13. W. A. Maltese, *FASEB J.* **4**, 3319 (1990).
14. S. L. Moores *et al.*, *J. Biol. Chem.* **266**, 14603 (1991).
15. J. F. Hancock, A. I. Magee, J. E. Childs, C. J. Marshall, *Cell* **57**, 1167 (1989).
16. W. R. Schafer *et al.*, *Science* **245**, 379 (1989).
17. L. A. Beck, T. J. Hosiak, M. Sinensky, *J. Cell Biol.* **107**, 1307 (1988).
18. H. Ellens, S. Doxsey, J. S. Glenn, J. M. White, *Methods Cell Biol.* **31**, 155 (1989).
19. J. S. Glenn, J. M. Taylor, J. M. White, *J. Virol.* **64**, 3104 (1990). SAG cells are identical to GAG cells.
20. J. S. Glenn, thesis, University of California, San Francisco (1992).
21. J. S. Glenn, J. A. Watson, C. M. Havel, J. M. White, unpublished data.
22. C. J. Wang, P. J. Chen, J. C. Wu, D. Patel, D. S. Chen, *J. Virol.* **65**, 6630 (1991); W.-S. Ryu, M. Bayer, J. Taylor, *ibid.*, in press; C. Sureau, personal communication.
23. W.-S. Ryu, J. S. Glenn, J. M. White, J. Taylor, in preparation.
24. Of 14 independent viral isolates sequenced, 13 code for Cys-Arg-Pro-Gln-COOH and 1 codes for Cys-Thr-Pro-Gln-COOH as the four terminal amino acids of large delta antigen [K.-S. Wang *et al.*, *Nature* **323**, 508 (1986); S. Makino *et al.*, *ibid.* **329**, 343 (1987); M. Y. P. Kuo *et al.*, *J. Virol.* **62**, 1855 (1988); J. A. Saldanha, H. C. Thomas, J. P. Monjardino, *J. Gen. Virol.* **71**, 1603 (1990); Y.-P. Xia, M.-F. Chang, D. Wei, S. Govindarajan, M. M. C. Lai, *Virology* **178**, 331 (1990); F. Imazeki, M. Omata, M. Ohto, *J. Virol.* **64**, 5594 (1990); Y.-C. Chao, C.-M. Lee, H.-S. Tang, S. Govindarajan, M. M. C. Lai, *Hepatology* **13**, 345 (1991); P. Deny *et al.*, *J. Gen. Virol.* **72**, 735 (1991)].
25. We have recently found that specific mutation of the COOH-terminal Gln of large delta antigen to Pro converted the protein from an inhibitor to an enhancer of genome replication (*20*).
26. J. B. Gibbs, *Cell* **65**, 1 (1991).
27. Y. Reiss, J. L. Goldstein, M. C. Seabra, P. J. Casey, M. S. Brown, *ibid.* **62**, 81 (1990).
28. R. F. Ramig, in *Virology*, B. N. Fields *et al.*, Eds. (Raven, New York, 1990), pp. 112–122.
29. M. Y.-P. Kuo, M. Chao, J. Taylor, *J. Virol.* **63**, 1945 (1989).
30. (*R,S*)-[5-^{3}H]mevalonate (4 to 18.8 Ci/mmol) was synthesized according to the method of R. K. Keller, *J. Biol. Chem.* **261**, 12053 (1986).
31. V. Bruss and D. Ganem, *J. Virol.* **65**, 3813 (1991).
32. We thank J.-J. Gonvers for providing human anti-delta antigen serum, A. Alberts for providing Lovastatin, D. Ganem for providing the anti-HBs and SV24H, and J. M. Bishop, H. Bourne, and D. Ganem for helpful discussions and critical reading of the manuscript. J.M.W. is a recipient of an NIH grant and J.S.G. was supported by the Medical Scientist Training Program.

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Nondissociation of GAL4 and GAL80 in Vivo After Galactose Induction

Kerstin K. Leuther and Stephen A. Johnston*

Transcription of galactose-inducible genes in yeast is regulated by interaction between the activator protein GAL4 and the negative regulatory protein GAL80. It has been suggested that GAL80 binds to and represses GAL4 under uninduced conditions and dissociates from GAL4 on induction. However, the possibility that GAL80 remains associated with GAL4 after induction has not been ruled out. Experiments to discriminate between these two models were performed and revealed that GAL80 stays bound after induction.

