGal4ps in the Δ*gal3* strain resulted from a small accumulation of Gal1p in cells due to the residual activities of miniGal4ps in noninducing medium. This amount of Gal4p could be sufficient to induce the system maximally through a positive feedback. To test this hypothesis, we disrupted *GAL1* in the YJ0Z80Δ*gal3* strain. We found that in a *gal1 gal3* double disruption strain, miniGal4ps were no longer inducible (Fig. 3A) (see Discussion), arguing that the normal induction pathway is operable in the mini*GAL4* background.

Galactose induction kinetics of miniGal4ps. A recent study indicates that a serine residue at aa 699 (Ser699) of Gal4p is important for the galactose induction process (52). Among the miniGal4ps, only miniGal4p-7 still contains Ser699. Although miniGal4p-2, -7, and -9 were able to be induced fully by galactose to steady-state levels (see above), we were interested in examining their induction kinetics. We focused on miniGal4p-2 and -7, as they had similar levels of activity yet miniGal4p-2 did not contain Ser699 (Fig. 4A). As shown in Fig. 4B, wild-type Gal4p and miniGal4p-2 and -7 were induced by galactose and reached full activity at 12 h after galactose addition. Figure 4C shows that the rates of induction are essentially indistinguishable for miniGal4ps and full-length Gal4p. These results suggest that Ser699 is not required for normal induction kinetics in the context of miniGal4p-2 (see Discussion).

MiniGal4ps respond normally to *GAL6*, *GAL11*, and *SUG1*. The results reported above indicate that the glucose repression, the *GAL80* regulation, and the *GAL3*/*GAL1* induction pathways operate normally through the mini*GAL4*s. In order to detect other possible alterations of wild-type function in the minGal4ps, they were tested against mutations in three genes that have undefined mechanisms for affecting Gal4p function: *GAL6*, *GAL11*, and *SUG1*.

(i) *GAL6*. Gal6p is a newly discovered DNA and RNA (75) binding protease and the yeast homolog of the mammalian bleomycin hydrolase (72). The expression of this abundant protein in yeast is regulated by Gal4p (76). Deletion of this protein is not lethal but accelerates the galactose induction and leads to

A

	Activity (%)			
	YJ0Z80		YJ0Z80Δgal3	YJ0Z80Δgal3Δgal1
	glycerol	glycerol + galactose	glycerol + galactose	glycerol + galactose
Full-length Gal4p	<0.5	100	<0.5	<0.5
miniGal4p-2	0.8	57	55	0.8
miniGal4p-7	1.0	64	61	1
miniGal4p-9	0.6	44	43	0.6

B

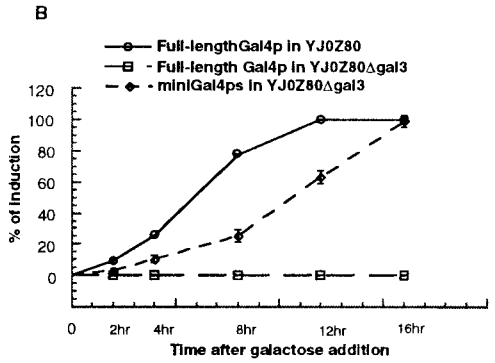

FIG. 3. Response of miniGal4ps to Gal80p repression and their relationship with Gal1p and Gal3p. Gal4ps were expressed from the yeast centromeric plasmid pSB32 under the control of the *GAL4* promoter. Activities of Gal4ps were measured by β-galactosidase assay. (A) MiniGal4ps were repressed by Gal80p and were induced by galactose. MiniGal4ps were induced by galactose in the absence of Gal3p but not in the absence of both Gal3p and Gal1p. (B) Time course of galactose induction in the absence of Gal3p. MiniGal4ps were induced with a small delay. Three miniGal4ps had indistinguishable induction curves, and the average of all three curves was plotted. Two percent galactose was added to a Gly-Lac culture at an OD_{600} of 0.3 to 0.4. Aliquots of the culture were taken after 2, 4, 8, 12, and 16 h for β-galactosidase assay.

higher accumulation of *GAL* gene mRNAs (75). We observed a similar response for miniGal4ps. In a *gal6* deletion strain, 8 h after the addition of galactose to the glycerol medium, there was a threefold increase in activities for miniGal4ps compared to that in a *GAL6* wild-type strain (Table 2).

(ii) *GAL11*. Like other transcriptional activators, Gal4p can also interact with components in the basal transcription apparatus. The activity of Gal4p is influenced by the presence of a component of the general transcription machinery, Gal11p (28). Mutation or deletion of *GAL11* is not lethal but decreases the overall transcriptional output of the cell (61). *gal11* deletion reduces Gal4p-dependent transcription four- to fivefold. When tested in a *gal11* deletion strain, the activities of miniGal4ps were lowered from 5- to 11-fold (Table 3). Overall, miniGal4ps, like full-length Gal4p, seem to depend on Gal11p for full activation of the *GAL* genes.

(iii) *SUG1*. The region between aa 841 and 875 of Gal4p (34-mer) is the core of the activation domain. A *gal4* mutant protein with a deletion of two-thirds of this region (gal4Dp) activates to only 3% compared to full-length Gal4p in vivo (63). *sug1-1* is a suppressor of gal4Dp, rescuing the activity to 60% of that of the full-length Gal4p (63). Sug1p has been identified as a component of the 19S regulatory subunit of the 26S proteasome (1, 51, 64, 65). It has been hypothesized that *sug1-1* affects transcription through altering Gal4p levels (51).

In order to test whether this suppression requires the context of the full-length Gal4p, we constructed 4D mutations (mini*gal4D*s) in the background of the mini*GAL4*s by removing the DNA sequence coding for aa 852 to 881. Expressing the minigal4D proteins under the control of the *GAL4* promoter from single-copy plasmids in both *SUG1* wild-type and *sug1-1* strains, we observed that *sug1-1* increased the activity of minigal4Dp-7 by 18-fold, very similar to the 20-fold increase for gal4Dp (Table 4). The activities of minigal4Dp-2 and -9 were also clearly rescued by *sug1-1*. However, it was difficult to estimate the degree of this rescue because the activities of minigal4Dp-2 and -9 were below our detection limit in a wild-type *SUG1* background (Table 4). By moderately increasing the expression of minigal4Dp-2 and minigal4Dp-9 from the multicopy plasmid YEp24 under control of the *GAL4* promoter, we were able to calculate the fold *sug1-1* rescue. The increases in transcriptional activity were 4- and 13-fold respectively (Table 4). Minigal4Dp-2 on YEp24 was rescued to 38% of the full-length Gal4p activity, about 60% of the activity of miniGal4p-2. This also fits with our knowledge that *sug1-1* can rescue the activity of a weak Gal4p to only 50 to 60% of the wild-type activity, regardless of its inherent activity (63).

