Gradient-directed cell migration (chemotaxis) and growth (chemotropism) are processes that are essential to the development and life cycles of all species. Cells use surface receptors to sense the shallow chemical gradients that elicit chemotaxis and chemotropism. Slight asymmetries in receptor activation are amplified by downstream signaling systems, which ultimately induce dynamic reorganization of the cytoskeleton. During the mating response of budding yeast, a model chemotropic system, the pheromone receptors on the plasma membrane polarize to the side of the cell closest to the stimulus. Although receptor polarization occurs before and independently of actin cable–dependent delivery of vesicles to the plasma membrane (directed secretion), it requires receptor internalization. Phosphorylation of pheromone receptors by yeast casein kinase 1 or 2 (Yck1/2) stimulates their internalization. We showed that the pheromone-responsive Gβγ dimer promotes the polarization of the pheromone receptor by interacting with Yck1/2 and locally inhibiting receptor phosphorylation. We also found that receptor phosphorylation is essential for chemotropism, independently of its role in inducing receptor internalization. A mathematical model supports the idea that the interaction between Gβγ and Yck1/2 results in differential phosphorylation and internalization of the pheromone receptor and accounts for its polarization before the initiation of directed secretion.

INTRODUCTION

Polarized cellular growth in response to a chemical gradient (chemotropism) is a fundamental process that is required for a broad range of phenomena, including angiogenesis, axon growth cone guidance, pollen tube guidance, fungal life cycles, and fungal pathogenicity. Similar to chemotactic cells, chemotroping cells must be able to determine the direction of dynamic chemical gradients, establish a stable axis of polarity, and realign that axis as they track changes in the gradient.

The ability of the budding yeast, *Saccharomyces cerevisiae*, to mate efficiently depends on what is to date the best-understood chemotropic response. During the sexual reproduction phase of their life cycle, haploid yeast cells of the opposite mating types *MATα* and *MATα* signal their position to one another by secreting peptide pheromones. The binding of pheromone to heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptors (GPCRs) on the surface of each cell type—Ste2 in *MATa* cells and Ste3 in *MATα* cells—and the consequent activation of their cognate G proteins induce cell cycle arrest, changes in gene expression, and the formation of mating projections, commonly called shmoo. Cells grow up the pheromone concentration gradient toward a potential mating partner, leading to the eventual fusion of the partners at their growth tips (1). The polarization of cell growth (morphogenesis) is accomplished by the directed movement of secretory vesicles along actin cables oriented toward the growth site (2, 3). The Gβγ subunit of the heterotrimeric G protein serves as a positional determinant for the chemotropic growth site by linking the pheromone receptor to the machinery that nucleates actin cables (4, 5).

As the most upstream elements in the mating response pathway, the pheromone receptor and its G protein are the primary gradient sensors. Unlike most chemotaxing cells, whose surface receptors are uniformly distributed on the plasma membrane, pheromone-stimulated yeast cells polarize their GPCR. In response to isotropic pheromone treatment, the receptor concentrates around the predetermined bud site, which is also known as the default polarity site. In mating mixtures, the receptor polarizes toward proximal mating partners, presumably where the pheromone concentration is greatest, and this phenomenon is detectable before morphogenesis occurs (6). The phenomenology and regulation of pheromone-induced receptor polarization in *MATα* cells is well documented. During vegetative growth, the *MATα*-specific receptor Ste2 appears uniformly distributed on the plasma membrane. When activated by ligand, however, the receptor is sequentially phosphorylated and ubiquitylated on its C-terminal cytoplasmic domain (7, 8). This stimulates its global internalization, after which it reappears as a polarized crescent on the cell surface (9, 10). Although receptor polarity could arise from the directed delivery of nascent receptors to the incipient shmoo site, several observations argue for a distinct mechanism. First, Ste2 becomes polarized before morphogenesis occurs. Second, establishment of receptor polarity does not depend on the directed delivery of vesicles along actin cables (6). Third, the genesis of receptor polarity absolutely depends on receptor internalization (6).
How does the receptor polarize toward the gradient source before and independently of actin cable-directed delivery of secretory vesicles? One possibility is that the pheromone-induced global internalization of the receptor is slower on the up-gradient side of the cell such that relative receptor density increases at the future shmoo site. This is consistent with the observation that the redistribution of the receptor depends on its internalization, but at the same time, it introduces a paradox: Because only activated receptors are phosphorylated, ubiquitylated, and internalized, the rate at which the receptor is removed from the cell surface should be greatest where the pheromone concentration is highest. In principle, therefore, the establishment of the chemotropic growth site through differential internalization of the receptor requires a mechanism to locally inhibit receptor internalization.