Transcriptional regulation in eukaryotes involves the interplay between positive and negative regulatory proteins. The yeast transcriptional activator GAL4 and its negative regulator GAL80 are said to be "poised" for activation. Under uninduced conditions (in the absence of galactose), GAL4 occupies its DNA binding site upstream of target genes, but its activation function is blocked by protein-protein interaction with GAL80. On induction, GAL4 becomes competent to activate the transcription of specific genes. The popular model (Fig. 1A) to explain this activation is that GAL80 dissociates from GAL4, allowing exposure of the GAL4 activation domain. Another possible model is that GAL80 remains bound after induction.

We devised a scheme (Fig. 1B) to discriminate between these two models in vivo. The basic components of the experiment were the following. First, a *GAL4* gene was used that encoded a protein that has reduced ability to activate transcription but still retains its normal affinity for the GAL80 protein (*1*). Second, a fusion was made between the GAL80 protein and the transcriptional activation domain of the VP16 protein of herpes simplex virus (*2*). The expectation was that the GAL80-VP16 fusion would be recruited to the enhancer region by GAL4 and activate transcription even in the uninduced state by virtue of the VP16 activation domain, because the acidic activation domain of VP16 is a strong activator in yeast (*3*) and a different activation domain had been fused to GAL80 to allow expression in the uninduced state (*4*).

We expected that the GAL4 proteins that were poor activators would produce high reporter gene expression in the uninduced state by binding the GAL80-VP16 protein. The question was what would happen on induction. If the GAL80-VP16 protein dissociates from GAL4, then the level of expression after induction should fall to the lower, GAL4 © mediated level. If, however, the GAL80-VP16 complex stays associated with GAL4 after induction, then the induced expression should be as high as or higher than that of the uninduced state. These two alternative outcomes are depicted in Fig. 1B.

As anticipated, the GAL80-VP16 hybrid stimulated high levels of transcription in noninducing media (Table 1). In inducing media, the level of expression was as high as or higher than uninduced for each of the GAL4 mutants tested, consistent with idea that GAL80 remains associated with the GAL4 after induction. We observed the same phenomenon for the wild-type GAL4. In this case, the level of expression is higher than that of the GAL80-VP16 plus GAL4 activation, suggesting that the GAL4 and VP16 activation domains are functioning synergistically under inducing conditions.

Several control experiments support the conclusion that GAL80 does not dissociate from GAL4 on induction. First, expression of the GAL80-VP16 hybrid or the VP16 domain itself in the absence of GAL4 gave no activation of the reporter gene (less than 1% of wild-type GAL4 activity), indicating that the VP16 stimulation of transcription is dependent on GAL4. To show that the VP16 stimulation is dependent on GAL4-GAL80 interaction, a *GAL4^c* allele was tested. GAL4^c mutants do not interact with GAL80 but retain their ability to activate transcription. The protein encoded by the *GAL4^c#18* allele (*5*) did not interact with

Table 1. A test of the dissociation versus transformation models. Yeast strain YJ0-Z that contained a deletion of *GAL4* and either a *GAL80* or *GAL80-VP16* integration was transformed with a CEN plasmid bearing the indicated *GAL4* allele. *LacZ* gene expression [β-galactosidase activity (β-Gal)] was measured in each strain under inducing (galactose) and noninducing (glycerol-lactate) conditions (*12*). The *GAL4-T858* (Thr^{858} → Leu; Tyr^{867} → Cys) and *GAL4-Y865* (Tyr^{865} → Ser; Tyr^{867} → Cys) alleles of *GAL4* have mutations in the COOH-terminal activation domain that decrease their activation but retain GAL80 interaction.

GAL4 derivative	GAL80 derivative	β-Gal activity (%)	
		Uninduced	Induced
GAL4 wild type	GAL80	<1	100
	GAL80-VP16	140	370
GAL4-T858	GAL80	<1	2
	GAL80-VP16	90	170
GAL4-Y865	GAL80	<1	70
	GAL80-VP16	190	220

Departments of Medicine and Biochemistry, University of Texas–Southwestern Medical Center, Dallas, TX 75235.

*To whom correspondence should be addressed.

