The Yeast G Protein α Subunit Gpa1 Transmits a Signal through an RNA Binding Effector Protein Scp160

Ming Guo,1,2 Christopher Aston,3 Scott A. Burchett,4 Christine Dyke,1 Stanley Fields,2,3 S. Johannes R. Rajarao,3 Peter Uetz,5,7 Yuqi Wang,1 Kathleen Young,2 and Henrik G. Dohlman 1,*
1Department of Biochemistry and Biophysics University of North Carolina at Chapel Hill Chapel Hill, North Carolina 27599
2Interdepartmental Neuroscience Program Yale University New Haven, Connecticut 06520
3Neuroscience Research Wyeth Research Princeton, New Jersey 08543
4National Institutes of Health Laboratory of Neural Connectivity Bethesda, Maryland 20892
5Departments of Genome Sciences and Medicine Howard Hughes Medical Institute University of Washington Seattle, Washington 98195

Summary

In yeast Saccharomyces cerevisiae the G protein βγ subunits (Ste4/Ste18) have long been known to transmit the signal required for mating. Here we demonstrate that GTPase-deficient mutants of Gx (Gpa1) directly activate the mating response pathway. We also show that signaling by activated Gpa1 requires direct coupling to an RNA binding protein Scp160. These findings suggest an additional role for Gpa1 and reveal Scp160 as a component of the mating response pathway in yeast.

Introduction

All cells have the capacity to respond to chemical and sensory stimuli in their environment. In many cases, signal detection occurs through cell surface receptors coupled to G proteins. In humans, receptors of this type can detect hormones, neurotransmitters, odors, taste, and light. In the yeast Saccharomyces cerevisiae, G protein-coupled receptors bind peptide ligands that promote haploid cell fusion, or mating. In this instance, haploid α and β cell types respond to pheromones secreted by cells of the opposite type. Receptor stimulation leads to activation of a G protein composed of an α subunit (Gpa1) and a tightly associated βγ dimer (Ste4/Ste18). Upon stimulation, Gx releases GDP, binds GTP, and dissociates from the βγ subunits, which in turn activate downstream signaling proteins. Events that precede mating include new gene transcription, morphological and cytoskeletal changes, and cell cycle arrest in G1 (Dohlman, 2002).

Gβγ propagates the mating signal through activation of at least three effector proteins: the Cdc42 GDP-GTP exchange factor Cdc24 (Zheng et al., 1994), a protein kinase Ste20 (Leeuw et al., 1998), and a kinase scaffolding protein Ste5 (Feng et al., 1998; Inouye et al., 1997; Whiteway et al., 1995). Activation of Cdc24 and Cdc42 is required for the morphological changes that accompany pheromone stimulation. Binding of Gβγ to Cdc24 occurs indirectly through Far1, a scaffold protein that is also required for pheromone-induced cell division arrest in G1. Activation of Ste20 initiates a signaling cascade that includes three other protein kinases associated with Ste5. The first is Ste11, which phosphorylates and activates a dual-specificity kinase Ste7, which phosphorylates and activates two mitogen-activated protein kinases (MAPKs) Fus3 and Kss1. Among the known MAPK substrates is the transcription factor Ste12, which is responsible for induction of most pheromone-inducible genes (reviewed in Dohlman and Thorner, 2001).

Gβγ has long been regarded as the sole signal-transmitting component of the G protein in yeast. Deletion of genes encoding either Gβ or Gγ results in a pheromone-insensitive sterile phenotype, while overexpression leads to constitutive signaling and growth arrest. Deletion of the Gx gene GPA1 leads to constitutive signaling and growth arrest, apparently due to uncontrolled signaling by free Gβγ. Conversely, overexpression of GPA1 leads to diminished signaling due to sequestration of free Gβγ. These findings demonstrated that the βγ moiety is sufficient to transmit the pheromone signal (reviewed in Dohlman and Thorner, 2001).