In a genetic screen for proteins that interact directly with Gβ (11), we identified yeast casein kinase 1 (Yck1), one of a pair of sister casein kinases (CKs) (12) that are essential for the phosphorylation of the pheromone receptor on its C-terminal cytoplasmic domain (8) and hence for its internalization (7, 8). Here, we showed that Gβ interacted with Yck1 at the plasma membrane and that Gβ locally inhibited phosphorylation of the receptor, consistent with the idea that differential phosphorylation and internalization of the receptor play a role in the establishment of polarity. In addition, imaging and genetic data suggest that differential receptor phosphorylation contributes to chemotropic function. A mathematical model that incorporates the Gβ-dependent inhibition of receptor phosphorylation mimics key aspects of gradient-induced receptor polarization.

RESULTS

Gβ interacts with Yck1 at the plasma membrane and is a candidate Yck substrate

To identify candidate interactors of Gβ, we previously conducted an allele-specific dosage suppressor screen in yeast in which we took advantage of the observation that the overexpression of Gβ induces the mating signal and thereby confers cell cycle arrest (13, 14). A high-copy yeast complementarity DNA (cDNA) library was screened for plasmids that could rescue the overexpression of wild-type Gβ, but not that of an adaptive-defective mutant form of Gβ, encoded by STE4A405V. Ten genes were identified, including those encoding Rho1 (11), Dse1 (15), and Yck1. In addition to genetic evidence of Gβ-Yck1 interaction, here we found that a myc-tagged form of Yck1 expressed in yeast specifically bound to a Gβ affinity column, as evidenced by both Western blotting and mass spectrometric analyses (Fig. 1, A and B, and Table 1). Moreover, a direct physical interaction between Gβ and Yck1 in yeast was shown by bimolecular fluorescence complementation (BiFC) assay (Fig. 1C and Table 2). The Gβ-Yck1 BiFC signal was detectable on the plasma membrane of pheromone-treated cells before morphogenesis occurred and was concentrated on the plasma membrane of mating projections in shmooing cells.

Because Gβ is phosphorylated on multiple sites in pheromone-treated cells (16) and because its sequence contains potential CK1 sites (17–19), we asked whether Gβ phosphorylation depended on Yck activity. Pheromone did not induce full phosphorylation of Gβ in cells lacking functional Yck1 and Yck2 (hereafter, Yck1/2) (Fig. 1D). Previous genetic analyses suggested that Gβ residues Thr230 (T320), Thr232 (T322), and Ser335 (S335) are phosphorylated and that full Gβ phosphorylation is dependent on the pheromone-responsive mitogen-activated protein kinase (MAPK) Fus3 (20). Mass spectrometric analysis of Gβ phosphorylated peptides purified from pheromone-treated cells confirmed these phosphorylation sites and revealed an additional site at Thr118 (Fig. 1E and fig. S1). Study of mammalian CK1s suggests that they require a phosphorylated substrate and...
that S/T²₅₀X/S/T (where n = 1 to 3 and X is any amino acid residue) is the consensus motif (17–19). If this holds true for yeast, the phosphorylation of Thr²₁₈ or Thr²₃₀ would convert Gβ to a Yck1 substrate (Fig. 1E). Conversely, unphosphorylated Gβ would be expected to interact with Yck1 to a lesser degree than would the partially phosphorylated species. We tested this with a mutant form of Gβ, stef²³₂₀₄ₛₛ₃₃₅₄ (henceforth Gβ²⁵), which cannot be phosphorylated (20). In pull-down experiments in which the relative amount of Yck1 that bound to Gβγ or Gβ²⁵γ beads was quantified by both Western blotting and mass spectrometric analyses (Fig. 1, A and B, and Table 1), the apparent affinity of Yck1 for Gβγ was less than that for Gβ. Note that endogenous Yck2 (that is, untagged native protein) was also identified by mass spectrometry as specifically binding to the Gβγ beads, and, like Yck1, it showed a weaker apparent affinity for Gβ²⁵ than for Gβ (Table 1).