MiniGal4ps interact with TBP. The 34-aa core of the Gal4p activation domain interacts with TBP in vitro (39). The strength of TBP binding to activation domains seems to correlate with their activation abilities (39). Conventionally, these in vitro binding assays rely on a fusion between an affinity tag and the activation domain of interest. The fact that miniGal4ps are more readily expressed and isolated than full-length Gal4p (data not shown) provides us with an opportunity to test this interaction in the context of fully functional activators rather than of an activation domain in isolation as usually done. We expressed miniGal4p-2, -7, and -9 in *E. coli* as GST fusion proteins. GST-miniGal4ps were immobilized on glutathione beads and incubated with in vitro-translated [^{35}S]Met-labeled TBP. With a GST–34-mer as the positive control, all three miniGal4ps retained TBP to similar degrees and to levels comparable to that of the GST–34-mer when the protein levels are normalized (Fig. 5).

The Gal4mCla protein is regulated normally. MiniGal4p-2, -7, and -9 possess high-level activity and function like wild-type Gal4p. In contrast, the small Gal4p derivative Gal4pmCla, containing just the assigned DNA binding and activation domains of Gal4p, is a weak activator, conditioning only 5% of wild-type Gal4p activity if expressed at physiological levels. It is possible that the regions deleted in Gal4pmCla relative to miniGal4ps would reveal other regulatory sites. However, when we tested Gal4pmCla for some of the key Gal4p functions such as Gal80p repression, galactose induction, and response to *gal6* or *gal11* deletion, it acted similarly to the wild-type Gal4p or miniGal4ps but at a very low activity level (Table 5).

The only significant difference between Gal4pmCla and miniGal4ps is the strength of transcriptional activity. Even when expressed from multicopy plasmids, the activity of Gal4pmCla is only 25% of the activity which full-length Gal4p achieves with a single-copy plasmid (Fig. 1). MiniGal4ps activated to 50 to 70% regardless of whether they were expressed from single- or multicopy plasmids (Fig. 1). However, the activity of overexpressed wild-type Gal4p is decreased 40 to 50% relative that of Gal4p expressed at normal levels (Fig. 1). Excessive amounts of strong activators, such as Gal4p, are thought to squelch transcription by titrating general transcription factors (3, 16). MiniGal4ps maintain a functional activation domain, yet, unlike wild-type Gal4p, they do not have a measurable squelching effect (Fig. 1). We do not know the basis of this difference in squelching response.

The difference in activities of miniGal4p and Gal4pmCla could be caused by different protein levels and/or DNA binding abilities. Western analysis showed that the steady-state levels of 2μm plasmid-overexpressed Gal4pmCla and miniGal4ps are very similar (Fig. 6A). For DNA binding, gel mobility shift assays with crude yeast extracts containing these overexpressed Gal4pmCla and miniGal4ps revealed comparable binding activities (Fig. 6B). In order to compare the DNA binding abilities of these different Gal4ps more accurately, protein concentrations were held constant while DNA concentrations were titrated from below to above saturation in gel mobility shift assays. Using full-length Gal4p as the reference point, we found that the difference in DNA binding abilities was small (less than 20%) (Table 6). Moreover, the strength of in vitro DNA binding does not seem to strictly correlate with the transcriptional activities of the Gal4ps tested. These results indicate that the difference in activities is not due to the difference in protein levels or in vitro DNA binding activities.

Defined deletions. To further define the function of the internal region of Gal4p, a series of nested deletions between aa 412 and 842 were made and fused to an N-terminal fragment containing either aa 1 to 237 or 1 to 412. The largest deletion terminates just before the core activation domain. These two sets of deletion mutants were expressed from the *GAL4* promoter and the single-copy plasmid pSB32. We found that all deletions containing aa 1 to 412 resulted in inactive protein (IntΔ1 to -5) (Fig. 7). By contrast, some of the same deletions combined with N-terminal aa 1 to 237 (IntΔ7 to -9) (Fig. 7) were active.

The contrast between IntΔ2 to -4 and IntΔ7 to -9 is dramatic and intriguing. Two simple explanations are that IntΔ2, -3, and -4 proteins are unstable in yeast cells or that they have lost DNA binding ability. We tested these possibilities by Western analysis and gel mobility shift assay. We expressed IntΔ2, -3, and -4 from multicopy plasmids for easier detection and found that at this expression level, IntΔ2, -3, and -4 are still inactive in transcription. Western analysis showed that IntΔ2, -3, and -4 are stable in cells. They seem to accumulate at a lower level (two- to threefold) than full-length Gal4p expressed from the same multicopy plasmid (Fig. 8A), which is probably caused by

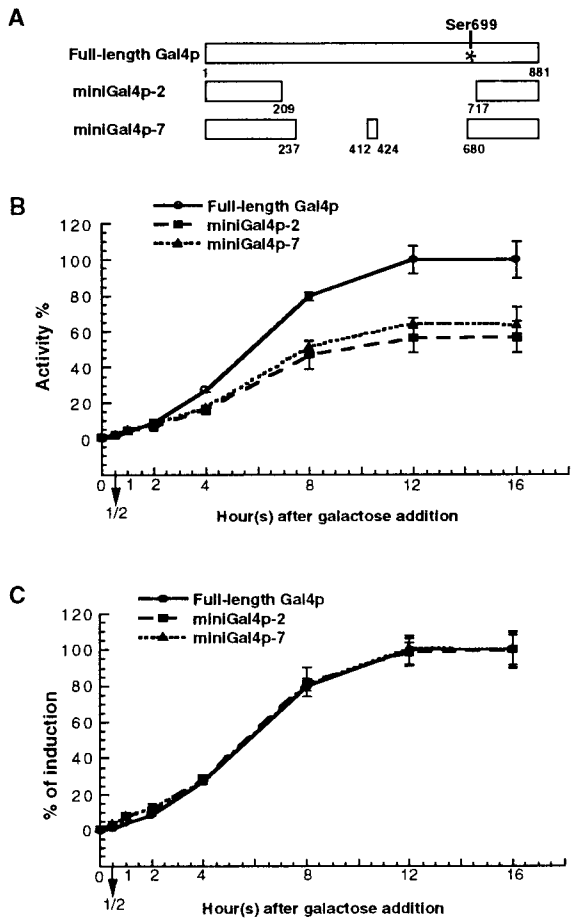

FIG. 4. MiniGal4ps display normal galactose induction kinetics. (A) Schematic representation of wild-type Gal4, miniGal4p-2, and miniGal4p-7 with respect to residue Ser699. (B) Time course of galactose induction of wild-type Gal4, miniGal4p-2, miniGal4p-7. β-galactosidase activities of these Gal4ps were assayed 1/2, 1, 2, 4, 8, and 12 h after Gly-Lac cultures (OD_{600} = 0.3 to 0.4) were induced with 2% galactose. Gal4ps were expressed from the yeast centromeric plasmid pSB32 under the control of the *GAL4* promoter. Yeast strain YJ0Z80 with an integrated copy of *GAL80* under control of its own promoter was used in these assays. (C) The β-galactosidase activities of these Gal4ps at different time points were calculated as the percentages of their own fully induced activities. The rates of induction for all of the Gal4ps tested were nearly identical. Means and standard deviations are shown.

the loss of a 3′ untranslated region of *GAL4* in the cloning process. This lowered protein level is at least fivefold higher than the physiological level of Gal4p. Therefore, we do not expect this to be the major cause of the transcriptional inactivity of IntΔ2, -3, and -4. We also tested the DNA binding abilities of IntΔ2, -3, and -4 by using the gel mobility shift assay as described in the previous section. IntΔ2, -3, and -4 appeared to be able to bind DNA (Fig. 8B) but with a slightly weaker affinity (difference of less than 0.4-fold) (Table 6) than full-length Gal4p. Again, we do not think that this weaker affinity alone is responsible for the inactivity of these mutants. A trivial explanation for the lack of activation is that these constructs have the C-terminal activation domain deleted. The presence of the DNA sequences was confirmed by PCR, and if the deletion was in the proteins, it would necessarily be very small because the proteins migrated to their predicted positions in the Western analysis (Fig. 8A).