Studies in other systems have revealed examples of cooperative (or in some cases antagonistic) regulation of signaling pathways by both Gx and Gβγ (Jordan et al., 2000). Here, we propose a positive signaling function for the Gx subunit in yeast. These findings are significant because Gpa1 has not previously been shown to transmit a signal to any known effector. Moreover, the effector in this case, Scp160, has not previously been recognized to act in the pheromone response pathway, and more generally, RNA binding proteins have not previously been identified as targets of G protein activation.

Results

GTPase-Deficient Gpa1 Mutants Activate the Mating Pathway

Our goal here was to identify an effector and signaling pathway activated by Gpa1. Any such effector will, by definition, bind and respond only to the GTP-bound form of the protein. Thus, our initial approach was to determine the functional consequences of a Gpa1 mutant that cannot hydrolyze GTP, since this would result in permanent activation of any effector. Such a mutant will not directly affect signaling by Gβγ, since Gβγ binds only to the inactive GDP-bound form of Gx (Sprang, 1997). To this end, we expressed in wild-type cells two

*Correspondence: h.dohlman@med.unc.edu
Present address: Institut für Genetik, Forschungszentrum Karlsruhe, D-76021 Karlsruhe, Germany.
Gpa1 mutations that block (Q323L) or slow (R297H) GTP hydrolysis (Apanovitch et al., 1998) and measured pheromone-dependent gene transcription using a reporter-transcription assay (FUS1 promoter, lacZ reporter). Because activation of the pheromone pathway can also trigger growth arrest, expression of the Gpa1 mutants was initially done using a galactose-inducible promoter that was activated shortly before performing the assay.

As shown in Figure 1A, both Gpa1 mutants produced a marked increase in signaling over the entire range of pheromone concentrations tested. Compared with the empty vector control, basal signaling was increased by 10-fold (Gpa1R297H) or 15-fold (Gpa1Q323L), and the maximum response was increased by ~40%. There was no difference in EC50. In contrast, overexpression of wild-type Gpa1 diminished the maximum response by more than half. This inhibitory effect was anticipated, since the wild-type protein in the GDP-bound state will help to sequester free GTP. As an additional control we tested the Gpa1E364K mutation, which was previously shown to inhibit pheromone-dependent growth arrest (Stratton et al., 1996). In this case we observed an inhibition of pheromone-dependent transcription nearly identical to that seen with overexpression of wild-type Gpa1 (data not shown). We also found that overexpression of Gpa1Q323L leads to increased phosphorylation of Ste4 (data not shown), an event that requires activation of the MAP kinases Fus3 and/or Kss1 (Li et al., 1998). These results indicate that the GTP-bound form of Gpa1 can activate the mating response pathway leading to new gene transcription and MAP kinase activation.

Another way to measure pheromone sensitivity is the growth inhibition plate assay (halo assay). In this method, a nascent lawn of cells is exposed to different amounts of pheromone spotted onto paper disks. The resulting zone of growth inhibition provides an indication of the cellular response to pheromone after several days. Thus, we compared growth arrest in cells that overexpress Gpa1Q323L or Ste4 versus the empty vector control. As shown in Figure 1B, Gpa1Q323L produced nearly normal-sized halos, indicating that activation of Gpa1 has no effect on the growth arrest response (Apanovitch et al., 1998). In contrast, overexpression of Ste4 completely inhibited growth such that no cells were visible even after prolonged incubation (data not shown).

Cells treated with high concentrations of pheromone
will eventually arrest growth and assume an enlarged and elongated “shmoo” morphology. Thus, we compared growth arrest in cells that overexpress Gpa1Q323L or the empty vector control. As shown in Figure 1C, overexpression of Gpa1Q323L produced an enlarged and elongated cell morphology; however, the cells continued to bud and divide. This is consistent with the results presented above, indicating that Gpa1 can initiate a signal leading to transcription but it does not affect the growth arrest response. A number of other signaling mutants (e.g., fus3, far1) have likewise been shown to simultaneously shmoo and divide in the presence of pheromone.