GAL80 protein in vitro (Fig. 2A). When GAL4^{c}#18 was tested in a GAL80-VP16 background, we observed no stimulation of transcription of the reporter (Fig. 2A), indicating that the GAL80-VP16–mediated activation was dependent on the interaction between GAL80 and GAL4. The decline in activity between uninduced and induced conditions is characteristic of *GAL4^{c}* alleles (6). It also is unlikely that the fusion of VP16 to GAL80 has altered normal interaction of GAL80 with GAL4, as a fusion of *Escherichia coli* β-galactosidase to the same position in GAL80 has no effect on inducibility (7). Furthermore, transformation of a strain containing both GAL4 and GAL80 with a plasmid containing GAL80-VP16 results in a constitutive phenotype, arguing that GAL80-VP16 interacts with GAL4. In addition, we were able to show that in vitro translated GAL80-VP16 binds the COOH-terminus of the GAL4 protein (Fig. 2B). These data demonstrate that the GAL80-VP16 fusion protein is functional.

In light of these results, we envision the

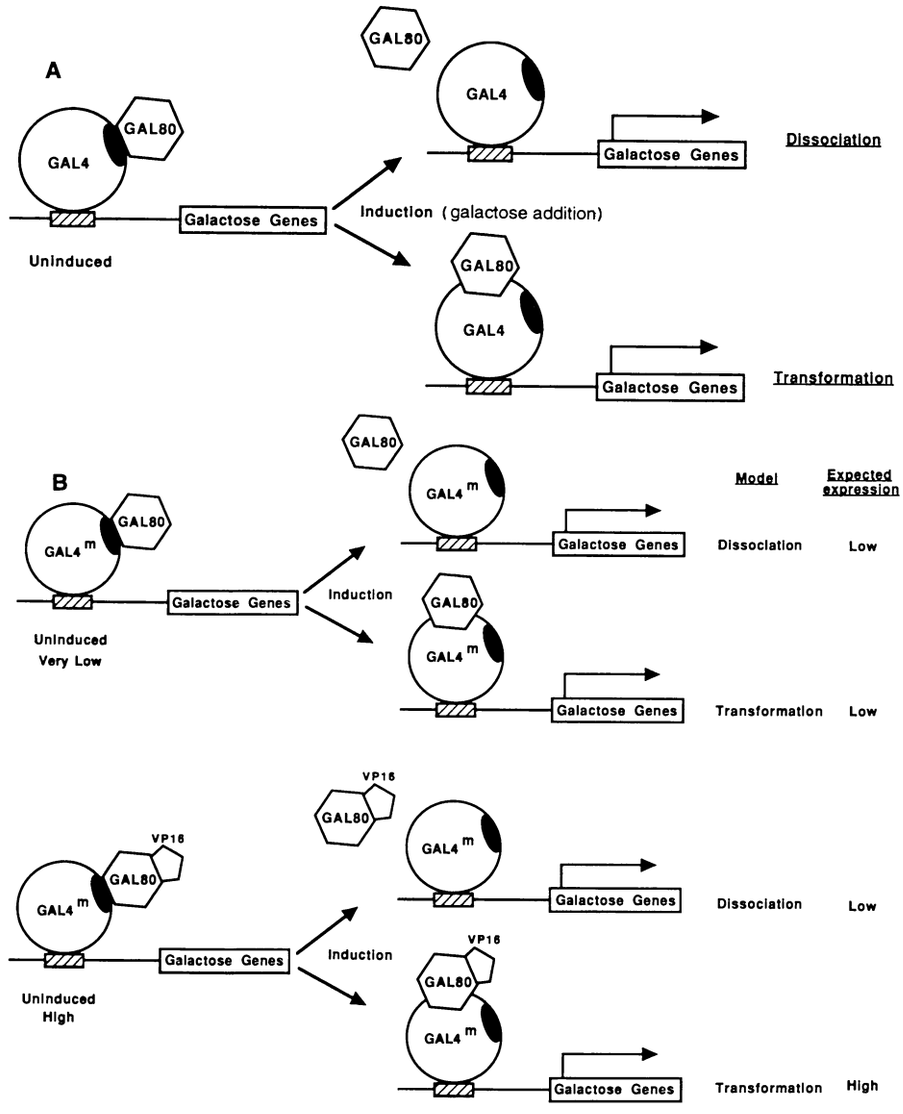
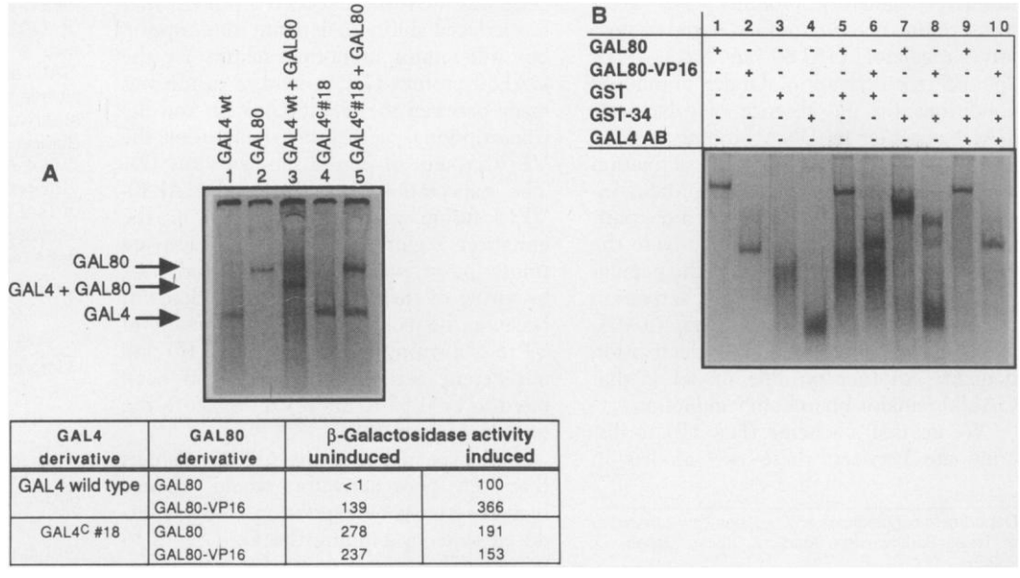