Gβ overexpression inhibits phosphorylation of the pheromone receptor and promotes its polarized localization

Because Gβ adopts a polarized localization before pheromone-induced morphogenesis occurs (21), its direct interaction with Yck1 raises the possibility that Gβγ promotes receptor polarization by locally inhibiting receptor phosphorylation. Consistent with this, a region of the plasma membrane enriched in hypophosphorylated receptor arises in response to isotropic pheromone treatment (6, 22). This was demonstrated with strain YDB111 (22), which expresses a green fluorescent protein (GFP)-tagged form of the regulator of G protein signaling (RGS) protein Sst2, and Ste²⁷₅₈, a mutant form of the Malα receptor that cannot be internalized. The Sst2-GFP reporter binds specifically to the unphosphorylated form of the Ste2 receptor (22). YDB111 cells treated with latrunculin A (LatA, which inhibits polymerization of actin) and pheromone cannot redistribute the receptor; nevertheless, they exhibit asymmetric receptor phosphorylation (6).

To determine whether Gβ could inhibit receptor phosphorylation, we examined pheromone- and LatA-treated YDB111 cells overexpressing either Gβ or Gβ²⁵, the weaker Yck1/2-binding form of Gβ (Fig. 2, A to F). Overexpression of Gβ correlated with an increase in both the amount of Sst2-GFP that was localized to the plasma membrane and the extent of Sst2-GFP polarity. In contrast, overexpression of Gβ²⁵ did not affect the plasma membrane localization of Sst2-GFP. To further test the effect of weakening the Gβ-Yck1/2 interaction on receptor phosphorylation, we replaced STE4 (Gβ) with stef²³₂₀₄ₛₛ₃₃₅₄ (Gβ²⁵) in the YDB111 strain. Treatment with pheromone and LatA stimulated statistically significantly less plasma membrane localization of Sst2-GFP in cells expressing the native amount of Gβ²⁵, compared to that in control cells (Fig. 2, F and G). Together, these data support the idea that Gβγ protects the pheromone receptor from Yck1/2-dependent phosphorylation, and that it does so most effectively in a discrete region of the plasma membrane.

Local inhibition of receptor phosphorylation by Gβγ helps to establish and maintain the chemotropic growth site

In time-lapse images of mating mixtures, we found that the polarized receptor crescent frequently relocated from the default polarity site to the chemotropic site in cells that had not yet shmooed (fig. S2). Could anisotropic receptor phosphorylation play a role in this process? In mating mixtures of YDB111 cells, the Sst2-GFP crescent was almost always visible before morphogenesis occurred and was either formed initially at the eventual shmoo site or relocated from the presumptive default site to an apparent chemotropic site before morphogenesis (Fig. 3). Thus, the position of minimal receptor phosphorylation anticipated the eventual site

### Table 1. Mass spectrometric quantification of Yck1 and Yck2 pull-downs by Gβγ affinity beads.

<table>
<thead>
<tr>
<th>Detected protein</th>
<th>Affinity bead type</th>
<th>Intensity, 70 to 55 kD</th>
<th>Intensity, 55 to 45 kD</th>
<th>Total intensity</th>
<th>Gβ/Gβ²⁵ ratio</th>
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<tbody>
<tr>
<td>Yck1</td>
<td>Gβγ</td>
<td>3.16 x 10⁷</td>
<td>2.25 x 10⁷</td>
<td>5.42 x 10⁷</td>
<td>2.25</td>
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<td></td>
<td>Gβ²⁵γ</td>
<td>6.88 x 10⁴</td>
<td>1.72 x 10⁷</td>
<td>2.41 x 10⁷</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Empty</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Yck2</td>
<td>Gβγ</td>
<td>2.39 x 10⁷</td>
<td>0</td>
<td>2.39 x 10⁷</td>
<td>2.09</td>
</tr>
<tr>
<td></td>
<td>Gβ²⁵γ</td>
<td>1.15 x 10⁷</td>
<td>0</td>
<td>1.15 x 10⁷</td>
<td></td>
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<tr>
<td></td>
<td>Empty</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 2. Quantification of BiFC results.