The ultimate purpose of pheromone stimulation is to promote mating. Thus, we compared the mating efficiency of cells that overexpress the Gpa1Q323L mutant with those that overexpress wild-type Gpa1 and cells that express normal levels of Gpa1. As shown in Figure 1D, Gpa1Q323L improved the mating frequency by more than 4-fold, while overexpression of wild-type Gpa1 diminished mating efficiency more than 10-fold, as compared with the control strain. These data mirror the results of the reporter-transcription, Ste4 phosphorylation, and cell morphology assays presented above, and support the model that GTP-bound Gpa1 can activate the mating-response pathway in parallel with Gβγ.

Genetic Analysis of Gpa1 Signaling

The data above suggest that Gα and Gβγ can each promote activation of the mating response pathway. Activation by each moiety could occur through separate effectors; one effector responds to Gβγ and promotes morphological changes, transcriptional induction, and growth arrest, while a second effector responds to Gα and promotes morphological changes and transcription only. An alternative possibility is that the Gpa1 effector acts in opposition to Ste4/Ste18 to inhibit the growth arrest response. These models are considered in more detail below.

To determine the point at which the two pathways converge, we examined Gpa1 signaling in a series of gene deletion mutants affecting known components of the pathway: these include the cell surface receptor (ste2Δ) and G protein βγ subunits (ste4Δ), as well as two downstream kinases (ste17Δ, ste7Δ). As shown in Figure 2A, Gpa1 transriptional activation was abolished in the ste4Δ, ste17Δ, and ste7Δ mutants. Transcriptional induction was still observed in the ste2Δ strain; however, the activity was diminished compared with that of the wild-type strain, presumably because unliganded receptor can promote a low level of GDP-GTP exchange on Gα (Siekhaus and Drubin, 2003). As expected, pheromone treatment had no additional stimulatory effect on signaling in any of these sterile mutants. Taken together, these results suggest that the Gpa1 effector acts downstream of the receptor, but at or upstream of Gβγ.

Since Gpa1 can stimulate transcription but fails to promote growth arrest, we considered whether it might act specifically through the MAPK Kss1. Deletion of KSS1 results in diminished transcription yet does not alter the growth arrest response. Deletion of the other MAPK in the pathway, Fus3, diminishes both activities (Cherkasova et al., 1999; Cook et al., 1997; Madhani et al., 1997). Moreover, Kss1 and/or Fus3 appear to phosphorylate a number of proteins that bind to Gpa1 (Ste3, Sat2, Ste4) and Ste4 (Ste5, Far1), consistent with a close association of Gpa1 and the MAPKs (Dohlman and Thorner, 2001; Metodiev et al., 2002). Thus, we compared signaling by Gpa1Q323L in strains lacking either Fus3 or Kss1. As shown in Figure 2B, pheromone-induced transcription was diminished to a similar extent in both the fus3Δ and kss1Δ gene deletion mutants. Gpa1Q323L–initiated signaling was also similar in both mutants, indicating that Gα signaling can occur through either kinase and not Kss1 specifically.

If Gα and Gβγ activate a common signaling pathway, they should induce the same set of gene transcripts. To test this, we transformed wild-type cells with Gpa1Q323L or an empty vector and grew these in the absence or presence of α factor for 1 hr. For these experiments we used a low dose of pheromone sufficient to trigger new gene transcription but not sufficient to invoke cell division arrest or induction of cell cycle-regulated genes (since Gpa1Q323L does not trigger arrest). Biotin-labeled cRNA was prepared from each strain and hybridized to an Affymetrix GeneChip representing ~7000 genes and open reading frames. Under these conditions pheromone induced 20 transcripts, 17 of which were also induced by Gpa1Q323L (Figure 2C). These data provide further evidence that Gpa1 and Ste4/Ste18 activate the same gene promoter elements, most likely through a common transcription factor Ste12.

The data presented in Figure 2A indicate that Gpa1 signals through Ste4/Ste18. If Gα and Gβγ are redundant, signaling by both components is likely to be non-additive. If they have distinct functions, signaling might appear additive or even synergistic. To distinguish between these possibilities, we compared the transcription response in cells that overexpress Gpa1Q323L, Ste4, or both. As shown in Figure 2D, overexpression of either protein produced a marked increase in signaling over the entire range of pheromone concentrations tested. Overexpression of both Ste4 and Gpa1Q323L further enhanced signaling across the entire dose-response profile. In the absence of added pheromone or when the effects of pheromone were subtracted, activation by Gpa1Q323L and Ste4 was additive. We conclude from these data that GTP-bound Gpa1 can contribute to signaling even when a cell is already activated by Gβγ. Stated differently, activation by both Gα-GTP and Gβγ is required for full activation of the pathway.