Fig. 1. Models for the interaction of GAL4 and GAL80. (**A**) The two models, dissociation and transformation, for the interaction of GAL4 and GAL80 proteins on induction. The hatched box indicates the GAL4 DNA binding site, and the filled oval indicates the GAL4 activation domain. (**B**) The scheme used to discriminate between the two models in (A). GAL4^{m} indicates an altered GAL4 protein that has reduced ability to activate transcription but maintains a normal GAL80-interactive function. GAL80-VP16 represents a fusion between GAL80 and the transcriptional activation domain of VP16 (*12*). The expected amounts of expression for the models are shown at the right.



GAL4 derivative	GAL80 derivative	β-Galactosidase activity uninduced	induced
GAL4 wild type	GAL80	< 1	100
	GAL80-VP16	139	366
GAL4^{C} #18	GAL80	278	191
	GAL80-VP16	237	153

Fig. 2. (**A**) Activation of transcription by GAL80-VP16 depends on the physical interaction of GAL4 and GAL80. In vitro translated GAL4 forms a new complex with in vitro translated GAL80 (*13*). When GAL4^{c}#18 was combined with GAL80, no such complex was formed. If we compare induced and uninduced levels of expression, the *GAL4^{c}#18* strain is not affected by the *GAL80-VP16* gene product, whereas the wild-type *GAL4* strain is. (**B**) GAL80-VP16 is a functional protein. In vitro translated GAL80-VP16 interacts with in vitro translated GST-34 (*13*). When GAL80 (lane 1) is mixed with GST (lane 3), no new complex is formed (lane 5). GAL80 and GST-34 (lane 4) combine to form a new complex (lane 7). This complex is removed with antibody specific for the GAL4 COOH-terminus (lane 9). Similarly, GAL80-VP16 (lane 2) does not interact with GST (lane 6) but does form a new complex when combined with GST-34 (lane 8), which can be removed with GAL4 antibody (GAL4 AB) (lane 10).