<table>
<thead>
<tr>
<th>% Cells with PM signal*</th>
<th>PM/Cyto ± SEM †</th>
<th>% PM ± SEM ≥ 1.25x Cyto ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-shmoo</td>
<td>Shmooed</td>
<td></td>
</tr>
<tr>
<td>Gβ²⁵–VF2 +VF1-Yck1</td>
<td>28.5</td>
<td>15.2</td>
</tr>
<tr>
<td>Gβ–VF2 + VF1</td>
<td>3.4</td>
<td>27.4</td>
</tr>
<tr>
<td>Gβ²⁵–VF2 + VF1-Yck1</td>
<td>n.d.</td>
<td>0.95 ± 0.03</td>
</tr>
<tr>
<td>Gβ–VF2 + VF1</td>
<td>n.d.</td>
<td>17.0 ± 1.9</td>
</tr>
</tbody>
</table>

*Cells with a clear plasma membrane signal were scored; n ≥ 45 cells. †Plasma membrane signal was normalized to the cytoplasmic signal in each cell by dividing the mean plasma membrane fluorescence by the mean cytoplasmic fluorescence. ‡Percentage of the plasma membrane that showed a signal at least 25% greater than the mean cytoplasmic signal in each cell was determined. §As compared to the corresponding measure for Gβ–VF2 + VF1 cells; P < 0.0001 (n ≥ 19 cells).
Fig. 2. Effects of Gβ and GβP− on receptor phosphorylation. (A to C) Overexpression of Gβ, but not GβP−, inhibits phosphorylation of the pheromone receptor. (A) YDB111 cells expressing native Gβ protein, excess Gβ, or excess GβP− (excess indicated by double arrows) were treated with pheromone and LatA at time zero. The cells were then imaged by epifluorescence microscopy to visualize the indicated proteins. The intensity of the Sst2-GFP signal on the plasma membrane corresponds to the location and quantity of the unphosphorylated pheromone receptor, whereas that of the Ste2-mCherry signal corresponds to the location and quantity of total pheromone receptor. The arrowhead indicates a polarized Sst2-GFP crescent. Relative signal intensities are indicated by the color bar. Images are representative of three or two (for the control strain) experiments. (B) Western blotting analysis of the relative amounts of Gβ and GβP− in lysates of 15Dau cells expressing the indicated constructs after 0 and 2 hours of exposure to galactose to induce GAL1 expression. The topmost band corresponds to Gβ; asterisks indicate Gβ degradation products. Western blots are representative of four experiments. (C) Normalized quantification of the amount of Sst2-GFP localized to the plasma membrane. The relative amount of Sst2-GFP localized to the plasma membrane at the indicated times after treatment with pheromone and LatA at time zero was normalized to total receptor abundance by dividing the mean plasma membrane Sst2-GFP signal by the mean plasma membrane Ste2-mCherry signal. Data are mean ratios ± SEM of three or two (for the GβP− overexpressing strain) experiments. *P ≤ 0.003 compared to control. n ≥ 44 cells for each time point for control and Gβ-overexpressing strains; n = 15 cells for each time point for the GβP−-overexpressing strain. (D and E) Overexpression of Gβ enhances polarity of unphosphorylated receptor in pheromone- and LatA-treated cells. (D) YDB111 cells expressing the native Gβ, excess Gβ, or excess GβP− (excess indicated by double arrows) were treated with pheromone and LatA at time zero. The cells were then imaged by epifluorescence microscopy to visualize Sst2-GFP. Arrowheads indicate polarized Sst2-GFP crescents. (E) The degree of polarization of Sst2-GFP to the plasma membrane of each cell is indicated by the polarity index: the mean fluorescence in the brightest third of the plasma membrane divided by the mean signal in the rest of the plasma membrane. Data are means ± SEM of at least two experiments per strain. *P < 0.02, **P = 0.002, compared to cells expressing excess Gβ. n ≥ 20 cells for each strain and time point. (F and G) The pheromone receptor is hyperphosphorylated in GβP− cells. (F) YDB111 cells expressing endogenous Gβ or GβP− were treated with pheromone and LatA at time zero. The cells were then imaged by epifluorescence microscopy to visualize the indicated proteins. (G) Quantification of the extent of Sst2-GFP localization to the plasma membrane. Data are means ± SEM of 15 cells for each strain and time point. *P = 0.005 for GβP− compared to Gβ cells at 90 min. Plasma membrane Ste2-mCherry amounts did not differ significantly in the control and experimental cells.
of polarized growth. Although the Sst2-GFP crescent in YDB111 cells expressing Gβ also anticipated the shmoo site, the Gβ cells shmooed about one time point earlier (15 min) than did the Gβ cells (n ≥ 30 cells; P = 0.05) and did so markedly closer to the presumptive default site (Fig. 3). These data suggest two conclusions. First, cells that are unable to internalize receptors, and thereby polarize receptor distribution, are nevertheless able to establish a chemotropic growth site by local inhibition of receptor phosphorylation. Second, the role Gβ plays in positioning and stabilizing the chemotropic growth site correlates with its effect on receptor phosphorylation.