A more likely explanation is that IntΔ2, -3, and -4 have a conformational change which allows DNA binding but not activation. The C-terminal activation domain of IntΔ2, -3, and -4 could be interacting with another part of Gal4p and the "trapped" C terminus inaccessible for activating transcription. To test this, we took an in vivo approach utilizing the interaction between the C-terminal activation domain and the Gal80 protein. Previously, a fusion protein was created by fusing the activation domain of the viral activator VP16 to the C terminus of full-length Gal80p (32). Gal4p-specific transcriptional output can be increased through the interaction of Gal80-VP16 with a weak Gal4p derivative in vivo (32). We did not observe any activity of the reporter gene when we coexpressed Gal80-VP16 and IntΔ-2, -3, and -4, both from 2μm plasmids. This suggests that the IntΔ-2, -3, and -4 proteins have structures which could have sequestered their C-terminal activation domains from Gal80p interaction. However, we cannot exclude the possibility that IntΔ-2, -3, and -4 might not be able to occupy Gal4p binding sites in vivo.

DISCUSSION

We have shown that Gal4p derivatives with large sections of the internal region deleted still retain the ability to qualitatively respond to known Gal4p regulatory signals, including glucose repression, *GAL80* inhibition, and induction kinetics. The only qualitative differences noted were the response to *GAL3* and the lack of squelching at high-level expression. This implies that the DNA binding and activation domains are sufficient for conveying most if not all external regulatory signals. Further, some of these deletion derivatives conditioned approximately 50% of the normal activation of *GAL* genes when expressed at physiological levels, indicating that the central region plays a role in the quantitative expression of the structural genes.

MiniGal4ps are bona fide Gal4ps. These nine internally truncated Gal4p mutants were termed miniGal4ps; three of them had high activity (47 to 66% of that of the full-length Gal4p for β-galactosidase activity). They were expressed from centromeric, single-copy plasmids and under the control of *GAL4*'s own promoter. We showed that these miniGal4ps were expressed at a level very close to the normal physiological level of full-length Gal4p.

The detailed characterization indicated that miniGal4ps were repressed by Gal80p, were induced by galactose with the same kinetics, responded normally to deletions of *gal6* or *gal11*, and interacted with TBP. Furthermore, miniGal4ps had sufficient context for *sug1-1* to rescue C-terminal truncations of Gal4p. All of these phenotypes and interactions show that miniGal4ps behave qualitatively like authentic full-length Gal4p.

It has been proposed by Stone and Sadowski that the internal region of Gal4p contains at least three inhibitory regions and a glucose-responsive domain (60). The central inhibitory regions could act independently to convey glucose repression, presumably by recruiting an inhibitory protein which affected DNA binding. This inhibition was counteracted in the absence of glucose by a C-terminal glucose-responsive domain. In contrast to this proposal, we find that all forms of Gal4p with the internal region deleted are fully glucose repressed, even ones with all of the inhibitory regions deleted. Our results are consistent with the model and evidence presented by Griggs and Johnston, where glucose repression is largely conveyed by regulation of *GAL4* transcription levels (17). As has been pointed out previously (19), it seems unlikely that there is another protein conveying glucose repression through inhibiting Gal4p DNA binding, since limited overexpression of Gal4p largely relieves repression (17, 24). Our method of analysis and that of

TABLE 2. MiniGal4ps respond to Δ*gal6*

Galp[a]	Activity[b] (%) after 8 h of galactose induction in:		Fold increase in activity
	YJ0Z80	YJ0Z80Δ*gal6*	
Full-length	100	268	3
MiniGal4p-2	57	158	3
MiniGal4p-7	64	168	3
MiniGal4p-9	44	122	3

[a] All of the Gal4ps were expressed from the yeast centromeric plasmid pSB32 and under the control of the *GAL4* promoter.
[b] The activities of Gal4ps were determined by β-galactosidase assay. Strains were cultured in noninducing medium supplemented with 3% glycerol and 2% lactic acid before the addition of 2% galactose for induction.

TABLE 4. The *sug1-1* allele rescues minigal4Ds

Gal4p[a]	Activity[b] (%) from single-copy plasmid in:		Fold increase in activity	Activity (%) from multicopy plasmid in:		Fold increase in activity
	YJ0Z	YJ0ZS		YJ0Z	YJ0ZS	
gal4D	3	60	20			
Minigal4Dp-2	<0.5	2	>4	10	38	4
Minigal4Dp-7	1	18	18			
Minigal4Dp-9	<0.5	1	>2	1	13	13

[a] All of the Gal4ps were expressed from the yeast centromeric plasmid pSB32 and under the control of the *GAL4* promoter.
[b] The activities of Gal4ps were determined by β-galactosidase assay. The activity of full-length Gal4p from pSB32 was set to be 100%.

Stone and Sadowski are different in that their Gal4p variants were expressed from strong promoters in a strain with *GAL80* deleted and on glucose medium (60).

***Gal3p* response and squelching differences.** There were two qualitative differences found between the miniGal4ps and the wild-type protein.

We observed that miniGal4ps were induced by galactose without Gal3p. When both Gal1p and Gal3p were disrupted, induction no longer occurred. This result showed that Gal1p, without overexpression, could substitute for Gal3p, consistent with the model proposed by Bhat and Hopper (4, 5). In fact, Gal1p was a reasonably good inducer in a *gal3* deletion background, and the induction was delayed only a few hours compared to that in *GAL3* wild-type cells. Nevertheless, full-length Gal4p can not be induced this quickly without Gal3p. Our reasoning is that the transcription of the *GAL1* gene is tightly controlled by Gal4p, which binds to the *GAL1* promoter (71). In the absence of galactose but presence of Gal80p, the activity of Gal4p is repressed and there is essentially no Gal1p to induce the *GAL* system when galactose enters the cell. In contrast, Gal3p has a basal expression level which is the key to the rapid induction. In the case of miniGal4ps, we believe that the residual activities of miniGal4ps escaping the Gal80p repression cause a small accumulation of Gal1p in the cells without galactose. This amount of Gal1p is enough to start inducing the system upon addition of galactose. Once the system is induced, higher levels of Gal1p can maintain the induction level. In this fashion, the *GAL* system was induced quite rapidly in the absence of Gal3p. Our results confirm that induction in the absence of Gal3p is dependent on Gal1p, as predicted by previous results (4, 5). The fact that a small change in the activity of Gal4p alters the induction profile points to the delicate balance between Gal4p activity and Gal4 and Gal80 protein levels in regulating gene expression. The induction phenotype observed with miniGal4ps mimics the induction system in the yeast *K. lactis*. *K. lactis* has only the galactokinase (Gal1p), which functions as both the inducer and the catalytic enzyme (41, 74). It uses a mechanism similar to that used by miniGal4ps to achieve galactose induction, in which Lac9p, the Gal4p homolog, has a higher basal level of expression (55) in the absence of galactose to ensure a certain level of the galactokinase. In essence, miniGal4ps convert the system in *S. cerevisiae* to that of *K. lactis* with regard to the galactose induction.