The experiments described above were performed in cells that contain endogenous wild-type Gpa1. We also examined the transcription response of Gpa1Q323L in a gpa1Δ mutant. Deletion of GPA1 will ordinarily result in constitutive growth arrest (through uncontrolled release of Gβγγ) but can be maintained by placing a downstream effector kinase under control of an inducible promoter (GAL1 promoter, STE7 gene). As shown in Figure 2E, overexpression of Gpa1Q323L produced an increase in Fus1-lacZ activity, similar to that observed in wild-type cells treated with pheromone (Figure 1A) or cells that lack Gpa1 altogether. Gpa1Q323L was also unable to suppress the constitutive (Gβγγ-mediated) growth arrest phenotype of the gpa1Δ strain (Apianovitch et al., 1998). These results show that mutationally activated Gpa1 can function in the absence or presence of endogenous wild-type Gpa1 and cannot sequester Gβγγ in vivo.
Figure 2. Gpa1 and Ste4/Ste18 Converge on a Common Signaling Pathway

(A and B) Plasmid pAD4M containing GPA1 Q323L (pADH-GPA1QL) or no insert (pADH) was transformed into wild-type and mutant strains, as indicated. The reporter transcription assay was performed as described in the legend to Figure 1, except that the cells were grown in the presence of dextrose.

(C) Wild-type cells were transformed with plasmid pAD4M containing no insert (vector) or GPA1 Q323L. Biotin-labeled cRNA was hybridized to an Affymetrix GeneChip. Data are from values generated by averaging three replicate chips per strain. Comparisons are Gpa1 Q323L versus vector (hatched bars, left axis) or vector plus pheromone versus no pheromone (black bars, right axis). Shown are genes induced by /H11022 3-fold after 2.5 nM /H9251 factor (20 total) that are also induced by Gpa1 Q323L.

(D) Reporter transcription activity was measured in wild-type cells transformed with plasmid pAD4M containing no insert or GPA1 Q323L, and plasmid pRS316-GAL containing STE4 (pGAL-STE4) or no insert (pGAL).

(E) Reporter transcription activity was measured in a gpa1/ste7/ mutant strain transformed with plasmid pAD4M containing no insert, GPA1, or GPA1 Q323L, and plasmid pYES containing STE7 and the GAL1 promoter. Cells were grown in dextrose and then shifted to galactose to induce STE7 (as indicated) for 6 hr.

Identification of a Gpa1 Effector by Genome-Wide Two-Hybrid Screening

One way to ascertain the Gpa1 effector is through the identification of associated proteins. To this end, we carried out a two-hybrid screen against an array of nearly all yeast open reading frames and identified Scp160 as a candidate binding partner (Uetz et al., 2000).

Scp160 Binds to the Active Conformation of Purified Gpa1

Any G protein effector will bind Gα in the active GTP-bound state, but not the inactive GDP-bound state. Thus, we examined whether Scp160 can interact with Gpa1 in a guanine-nucleotide-dependent manner. To this end, we coexpressed full-length Scp160 fused to
Over the past fifteen years the paradigm of Gα signaling in yeast has held firm. Here, we propose a plasmid-expressing component of the Gpa1 signaling pathway. No RNA We have shown here that Gpa1Q323L amplifies the pheromone signaling function for the Gα protein, and this activity requires Scp160. Coexpression of Gβγ further amplifies the signal, but with some distinct properties. Whereas Gpa1E364K signaling requires expression of SCP160, Gβγ does not. Whereas Gβγ promotes growth arrest, Gpa1E364K does not. Perhaps the Gpa1E364K signal falls below a threshold needed to induce cell cycle arrest. Alternatively, Scp160 might regulate a component of the cell cycle machinery that diminishes the growth arrest response.