To further examine the possibility that receptor phosphorylation plays a role in chemotropism apart from triggering receptor internalization, we replaced native STE2 with STE2XR, STE2XR 6SA, or STE2XR 6SD in an otherwise wild-type MATa strain. These alleles all encode forms of the receptor that cannot be internalized (7XR) (23) and that either are barely phosphorylated (6SA) or have phosphomimetic substitutions (6SD) (24). We then assessed the effects of the mutant receptors on chemotropism in time-lapse images of mating mixtures (Fig. 4). Cells that were fully competent to mate typically shmooed directly toward their mating partner, grew chemotropically about the same distance to their point of contact, and ultimately fused at an angle near 0° (Fig. 4, B and D). The resulting zygotes were straight and symmetrical. In contrast, cells defective in gradient sensing or chemotropism, or wild-type cells confused by exogenous pheromone (fig. S3), did not orient directly toward their partners and ultimately formed angled zygotes (21).

When chemotropism was assayed by observing mating pairs directly rather than by quantifying the efficiency of diploid formation using genetic markers, as performed by McClure et al. (25), it was apparent that STE2XR conferred a defect in initial orientation (Fig. 4B). The mean orientation angle of the mutant cells was considerably smaller than that of the control cells, and the mean fusion angle and the mean time to fusion were markedly greater in the wild-type MATa × MATa STE2XR mating mixtures than in the wild-type × wild-type crosses (Fig. 4, C to E). Note that STE2XR conferred an increase in the mean fusion angle that was equivalent to the addition of exogenous pheromone to wild-type mating mixtures (fig. S3). We infer that the inability to internalize and thereby redistribute the mutant receptor compromised directional sensing. When cells could neither polarize the distribution of the pheromone receptor nor locally affect its phosphorylation state, they were unable to grow chemotropically. In mating mixtures, the MATa STE2XR 6SA cells that ultimately formed zygotes with wild-type MATa cells always polarized their growth adjacent to their last bud site (Fig. 4B). Similarly, all MATa STE2XR 6SA cells that ultimately formed zygotes polarized their growth—or mated without undergoing morphogenesis—proximal to their last bud site. Thus, MATa STE2XR 6SA and MATa STE2XR 6SD cells could not switch from the presumptive default polarity site to a chemotropic growth site regardless of the position or proximity of potential mating partners (Fig. 4C).

In addition to eliminating gradient sensing in situ, the STE2XR 6SA and STE2XR 6SD alleles conferred pronounced defects in mating projection formation (Fig. 4F). Although most of the mating MATa STE2XR 6SA cells and all of the mating MATa STE2XR 6SD cells exhibited robust polarized growth, a substantial fraction of the mutants apparently could not stabilize their axis of polarity well enough to generate a tapered shmoo tip. Such cells elongated along very broad growth zones, the shape of which was easily distinguished from that of a normal mating projection. Notably, the broadly polarized MATa STE2XR 6SA and MATa STE2XR 6SD cells persistently turned in one direction as they elongated (Fig. 4B and fig. S4), a behavior also seen in sst2Δa cells (26). Because some of these cells eventually mated, albeit over abnormally long times (Fig. 4E), their curving growth might be mistaken for chemotropism. Is the persistent turning toward the eventual fusion site a marked example of reorientation? Our data indicate that it is not. The broadly polarized and curving MATa STE2XR 6SA and MATa STE2XR 6SD cells showed no sign of gradient sensing. Their initial direction of growth was determined by default polarity (Fig. 4C), and they continued to elongate and turn until they encountered a mating-competent MATa cell directly in their path, often after having failed to mate with nearer potential partners (Fig. 4B). Only 26% of the MATa STE2XR 6SA and MATa STE2XR 6SD cells that elongated and turned, and 42% of those that were initially positioned within 1 μm of one or more potential partners, formed zygotes within the 6-hour time course (fig. S4). On the basis of our analysis of cells expressing mutant receptors in physiological gradients, we conclude that receptor internalization is required for full chemotropic function and that receptor phosphorylation plays a critical role in chemotropism that is distinct from its established function as a stimulus for internalization.