The second qualitative difference is the failure of the mini Gal4ps to manifest squelching, i.e., the decrease in expression of regulated genes on overexpression of the regulatory protein. This presumably results from titrating some transcription factor. At this point we do not know the basis for this difference. One possibility is that the internal region interacts with this general transcription factor so the miniGal4ps do not titrate it. However, there is no evidence to date that this region of Gal4p interacts with another protein. A second possibility is that the internal region provides a structural flexibility that allows wild-type Gal4 protein to activate from unnatural sites more readily

TABLE 3. MiniGal4ps respond to Δ*gal11*

Galp[a]	Activity[b] (%) in:		Fold reduction in activity
	YJ0Z	YJ0Z80Δ*gal11*	
Full-length	100	21	5
MiniGal4p-2[a]	56	7	8
MiniGal4p-7[a]	66	6	11
MiniGal4p-9[a]	47	9	5

[a] All of the Gal4ps were expressed from the yeast centromeric plasmid pSB32 and under the control of the *GAL4* promoter.
[b] The activities of Gal4ps were determined by β-galactosidase assay. Strains were cultured in noninducing medium containing 3% glycerol and 2% lactic acid.

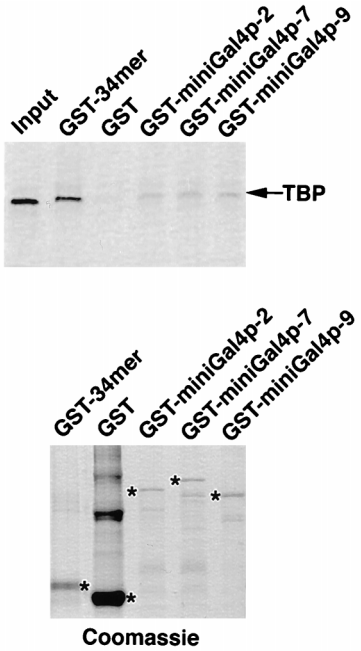

FIG. 5. MiniGal4ps interact with TBP in vitro. GST-miniGal4p fusion proteins immobilized on beads were incubated with [^{35}S]Met-labeled, in vitro-translated TBP. The actual amounts of fusion proteins are shown in the Coomassie blue-stained gel. Asterisks indicate the bands of GST fusion proteins at their predicted sizes.

TABLE 5. Response of Gal4pmCla to Gal80p repression, galactose induction, Δ*gal6*, and Δ*gal11*

Gal4p[a]	Activity[b] (%) in:				
	YJ0Z80		YJ0Z80Δ*gal6* with glycerol plus galactose	YJ0Z	YJ0ZΔ*gal11*
	With glycerol	With glycerol plus galactose			
Full-length	<0.5	100	268	100	21
Gal4pmCla	<0.5	1	3	5	1

[a] Full-length Gal4p and Gal4pmCla were expressed from the yeast centromeric plasmid pSB32 and under the control of the *GAL4* promoter.

[b] The activities of Gal4ps were determined by β-galactosidase assay. The absolute activity with Gal4p in YJ0Z is about twofold higher than that with Gal4p in YJ0Z80.

TABLE 6. Relative DNA binding abilities of Gal4p derivatives compared to full-length Gal4p

Gal4p[a]	Ratio[b] (relative K_D)
Full-length	1
MiniGal4p-2	1.2 ± 0.1
MiniGal4p-7	1.1 ± 0.1
MiniGal4p-9	1.0 ± 0.1
Gal4pmCla	0.9 ± 0.1
IntΔ-2	1.3 ± 0.2
IntΔ-3	1.3 ± 0.2
IntΔ-4	1.4 ± 0.2

[a] The Gal4p and all derivatives were expressed from the 2μm plasmid YEp351 and under the control of the *GAL4* promoter.

[b] The DNA concentrations with which half-maximal binding was reached ($[DNA]_{50}$) were used to represent the K_ds (dissociation constants) of the Gal4p derivatives. The ratio is calculated as the $[DNA]_{50}$ of the derivative divided by the $[DNA]_{50}$ of the full-length Gal4p in the same set of gel mobility shift assay. A ratio of >1 reflects a weaker binding ability, and a ratio of <1 reflects a stronger binding ability, compared to that of full-length Gal4p. Results are means and standard deviations from at least three gel mobility shift assays.

than the miniGal4ps. Understanding this difference is probably central to understanding the squelching phenomenon.

Phosphorylation and galactose induction. Phosphorylation has been suggested to play a role in Gal4p function (42, 43, 49, 53). In a recent study to examine phosphorylation sites in Gal4p, a cluster of phosphorylatable serines near aa 700 was discovered (52). When Ser699 was mutated to alanine, this mutant retained full activity in the absence of Gal80p. However, in the presence of Gal80p, the rate of induction is much slower, up to 8 h after galactose addition. It was suggested, therefore, that the phosphorylation of Ser699 is necessary for induction (52).

Only one of the miniGal4ps isolated in this study contains Ser699. We find that miniGal4ps lacking Ser699 can be fully induced and that the induction kinetics is the same as for full-length Gal4p. Therefore, Ser699 does not seem to be required for normal induction in the context of a Gal4 protein such as miniGal4p-2.

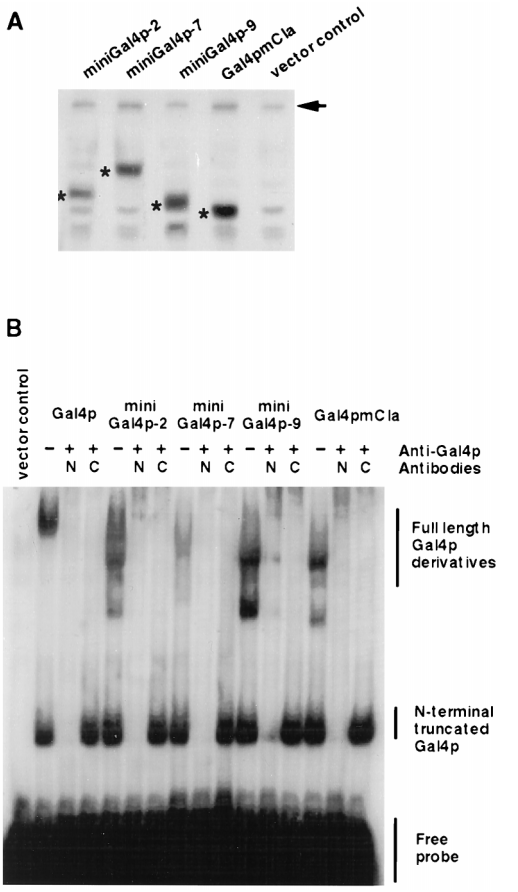

FIG. 6. Protein levels and DNA binding of miniGal4ps and Gal4pmCla. (A) Western blot of crude yeast extracts expressing miniGal4ps and Gal4pmCla from the 2μm plasmid YEp351 under the control of the *GAL4* promoter in the yeast strain YJ0Z80. Levels of expression were very similar. Asterisks indicate the bands of Gal4ps according to their predicted size. The arrow points to a nonspecific band of protein cross-reacting with the antibody, which served as an approximate loading control. (B) Gel mobility shift assay of miniGal4ps and Gal4pmCla with crude yeast extracts. Gal4p-containing complexes were identified by incubation with an antibody directed against the DNA binding domain of Gal4p (lanes N). The truncated N-terminal Gal4p fragment was distinguished by incubation with an antibody directed against the last 146 aa of Gal4p (lanes C).

