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.

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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*).
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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-

olar membranes. Furthermore, in plant and fungal cells, vacuolar mechanosensitive high-conductance channels could possibly function as osmoregulators to maintain isotonicity between the vacuole and the cytoplasm.

Ashley Garrill
Roger R. Lew
I. Brent Heath
Department of Biology,
York University,
4700 Keele Street,
North York, Ontario, Canada M3J IP3

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Response: The evidence that in our study (*1*) we were measuring mechanosensitive channels in the plasma membrane and not in vacuolar membrane–bounded spherical bodies is as follows.

1) All protoplasts produced from urediospore germlings of *Uromyces appendiculatus*, whether rich with cytoplasmic contents or devoid of such contents ("uniformly dark, smooth, and empty"), were formed only from germling apices. Most were formed from the apical dome of the germ tube apex; a few formed as far back as 15 μm from the most extreme point of the apex (about 10% of the length of the cell cytoplasm), but not further back.

2) In light and electron microscopy observations, we found that the first 30 to 40 μm of the *Uromyces* germling apex did not contain vesicles (vacuoles) larger than 0.3 μm in diameter (*2*, *3*). Such vesicles would be extremely difficult to patch-clamp. Larger vacuoles are formed only in "the region behind the nuclei where vacuolation occurs" (*3*). Two nuclei are normally positioned in the midcytoplasmic region of germlings that are about 3 hours old, a time when all of the protoplasm has just migrated out of the spore. We used germlings less than 2 hours old and 40 to 70 μm in length. The nuclei in such germlings would just be entering the germ tube from the spore, and vacuoles of any significant size would not yet have formed in the germ tube.

3) The temporal aspects of protoplast development under various environmental conditions are most important. Most of the protoplasts formed from *Uromyces* germlings were rich in cytoplasm when produced in 0.6 M sorbitol buffered with 17 mM MES, pH 6.0, containing Novozyme (5 mg/ml), and more so when the osmoticum was changed to 0.55 M (Fig. 1A). "Uniformly dark, smooth, and empty" protoplasts were produced in abundance when germlings were initially treated with, and left in, 0.5 to 0.525 M osmoticum containing Novozyme (Fig. 1B). Such protoplasts began forming within 1 min from the time the osmoticum with Novozyme was added and continued to enlarge over the next 5 to 10 min. Occasionally, with additional time, cytoplasmic organelles migrated or streamed into some of these "empty" protoplasts. Many germlings ceased forming protoplasts after the initial production of the "empty" ones, although many of the same germlings also subsequently produced typical protoplasts filled with cytoplasm. It seems unlikely that vesicles bounded by the vacuolar membrane would be similarly filled. We never observed the most proximal one-third of germling protoplasm, which normally contains vacuoles, exit the germ tube during the formation of protoplasts.

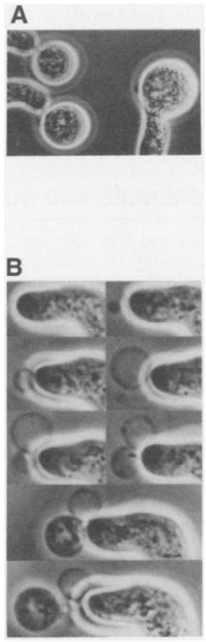

Fig. 1. Germling apices of *Uromyces*. (**A**) Protoplasts filled with cytoplasm after treatment. (**B**) Apex with plasma membrane–bounded, "empty" protoplasts. The protoplast formed first (left to right, then down) is devoid of cytoplasmic organelles, and the protoplast formed next is filled with a few organelles, possibly mitochondria.

Different fungal protoplasts can have different appearances (*1*, *4*, *5*), and the same fungus can generate different objects covered with plasma membrane. If zymolyase is used to digest the cell wall of *Schizosaccharomyces pombe*, protoplast protuberances are formed at the cell apex. If Novozyme is used (*6*), protoplasts are generated by global wall digestion. They look shiny and appear to have surface invaginations. We were able to form gigaseals in all forms of protuberances and protoplasts of *S. pombe*. Some types of ion channels (as measured by identical conductance, voltage dependence, and kinetics), including a mechanosensitive channel, were found in all these forms. Like the spheres (protoplasts) from *Uromyces* after Novozyme treatment, the protuberances of *S. pombe* appear smooth, dark, and empty (*5*, *7*) and can become filled with cytoplasmic organelles.

Garrill *et al.* hypothesize that there might be high-conductance mechanosensitive channels in vacuolar membranes of plant and fungal cells. We have patch-clamped more than 200 vacuoles of *Saccharomyces cerevisiae* (*8*) and encountered voltage-gated or Ca^{2+}-gated channels, but not mechanosensitive channels. Every one of hundreds of plasma membrane patches of *S. cerevisiae* contained mechanosensitive channel activities.

Contrary to the implication of Garrill *et al.*, Bertl and Slayman, who have studied recently the kinetics of the K^+ channel of this membrane (*9*), stated that they formed seals of higher than 20 gigaohms on the plasma membrane of *S. cerevisiae*. For the reasons stated above, we are confident that we have demonstrated the presence of mechanosensitive channels in the plasma membrane of *Uromyces*.

H. C. Hoch
Department of Plant Pathology,
Cornell University,
New York State Agricultural Experiment Station,
Geneva, NY 14456
C. Kung
X.-L. Zhou
Laboratory of Molecular Biology and *Department of Genetics,*
University of Wisconsin,
Madison, WI 53706
M. A. Stumpf
413 Dovercourt Road,
Toronto, Ontario M6J 3E8,
Canada

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