The pheromone receptor becomes polarized before Cdc42 is activated

In pheromone-treated cells that were first allowed to internalize the receptor for 15 min and then treated with LatA, a polarized receptor crescent subsequently formed on the plasma membrane within about 30 min, even though ongoing actin-dependent secretion and endocytosis were blocked (Fig. 6, A and B) (6, 27). A likely explanation for this is that, at the time of LatA treatment, a preexisting polarity site—in this case, one that was generated as the receptor was internalized—was amplified by uniform delivery of secretory vesicles together with biased docking and fusion at the plasma membrane, as proposed by Sahin et al. (28). The receptor itself could be the primary determinant of this site. Although the membrane localization of the Ste2-GFP reporter was undetectable after 15 min of induced internalization (6), a concentrated receptor patch was seen when such cells were labeled with Alexa Fluor 594–tagged pheromone (fig. S5). Alternatively, cortical polarity could be generated by actin-independent, Cdc42-dependent exocytosis, as described by Bendezú and Martin (29), or by endocytosis and recycling of Cdc42, as proposed by Yamamoto et al. (30). To distinguish between these possibilities, we investigated whether pheromone-induced receptor (Ste2-GFP) polarity was detectable before
Fig. 4. Effects of receptor internalization and phosphorylation on chemotropism. (A) Illustration of how the initial orientation angles (left, δ) and the fusion angles (right, γ) were measured. Polarized growth initiated within 45° of the cytokinesis site (CKS, red bar) was presumed to be at the default polarity site, demarcated by dotted blue lines. (B) Time-lapse DIC and fluorescent images of the indicated mating mixtures taken at 15-min intervals after spreading on agarose pads. The GFP-Bud1 signal at the plasma membrane was used to distinguish the MATα cells and as a marker for cell fusion. The initial orientation, reorientation, and fusion angles are indicated by δ, δ', and γ, respectively. (i) Representative wild-type (WT) × WT mating. The MATα cell shmooed opposite to its CKS. (ii) Example of a mating cell reorienting by initiating a second projection in a WT × WT cross. (iii) Representative WT MATα × MATα STE27XR mating showing defective initial orientation and the formation of an angled zygote after 90 min. (iv) Two representative WT MATα × MATα STE27XR 6SA matings showing a MATα cell fusing adjacent to the CKS of a mutant cell that failed to polarize its growth (top) and a mutant cell that broadly polarized its growth in the default region but failed to fuse with an adjacent partner until it had elongated over an abnormally long time and distance (bottom). (v) Two representative WT MATα × MATα STE27XR 6SD matings showing mutant cells that broadly polarized their growth at the default site (top) or presumptive default site (bottom) and elongated while persistently turning until their growth zones were apposed to a partner. (C) Quantification of initial orientation angles [purple lines in (A)]. *P < 0.06, **P = 0.003, ***P < 0.0001. (D) Quantification of fusion angles [aquamarine lines in (A)]. *P < 0.0001. (E) Time to fusion. Diffusion of GFP-Bud1 from the MATα partner to the MATα partner was used as a marker for the completion of cell fusion. *P = 0.01, **P < 0.0001. (F) Effect of receptor mutants on morphogenesis. MATα cells that mated were placed in three classes on the basis of their ability to form mating projections: tapered growth (normal shmoo), broadly polarized growth, and no polarized growth, as indicated. The proportion of normal shmooes was compared by χ² test. *P < 0.0001. The indicated n values represent two experiments for the STE27XR 6SA and STE27XR 6SD strains and one experiment for the WT and STE27XR strains. NS, not significant.
Cdc42–guanosine 5′-triphosphate (GTP) [Gic2–protein binding domain (PBD)–red fluorescent protein (RFP)] polarity occurred or vice versa. Before they were treated with pheromone, about half of the cells in G1 exhibited Gic2-PBD-RFP polarity, but not Ste2-GFP polarity, whereas none showed receptor polarization alone, consistent with the essential role of Cdc42-GTP in bud emergence (Fig. 5, A and B). Conversely, about half of the cells in G1 that were not yet shmingo after 60 min of pheromone treatment exhibited crescents before Cdc42-GTP polarity was detectable, even though the reporter signals were of similar intensity. No pheromone-treated cells were observed that exhibited polarized Gic2-PBD-RFP before exhibiting polarized Ste2-GFP (Fig. 5, A and B).