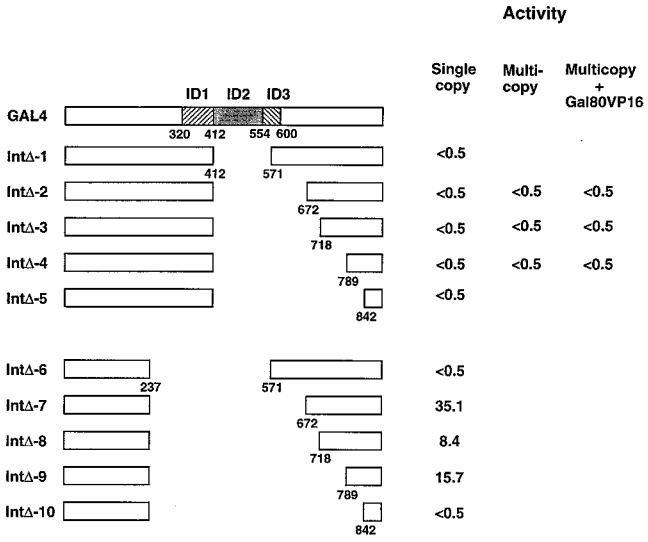

FIG. 7. Schematic representations and activities of the full-length Gal4p and the internal deletion mutants. A chromosomal integrated β-galactosidase gene driven by the *GAL1/10* promoter was used as the reporter gene. Activities of Gal4ps were measured by β-galactosidase assay. All Gal4ps were under the control of *GAL4*'s own promoter. Single-copy Gal4ps were expressed from the centromeric plasmid pSB32. Multicopy Gal4ps were expressed from the 2μm plasmid YEp351. The Gal80-VP16 fusion protein was expressed from a 2μm plasmid and under the control of the *ADH1* promoter.

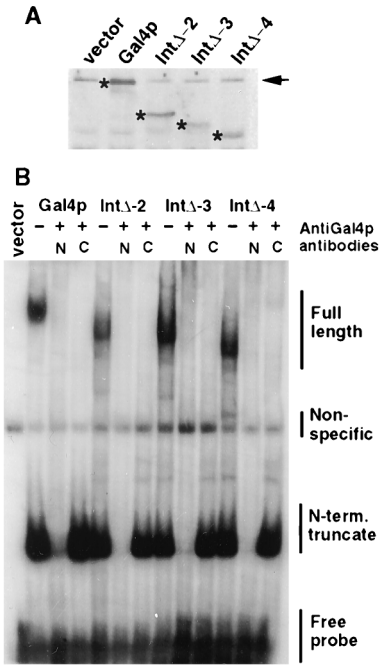

FIG. 8. Protein levels and DNA binding of internal deletion mutants. (A) Western blot of crude yeast extracts expressing internal deletion mutants IntΔ-2, -3, and -4 and full-length Gal4p from the 2μm plasmid YEp351 under the control of the *GAL4* promoter in the yeast strain YJ0Z80. Asterisks indicate the bands of Gal4ps according to their predicted sizes. The arrow points at a nonspecific band of protein cross-reacting with the antibody, which served as an approximate loading control. (B) Gel mobility shift assay of internal deletion mutants IntΔ-2, -3, and -4 and full-length Gal4p with crude yeast extracts. Gal4p-containing complexes were identified by incubation with an antibody directed against the DNA binding domain of Gal4p (lanes N). The truncated N-terminal Gal4p fragment was distinguished by incubation with an antibody directed against the last 146 aa of Gal4p (lanes C).

We cannot exclude the possibility that Ser699 is required for induction in the context of full-length Gal4p. However, the interpretation that phosphorylation of Ser699 is required for normal induction kinetics awaits further experimentation, as Ser699 was changed only to alanine (52). An earlier example showed that in ADR1p, the phenotype caused by a serine-to-alanine mutation could be reverted by changing alanine to other nonphosphorylatable amino acids (11, 13). We should also not overlook the possibility that the Ser699-Ala mutation caused conformational changes to the Gal4p C-terminal Gal80p interaction.

Levels of expression. It is known that the level of expression of Gal4p can influence the transcriptional output. Griggs and Johnston showed that a modest reduction in *GAL4* expression led to very large reduction in *GAL1* expression (17). This phenomenon is explained by the cooperative binding of Gal4p to its multiple binding sites in the *GAL1* promoter. The relationship between the Gal4p level and *MEL1*, which has a single binding site, is very different. Without the cooperativity effect, a small change in Gal4p level will not cause dramatic changes of *MEL1* expression. We have examined the abilities of miniGal4ps to activate the *MEL1* gene by α-galactosidase assays. We found that miniGal4p-2, -7, and -9 activated to 47, 51, and 44%, respectively, indicating that these Gal4ps are truly good activators.

The main difference between the miniGal4ps obtained in this study and other artificially constructed fusion proteins is that miniGal4ps are strong activators, whereas most fusion proteins are transcriptionally weak or inactive if expressed at physiological levels (40). Thus, miniGal4ps could provide an opportunity for the biochemical purification of biologically active Gal4ps. It has been difficult to purify active, full-length Gal4p even in small amounts. Gal4p active for DNA binding has been purified from yeast only in a complex with Gal80p (10, 48). Due to this limitation, the biochemical aspects of transcriptional activation by Gal4p or the mechanism of galactose induction could not be explored by using in vitro experiments. MiniGal4ps perform all of the known functions of Gal4p and therefore could be used as surrogates for full-length Gal4p in biochemical studies. Also, structural studies of Gal4p have been done only with the minimal DNA binding domain (aa 1 to 66) (29, 38) and the 34-aa core of the activation domain separately (70). It will be important to determine the structural configuration of the activation domain in the context of an actively DNA binding form of Gal4p, such as miniGal4ps. Preliminary experiments indicate that the miniGal4ps are more readily purified than full-length Gal4p.

What is the functional role of the internal region of Gal4p? Does the internal region of Gal4p play any role other than modulating the level of transcriptional activation? The most extensive study of this question was a deletion analysis conducted by Stone and Sadowski (60). Our deletion analysis is consistent with the prediction of Stone and Sadowski that there are multiple inhibitory domains (IDs) in the internal region of Gal4p (60). As presented in Fig. 6, the central homology region (ID1) and aa 571 to 632 (ID3) can act as negative elements. We did not directly test for the function of ID2. However, the interactions must be more complicated than two independent repressing domains, as the central homology region (ID1) is also required for function in the context of the rest of Gal4p (Fig. 1).