following model for GAL4-GAL80 interaction. As originally proposed (*8*, *9*), GAL4 is poised at its DNA binding site in the uninduced state with bound GAL80 preventing the function of the activation domain. In the presence of galactose, an as yet unidentified induction signal is transmitted to GAL80 (or possibly GAL4). This signal elicits a transformation of the GAL4-GAL80 complex that exposes the GAL4 activation domain. This model is consistent with several other observations. First, deleting the *GAL80* gene leads to higher induced expression (*10*), as if the GAL80 protein still had a slight repressive effect when in the induced form. Second, electrophoretic mobility-shift experiments performed with extracts from either induced or uninduced strains showed no difference in the amount of GAL4-GAL80 complex (*7*). Third, we and others (*11*) have been unable to dissociate the GAL4-GAL80 complex in vitro with potential inducers. Each of these observations is readily explained under the transformation model. Our experiments do not distinguish between the parsimonious model, in which GAL80 stays associated with GAL4 on induction, and one in which GAL80 transfers to some other protein in the promoter region.

The genetic and molecular manipulability of yeast and the evolutionary conservation of eukaryotic transcription mechanisms have made the galactose system, and GAL4 in particular, a paradigm for studying gene regulation. It is becoming increasingly clear that, as with GAL4, interactions between positive and negative regulators play an essential role in modulating transcription. The GAL4-GAL80 interaction is more complicated and interesting than originally envisioned and should provide lessons in protein-protein interaction.

REFERENCES AND NOTES

1. K. K. Leuther and S. A. Johnston, in preparation.
2. S. J. Triezenberg, R. C. Kingsbury, S. L. McKnight, *Genes Dev.* **2**, 718 (1988).
3. D. I. Chasman, J. Leatherwood, M. Carey, M. Ptashne, R. D. Kornberg, *Mol. Cell. Biol.* **9**, 4746 (1989).
4. J. Ma and M. Ptashne, *Cell* **55**, 443 (1988).
5. This GAL4 protein has the following amino acid substitutions: $Asn^{857} \rightarrow Ile$, $Asp^{862} \rightarrow Gly$, $Asn^{866} \rightarrow His$, and $Leu^{868} \rightarrow Arg$. Details will be described elsewhere (*1*).
6. K. Matsumoto, Y. Adachi, A. Toh-e, Y. Oshima, *J. Bacteriol.* **141**, 508 (1980).
7. S. D. Langdon and S. A. Johnston, unpublished data.
8. S. A. Johnston and J. E. Hopper, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6971 (1982).
9. E. Giniger, S. M. Varnum, M. Ptashne, *Cell* **40**, 767 (1985); J. Ma and M. Ptashne, *ibid.* **50**, 137 (1987); S. B. Selleck and J. E. Majors, *Mol. Cell. Biol.* **7**, 3260 (1987).
10. T. E. Torchia, R. W. Hamilton, C. L. Cano, J. E. Hopper, *Mol. Cell. Biol.* **4**, 1521 (1984); R. R. Yocum and M. Johnston, *Gene* **32**, 75 (1984).
11. N. F. Lue, D. I. Chasman, A. R. Buchman, R. D. Kornberg, *Mol. Cell. Biol.* **7**, 3446 (1987); M. R. Parthun and J. A. Jaehning, *J. Biol. Chem.* **265**, 209 (1990).
12. The GAL80-VP16 fusion was constructed by ligating the Bgl II–Bam HI fragment of pCRF3 encoding the COOH-terminal 78 amino acids of VP16 into a Bam HI site created at the last codon (amino acid position 435) of GAL80. The *GAL4* alleles were carried on the single-copy shuttle vector pSB32. *GAL80* and *GAL80-VP16*, respectively, were integrated at the *ura3* locus. Yeast strain YJ0-Z (this study) was used, which is *a gal4$^{\Delta}$ gal80$^{\Delta}$ ura3-52 leu2-3,112 his3 ade1 MEL1 GAL1::lacZ*. β-Galactosidase is a product of the chromosomally integrated *Escherichia coli lacZ* gene under *GAL1* promoter regulation. Carbon sources were 3% glycerol and 2% lactic acid for uninduced cultures and 3% glycerol, 2% lactic acid, and 2% galactose for induced cultures. Whole-cell yeast extracts were prepared by the glass bead method (*8*). Values shown are the average from at least three independent assays. Values from independent assays varied by <15%.
13. The *GAL4, GAL80, GAL80-VP16, GST*, and *GST-34* alleles were transcribed in vitro with T7 RNA polymerase (Promega, Madison, WI) and translated in the presence of ^{35}S-labeled methionine (Amersham, Arlington Heights, IL) in rabbit reticulocyte lysates according to protocols supplied by the manufacturer (Promega). The *GAL4* alleles were deleted for the internal two-thirds of the coding sequence to facilitate translation. These truncated GAL4 proteins are active in vivo and yield an inducible phenotype. GST is glutathione *S*-transferase [D. B. Smith and K. S. Johnson, *Gene* **67**, 31 (1988)]. GST-34 contains 34 COOH-terminal amino acids (amino acid positions 841 to 874) of GAL4 fused to glutathione *S*-transferase. Binding reactions contained A(50) buffer [25 mM tris (pH 7.5), 5 mM $MgCl_2$, 0.1 mM EDTA, 10% glycerol, 50 mM KCl], UAS_G (1 ng) [J. C. Corton and S. A. Johnston, *Nature* **340**, 724 (1989)] and in vitro translated proteins (2 μl) in a total volume of 15 μl. Reactions were incubated for 15 min at room temperature before loading on a 5% nondenaturing polyacrylamide gel (acrylamide:bis = 29:1) in 0.5× TBE (45 mM tris, 45 mM boric acid, and 1 mM EDTA, pH 8.0). Electrophoresis was performed for 3 hours at 150 V. After electrophoresis, the gel was fixed for 30 min in 50% methanol, 10% acetic acid, and dried under vacuum. The dried gel was exposed to Kodak X-Omat film for 2 hours. Values shown for β-galactosidase activity were determined as described (*12*).