Gα proteins have traditionally been regarded as regulators of enzymes that produce some form of chemical second messenger. Recent studies have revealed new types of G protein effectors that are not necessarily...
Figure 4. Scp160 Binds to the Active State of Gpa1

Cells expressing Scp160-Myc (in plasmid pYES) and either Gpa1-GST or GST alone (plasmid pAD4M) were grown to mid-log phase and then lysed in the presence of GDP, GDP\textsuperscript{\textbeta}AlF\textsubscript{4}, or GTP\textgammaS as indicated. Detergent-solubilized lysates were immobilized on glutathione-Sepharose, washed, and eluted with SDS-PAGE sample buffer. Retained protein (Bound) was detected by immunoblotting with antibodies against (B) GST, (D) Myc, and (F) Ste4. Samples of the soluble cell lysate (Applied) were similarly probed with antibodies against (A) GST, (C) Myc, and (E) Ste4 to confirm equivalent levels of protein expression. Arrows indicate the protein specifically recognized by the antibody.

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enzymes but which indirectly regulate such enzymes. The G\alpha\textgamma effector Eya2 functions as a transcription co-activator (Fan et al., 2000). G\alpha\textgamma and G\alpha\textbeta have been reported to activate Rap1GAP, a GTPase accelerating protein for the monomeric G protein Rap1 (Meng et al., 1999; Mochizuki et al., 1999). G\alpha\textgamma and G\alpha\textbeta bind cadherin and promote dissociation of the transcriptional activator \beta-catenin (Meigs et al., 2001). G\alpha\textdelta and G\alpha\textgamma activate p115RhoGEF, which is a guanine nucleotide exchange factor for RhoA (Hart et al., 1998; Kozasa et al., 1998). Our findings suggest that Scp160 is an RNA binding effector that is activated by the G protein \alpha subunit. These findings propose that the signaling network is larger and more complex than previously recognized, and raise the intriguing prospect of RNA as a form of "second messenger."

Experimental Procedures

Strains and Plasmids

Standard methods for the growth, maintenance, and transformation of yeast were used throughout (Ausubel et al., 1987). The yeast S. cerevisiae strains used in this study were BJ2168 (MATa ura3-52 leu2-3,112 trp1-1122看他1-407 pep4-3) (from E. Jones, Carnegie-Mellon University), BY4741 (MATa leu2-3,112 met15-423 his3-13 ura3-52), and BY4741-derived mutants lacking YBR116c, SCP160, STE2, STE4, STE11, STE7, FUS3, and KSS1 (Research Genetics). pYES-SCP160, pYES-SCP160-MYC, and pYES-STE7 were constructed by subcloning PCR-amplification products into pYES2.1 TOPO TA (Invitrogen Corporation). Other plasmids used here have been described previously (Dohlman et al., 1995, 1996; Song et al., 1996).

Functional Assays

The functional assays described previously include microarray analysis (Burchett et al., 2002) and mating efficiency (Dohlman et al.,...
as well as halo and reporter-transcription assays (Hoffman et al., 2002). RNA for microarray was isolated from BY4741 cells transformed with either pADAM-GPA1 or pADAM and treated at mid-log phase with 0 or 2.5 mM α factor for 1 hr. Binding was performed as described earlier for Gpa1 binding to Gβγ, with minor modifications (Song et al., 1996). Samples were resolved using 6% (for Scp160-Myc) or 10% SDS-PAGE, and detected with antibodies to GST (from J. Steitz, Yale University), Myc, or Ste4 (from D. Jenness, University of Massachusetts), and the ECL chemiluminescence system (Amersham Pharmacia Biotech).

Acknowledgments

This work was supported by NIH grants P41 RR11823 (to S.F.), GM59167, and GM065533 (to H.G.D.). M.G. is a graduate student in the Yale Interdepartmental Neuroscience Program. S.F. is an investigator of the Howard Hughes Medical Institute.

Received: June 5, 2002
Revised: May 7, 2003
Accepted: May 28, 2003
Published: August 28, 2003

References