It was suggested that the IDs worked through recruiting another protein which could inhibit Gal4p DNA binding (60). We think that this is unlikely for two reasons. First, a genetic selection for mutations that might relieve the inhibition of the central homology region deletion revealed only further deletions in Gal4p. Unless the putative protein was essential, it probably would have been evident in this selection. More directly, we find that internal deletions IntΔ-2, -3, and -4, which retain ID1, are able to bind DNA in crude extracts. The defect in these proteins appears to be the inaccessibility of the activation domain.

The major function of the internal region appears to be with respect to the level of transcription. As shown, the difference in activities is probably not due to differences in protein levels of these derivatives. This suggests that the internal region of Gal4p does not affect protein stability, since different small Gal4p derivatives contain different amounts of this region yet accumulate to very similar levels in vivo. The DNA binding activities of these Gal4ps were tested and shown to be quite similar to that of the full-length Gal4p. Therefore, their transcriptional activity is not directly proportional to their in vitro affinity to DNA. However, the in vitro DNA binding ability as determined by gel mobility shift assays may not reflect how these proteins occupy their binding sites in vivo.

Comparing the sequences of all of the miniGal4ps obtained in this study, we did not find a clear sequence boundary between the stronger or weaker activators. The only trend observed was the increasing strength with increasing length of miniGal4ps (Fig. 9). On the surface, this contradicts the view of transcriptional activators as being modular. However, both the DNA binding and activation domains are required to give activity. Moreover, this trend suggests the possible role of the internal region as a spacer between the DNA binding domain

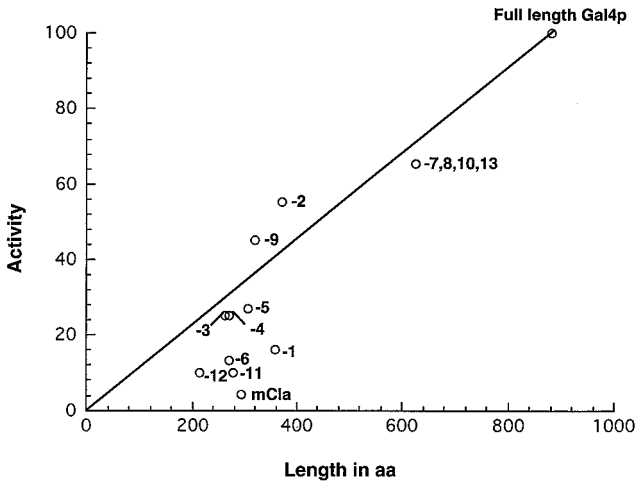

FIG. 9. Potential relationship between the lengths and the activities of Gal4ps. The lengths (in amino acids) of different miniGal4ps and full-length Gal4p were plotted against their activities when expressed at the single-copy level, as shown in Fig. 1. The numbers represent the isolates of the miniGal4ps. mCla, Gal4pmCla.

and the activation domain to promote the transcription potency of Gal4p. Supporting the spacer proposal, the λ repressor linker region was shown to increase the transactivation potency of a Gal4-VP16 fusion protein in vitro (47). Deletion analysis with another yeast activator, ADR1p, suggests four activation domains within the 1,323-aa protein (12). There is a stepwise decrease in activity when those domains are sequentially removed. It has also been observed that when fused to the minimal DNA binding domain of Gal4p, longer activation domains activate more strongly (40). Possibly, with a longer spacer the activation domain can be more effectively positioned to contact the basal machinery.

The internal region could also be involved in intramolecular interactions and conformational changes, as implied by the result that deletion mutants IntΔ-2, -3, and -4 may be inaccessible to Gal80p. There is an increasing appreciation of the importance of intramolecular conformations in regulatory protein function. The most extensive mutational study in this regard is that of the glucocorticoid receptor. On binding of specific DNA sites, conformational changes in the protein which control the activation and repression functions of specific domains are proposed to take place (59). The Ets-1 protein is probably the most characterized structurally as to such conformational changes (15, 27, 50). Ets-1 is predicted to have two inhibitory domains that act cooperatively to control DNA occupancy. This is in contrast to Gal4p, where the inhibitory domains act independently. The recent structural study on Ets-1 suggests that a C-terminal domain interacts within an N-terminal inhibitory region (15). In yeast, Leu3p is transcriptionally active only in the presence of α-isopropylmalate (66). A model has been proposed in which interaction of the central part with the C-terminal activation domain prevents activation of Leu3p until an α-isopropylmalate-induced conformational change occurs (77). Also implying intramolecular interaction, a point mutation in the internal region of Put3p was found to be suppressed by a second point mutation in another part of Put3p (14). Although there has not been evidence for conformational changes in Gal4p, it is reasonable to speculate that intramolecular interactions between the activation domain and other parts of Gal4p could exist. In addition to the Gal80p repression mechanism, the activation domain may be masked when Gal4p is not activating transcription. We can also envision multiple regions in Gal4p working in concert to mask or unmask the activation domain when necessary.

It is clear that Gal4p is structurally a more complex protein than is often envisioned, yet its known functions are carried out largely through the N-terminal DNA binding and C-terminal activation domains. The complexity and the role of its large internal region remain to be unraveled. Our results suggest that its role is as a spacer and/or a surface for intramolecular or Gal4p-Gal4p interactions. Knowledge of the detailed conformation of the protein awaits breakthroughs in structural analysis that may be facilitated by using miniGal4ps.