14. Plasmid pCRF3 was provided by S. Triezenberg. This work was supported by grants from NIH and the Human Frontier Science Program to S.A.J. and funds from the Perot Family Foundation and the Moss Heart Trust.

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TECHNICAL COMMENTS

Measuring Mechanosensitive Channels in *Uromyces*

X.-L. Zhou *et al.* report (*1*) the existence of a mechanosensitive ion channel in the plasma membrane of the phytopathogenic fungus *Uromyces appendiculatus*. They suggest that, by transducing plasma membrane stress induced by leaf topography into cation influx, the channel may play a role in triggering differentiation of the tips of germlings into appressoria, structures that are required for infecting a host plant.

Uromyces, like all fungi and many other cell types, extends by the process of tip growth, a highly polarized mode of cellular extension (*2*). In view of studies of transhyphal electrical currents and apically high Ca^{2+} gradients, it is likely that the distribution of ion transport proteins along a fungal hypha is far from random (*3*), as we have recently found in studies of Ca^{2+}-activated K^+ and stretch-activated channels in *Saprolegnia ferax* (*4*). Thus any study of ion transport proteins located on the membrane of a growing tip should give detailed information about the origin of the membranes or protoplasts from which they were obtained.

Zhou *et al.* report (*1*) that all protoplasts used in their study were formed from the germling apices, which appears to mean that they were liberated from the apex rather than having been generated from apical regions of the cell. Protoplasts selected for patch-clamping were "uniformly dark, smooth, and empty when viewed with phase contrast optics" (*1*). These protoplasts differ markedly from those we have derived from the hyphal tip of *S. ferax* (*4*). We found protoplasts produced in distinct linear arrays, which enabled us to select for patch-clamping protoplasts that originated from different regions of the fungal hypha. Without exception, protoplasts from the tip region appeared cytoplasmically rich. We found more vacuolation in protoplasts derived from more distal regions of the hypha. Cytologically, vacuolated cells may exhibit more detail (because of cytoplasmic strands and the cytoplasmic rim) than cytoplasm-rich cells, which show uniformity (but are also refractile). In contrast, vacuoles are uniformly "dark, smooth, and empty." Our in situ protocol differed from the protocol of Zhou *et al.* (*1*), in which information about provenance was not provided.

Zhou *et al.* do not appear to account for the possibility that they may have been using vacuoles liberated from the highly vacuolate (*5*) germlings or protoplasts during growth and wash treatments. Bertl and Slayman (*6*), who studied the functional expression of membrane proteins, overcame gigaseal formation problems when attempting patch-recording of yeast plasma membrane by using vacu-