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REFERENCES

1. **Akiyama, K., K. Yokota, S. Kagawa, N. Shimbara, G. N. DeMartino, C. A. Slaughter, C. Noda, and K. Tanaka.** 1995. cDNA cloning of a new putative ATPase subunit p45 of the human 26S proteasome, a homolog of yeast transcriptional factor Sug1p. FEBS Lett. **363:**151–156.
2. **Bajwa, W., T. E. Torchia, and J. E. Hopper.** 1988. Yeast regulatory gene *GAL3*: carbon regulation; UAS_{GAL} elements in common with *GAL1*, *GAL7*, *GAL10*, *GAL80*, and *MEL1*; encoded protein strikingly similar to yeast and *Escherichia coli* galactokinases. Mol. Cell. Biol. **8:**3439–3447.
3. **Baker, S. M., S. A. Johnston, J. E. Hopper, and J. A. Jaehning.** 1987. Transcription of multiple copies of the yeast GAL7 gene is limited by specific factors in addition to GAL4. Mol. Gen. Genet. **208:**127–134.
4. **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.
5. **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.
6. **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.
7. **Bram, R. J., N. F. Lue, and R. D. Kornberg.** 1986. A GAL family of upstream activating sequences in yeast: roles in both induction and repression of transcription. EMBO J. **5:**603–608.
8. **Brent, R., and M. Ptashne.** 1985. A eukaryotic transcriptional activator bearing the DNA specificity of a prokaryotic repressor. Cell **43:**729–736.
9. **Carey, M., H. Kakidani, J. Leatherwood, F. Mostashari, and M. Ptashne.** 1989. An amino-terminal fragment of GAL4 binds DNA as a dimer. J. Mol. Biol. **209:**423–432.
10. **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.
11. **Cherry, J. R., T. R. Johnson, C. Dollard, J. R. Shuster, and C. L. Denis.** 1989. Cyclic AMP-dependent protein kinase phosphorylates and inactivates the yeast transcriptional activator ADR1. Cell **56:**409–419.
12. **Cook, W. J., D. Chase, D. C. Audino, and C. L. Denis.** 1994. Dissection of the ADR1 protein reveals multiple, functionally redundant activation domains interspersed with inhibitory regions: evidence for a repressor binding to the ADR1c region. Mol. Cell. Biol. **14:**629–640.
13. **Denis, C. L., S. C. Fontaine, D. Chase, B. E. Kemp, and L. T. Bemis.** 1992. ADR1c mutations enhance the ability of ADR1 to activate transcription by a mechanism that is independent of effects on cyclic AMP-dependent protein kinase phosphorylation of Ser-230. Mol. Cell. Biol. **12:**1507–1514.
14. **Desetages, S. A. G., D. A. Falvey, R. J. Reece, and M. C. Brandriss.** 1996. Functional analysis of the Put3 transcriptional activator of the proline utilization pathway in Saccharomyces cerevisiae. Genetics **142:**1069–1082.
15. **Donaldson, L. W., J. M. Petersen, B. J. Graves, and L. P. McIntosh.** 1996. Solution structure of the ETS domain from murine Ets-1: a winged helix-

turn-helix DNA binding motif. EMBO J. **15:**125–134.
16. **Gill, G., and M. Ptashne.** 1988. Negative effect of the transcriptional activator GAL4. Nature **334:**721–724.
17. **Griggs, D. W., and M. Johnston.** 1991. Regulated expression of the GAL4 activator gene in yeast provides a sensitive genetic switch for glucose repression. Proc. Natl. Acad. Sci. USA **88:**8597–8601.
18. **Hashimoto, H., Y. Kikuchi, Y. Nogi, and T. Fukusawa.** 1983. Regulation of expression of the galactose gene cluster in Saccharomyces cerevisiae. Mol. Gen. Genet. **191:**31–38.
19. **Huibregtse, J. M., P. D. Good, G. T. Marczynski, J. A. Jaehning, and D. R. Engelke.** 1993. Gal4 protein binding is required but not sufficient for derepression and induction of GAL2 expression. J. Biol. Chem. **268:**22219–22222.
20. **Ito, H., Y. Fukuda, K. Murata, and A. Kimura.** 1983. Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. **153:**163–168.
21. **Johnston, M.** 1987. A model fungal gene regulatory mechanism: the *GAL* genes of *Saccharomyces cerevisiae*. Microbiol. Rev. **51:**458–476.
22. **Johnston, M., and J. Dover.** 1988. Mutational analysis of the GAL4-encoded transcriptional activator protein of Saccharomyces cerevisiae. Genetics **120:**63–74.
23. **Johnston, M., J. S. Flick, and T. Pexton.** 1994. Multiple mechanisms provide rapid and stringent glucose repression of *GAL* gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **14:**3834–3841.
24. **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.
25. **Johnston, S. A., J. J. Salmeron, and S. S. Dincher.** 1987. Interaction of positive and negative regulatory proteins in the galactose regulon of yeast. Cell **50:**143–146.
26. **Johnston, S. A., M. J. Zavortink, C. Debouck, and J. E. Hopper.** 1986. Functional domains of the yeast regulatory protein GAL4. Proc. Natl. Acad. Sci. USA **83:**6553–6557.
27. **Jonsen, M. D., J. M. Petersen, Q. P. Xu, and B. J. Graves.** 1996. Characterization of the cooperative function of inhibitory sequences in Ets-1. Mol. Cell. Biol. **16:**2065–2073.
28. **Kim, Y. J., S. Bjorklund, Y. Li, M. H. Sayre, and R. D. Kornberg.** 1994. A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:**599–608.
29. **Kraulis, P. J., A. R. C. Raine, P. L. Gadhavi, and E. D. Laue.** 1992. Structure of the DNA-binding domain of zinc GAL4. Nature **356:**448–450.
30. **Lamphier, M. S., and M. Ptashne.** 1992. Multiple mechanisms mediate glucose repression of the yeast GAL1 gene. Proc. Natl. Acad. Sci. USA **89:**5922–5926.
31. **Leuther, K. K.** 1993. Ph.D. thesis. University of Texas Southwestern Medical Center, Dallas.
32. **Leuther, K. K., and S. A. Johnston.** 1992. Nondissociation of GAL4 and GAL80 in vivo after galactose induction. Science **256:**1333–1335.
33. **Leuther, K. K., J. M. Salmeron, and S. A. Johnston.** 1993. Genetic evidence that an activation domain of GAL4 does not require acidity and may form a beta sheet. Cell **72:**575–585.
34. **Lue, N. F., D. I. Chasman, A. R. Buchman, and R. D. Kornberg.** 1987. Interaction of *GAL4* and *GAL80* gene regulatory proteins in vitro. Mol. Cell. Biol. **7:**3446–3451.
35. **Ma, J., and M. Ptashne.** 1987. The carboxy-terminal 30 amino acids of GAL4 are recognized by GAL80. Cell **50:**137–142.
36. **Ma, J., and M. Ptashne.** 1987. Deletion analysis of GAL4 defines two transcriptional activating segments. Cell **48:**847–853.
37. **Marczak, J. E., and M. C. Brandriss.** 1991. Analysis of constitutive and noninducible mutations of the PUT3 transcriptional activator. Mol. Cell. Biol. **11:**2609–2619.
38. **Marmorstein, R., M. Carey, M. Ptashne, and S. C. Harrison.** 1992. DNA recognition by GAL4: structure of a protein-DNA complex. Nature **356:**408–414.
39. **Melcher, K., and S. A. Johnston.** 1995. GAL4 interacts with TATA binding protein and coactivators. Mol. Cell. Biol. **15:**2839–2848.
40. **Melcher, K., and S. A. Johnston.** Unpublished data.
41. **Meyer, J., A. Walker-Jonah, and C. P. Hollenberg.** 1991. Galactokinase encoded by *GAL1* is a bifunctional protein required for the induction of the *GAL* genes in *Kluyveromyces lactis* and able to suppress the *gal3* phenotype in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**5454–5461.
42. **Mylin, L. M., J. P. Bhat, and J. E. Hopper.** 1989. Regulated phosphorylation and dephosphorylation of GAL4, a transcriptional activator. Genes Dev. **3:**1157–1165.
43. **Mylin, L. M., M. Johnston, and J. E. Hopper.** 1990. Phosphorylated forms of GAL4 are correlated with ability to activate transcription. Mol. Cell. Biol. **10:**4623–4629.
44. **Nehlin, J. O., M. Carlberg, and H. Ronne.** 1991. Control of yeast GAL genes by MIG1 repressor: a transcriptional cascade in the glucose response. EMBO J. **10:**3373–3377.
45. **Nishizawa, M., Y. Suzuki, Y. Nogi, K. Matsumoto, and T. Fukasawa.** 1990. Yeast Gal11 protein mediates the transcriptional activation signal of two different transacting factors, Gal4 and general regulatory factor I/repressor/activator site binding protein 1/translation upstream factor. Proc. Natl. Acad. Sci. USA **87:**5373–5377.
46. **Nogi, Y., and T. Fukusawa.** 1983. A novel mutation that affects utilization of galactose in Saccharomyces cerevisiae. Curr. Genet. **195:**115–120.
47. **Ohashi, Y., J. M. Brickman, E. Furman, B. Middleton, and M. Carey.** 1994. Modulating the potency of an activator in a yeast in vitro transcription system. Mol. Cell. Biol. **14:**2731–2739.
48. **Parthun, M. R., and J. A. Jaehning.** 1990. Purification and characterization of the yeast transcriptional activator GAL4. J. Biol. Chem. **265:**209–213.
49. **Parthun, M. R., and J. A. Jaehning.** 1992. A transcriptionally active form of GAL4 is phosphorylated and associated with GAL80. Mol. Cell. Biol. **12:**4981–4987.
50. **Petersen, J. M., J. J. Skalicky, L. W. Donaldson, L. P. McIntosh, T. Alber, and B. J. Graves.** 1995. Modulation of transcription factor Ets-1 DNA binding: DNA-induced unfolding of an alpha helix. Science **269:**1866–1869.
51. **Rubin, D. M., O. Coux, I. Wefes, C. Hengartner, R. A. Young, A. L. Goldberg, and D. Finley.** 1996. Identification of the gal4 suppressor Sug1 as a subunit of the yeast 26S proteasome. Nature **379:**655–657.
52. **Sadowski, I., C. Costa, and R. Dhanawansa.** 1996. Phosphorylation of Gal4p at a single C-terminal residue is necessary for galactose-inducible transcription. Mol. Cell. Biol. **16:**4879–4887.
53. **Sadowski, I., D. Niedbala, K. Wood, and M. Ptashne.** 1991. GAL4 is phosphorylated as a consequence of transcriptional activation. Proc. Natl. Acad. Sci. USA **88:**10510–10514.
54. **Salmeron, J. J., and S. A. Johnston.** 1986. Analysis of the Kluyveromyces lactis positive regulatory gene LAC9 reveals functional homology to, but sequence divergence from, the Saccharomyces cerevisiae GAL4 gene. Nucleic Acids Res. **14:**7767–7781.
55. **Salmeron, J. J., S. D. Langdon, and S. A. Johnston.** 1989. Interaction between transcriptional activator protein LAC9 and negative regulatory protein GAL80. Mol. Cell. Biol. **9:**2950–2956.
56. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Laboratory course manual for methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
57. **Silver, P. A., L. P. Keegan, and M. Ptashne.** 1984. Amino terminus of the yeast GAL4 gene product is sufficient for nuclear localization. Proc. Natl. Acad. Sci. USA **81:**5951–5955.
58. **Spiegelman, S., R. Rotman-Sussman, and E. Pinska.** 1950. On the cytoplasmic nature of "long-term adaptation" in yeast. Proc. Natl. Acad. Sci. USA **36:**591–606.
59. **Starr, D. B., W. Matsui, J. R. Thomas, and K. R. Yamamoto.** 1996. Intracellular receptors use a common mechanism to interpret signaling information at response elements. Genes Dev. **10:**1271–1283.
60. **Stone, G., and I. Sadowski.** 1993. GAL4 is regulated by a glucose-responsive functional domain. EMBO J. **12:**1375–1385.
61. **Suzuki, Y., Y. Nogi, A. Abe, and T. Fukasawa.** 1988. GAL11 protein, an auxiliary transcription activator for genes encoding galactose-metab olizing enzymes in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8:**4991–4999.
62. **Suzukifujimoto, T., M. Fukuma, K. Yano, H. Sakurai, A. Vonika, S. A. Johnston, and T. Fukasawa.** 1996. Analysis of the galactose signal transduction pathway in *Saccharomyces cerevisiae*: interaction between Gal3p and Gal80p. Mol. Cell. Biol. **16:**2504–2508.
63. **Swaffield, J. C., J. F. Bromberg, and S. A. Johnston.** 1992. Alterations in a yeast protein resembling HIV Tat-binding protein relieve requirement for an acidic activation domain in GAL4. Nature **360:**698–700.
64. **Swaffield, J. C., K. Melcher, and S. A. Johnston.** 1996. Correction: a highly conserved ATPase protein as a mediator between acidic activation domains and the TATA-binding protein. Nature **379:**658.
65. **Swaffield, J. C., K. Melcher, and S. A. Johnston.** 1995. A highly conserved ATPase protein as a mediator between acidic activation domains and the TATA-binding protein. Nature **374:**88–91.
66. **Sze, J. Y., M. Woontner, J. A. Jaehning, and G. B. Kohlhaw.** 1992. In vitro transcriptional activation by a metabolic intermediate: activation by Leu3 depends on alpha-isopropylmalate. Science **258:**1143–1145. (Erratum, **262:**492, 1993.)
67. **Torchia, T. E., R. W. Hamilton, C. L. Cano, and J. E. Hopper.** 1984. Disruption of regulatory gene *GAL80* in *Saccharomyces cerevisiae*: effects on carbon-controlled regulation of the galactose/melibiose pathway genes. Mol. Cell. Biol. **4:**1521–1527.
68. **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.
69. **Tsai, M. J., and B. W. O'Malley.** 1994. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. Annu. Rev. Biochem. **63:**451–486.
70. **Van Hoy, M., K. K. Leuther, T. Kodadek, and S. A. Johnston.** 1993. The acidic activation domains of the GCN4 and GAL4 proteins are not alpha helical but form beta sheets. Cell **72:**587–594.

71. **West, R. J., R. R. Yocum, and M. Ptashne.** 1984. *Saccharomyces cerevisiae GAL1-GAL10* divergent promoter region: location and function of the upstream activating sequence UASG. Mol. Cell. Biol. **4:**2467–2478.
72. **Xu, H. E., and S. A. Johnston.** 1994. Yeast bleomycin hydrolase is a DNA-binding cysteine protease. J. Biol. Chem. **269:**21177–21183.
73. **Xu, H. E., A. Vonica, and S. A. Johnston.** Unpublished data.
74. **Zenke, F. T., R. Engels, V. Vollenbroich, J. Meyer, C. P. Hollenberg, and K. D. Breunig.** 1996. Activation of Gal4p by galactose-dependent interaction of galactokinase and Gal80p. Science **272:**1662–1665.
75. **Zheng, W., and S. A. Johnston.** Unpublished data.
76. **Zheng, W., H. E. Xu, and S. A. Johnston.** Unpublished data.
77. **Zhou, K. M., and G. B. Kohlhaw.** 1990. Transcriptional activator LEU3 of yeast. Mapping of the transcriptional activation function and significance of activation domain tryptophans. J. Biol. Chem. **265:**17409–17412.