Changes in the localization of the receptor also preceded the redistribution of active Cdc42 and morphogenesis in gradient-stimulated cells (Fig. 5, C and D). In time-lapse images of MatA cells that switched from their presumptive default polarity site to a presumptive chemotrophic growth site in mating mixtures, both Ste2-GFP and Gic2-PBD-RFP moved slowly from their initial sites to final sites of polarization, consistent with results reported by Hegemann et al. (31). The pheromone receptor was clearly detectable and centered around the eventual polarized growth site before Gic2-PBD-RFP was similarly redistributed in 13 of 19 scored cells (68%) and was the first to fully relocalize in 13 of 16 scored cells (81%). Redistribution of Gic2-PBD-RFP was never seen to precede that of Ste2-GFP. These data suggest that pheromone-induced polarity of the receptor determines the position of the chemotrophic shmoo site upstream of active Cdc42.

**GβpR cells are defective in receptor polarization**

Our biochemical data suggest that GβpR has a reduced affinity for Yck1/2 compared to that of Gβ. We also previously showed that the pheromone-induced polarization of GFP-GβpR before morphogenesis occurs is moderately defective (21). If either early Gβ polarization or the Gβ–Yck1 interaction plays an important role in establishing receptor polarity, then GβpR would be expected to adversely affect the formation of receptor crescents. To test this, we compared the localization of GFP-tagged receptor in Gβ and GβpR cells treated with LatA 15 min after pheromone stimulation. We found that GβpR cells exhibited reduced receptor polarization as compared to that of Gβ cells (Fig. 6, A and B), suggesting a role for Gβ phosphorylation in the establishment of the polarity site as the receptor undergoes global internalization. A similar result was observed under physiological conditions: GβpR cells exposed to pheromone gradients in mating mixtures also exhibited a defect in receptor polarization (Fig. 6, C and D).

**Pheromone-induced internalization can result in the polarization of activated receptors**

To follow the internalization of ligand-bound receptors as distinct from the trafficking of inactive receptors, we treated cells with fluorescent pheromone. Cells were labeled on ice in medium lacking essential nutrients and upon their release from the pheromone gradient, which was expected because of the higher receptor occupancy in the cell facing the gradient immediately after it was stimulated with pheromone, which was expected because of the higher receptor occupancy in this area (fig. S8). Within 12 min (network 3) or 15 min (network 2), however, iteration of the feedback loops reversed the differential so that receptor internalization was slowest at the front of the cell, where the receptor eventually became polarized. Output from the mathematical model also yielded testable predictions about the spatiotemporal dynamics of other key signaling elements in the response networks. Whereas the heterotrimeric G protein remained largely uniform in distribution on the surface of the gradient-stimulated computational cell, activated Gu and free Gβγ polarized together with the receptor (fig. S9). At the same time, inverse intracellular gradients of free Yck (back) and Gβγ-bound Yck (front) were generated (fig. S10).

**DISCUSSION**

Although both chemotactic and chemotropic cells use GPCRs to detect and respond to chemical gradients, they differ in how they deploy their surface receptors. With few exceptions, chemotactic cells exhibit a uniform surface distribution of receptors. This enables them to rapidly sense changes in ligand concentration in three dimensions. Although the surface
Fig. 5. Localization of receptor and Cdc42-GTP reporters in vegetative and pheromone-stimulated \( G_1 \) cells. (A and B) Sequential polarization of the receptor and active Cdc42 in cultured cells. Strain AIY301 was grown to log phase, and \( G_1 \) (round, unbudded) cells were scored for polarization of the Ste2-GFP (receptor) and Gic2-PBD-RFP (Cdc42-GTP) reporters before and after pheromone treatment. (A) Representative images 60 min after treatment with pheromone. The exposures and processing were the same for all images. (B) Relative proportions of cells showing Ste2-GFP and Gic2-PBD-RFP polarity before and at the indicated times after pheromone-induced morphogenesis. RNP: round cell, neither reporter polarized; RAC: round cell, activated Cdc42 polarized; RR: round cell, receptor reporter polarized; RB: round cell, both reporters polarized; SB: shmooing cell, both reporters polarized. \( n = 20, 35, \) and 56 cells at the 0-, 1-, and 2-hour time points, respectively, from two experiments. (C and D) Sequential polarization of the receptor and active Cdc42 in mating cells. (C) Time-lapse images of representative \( MA\) cells that switched from their presumptive default polarity site to a presumptive chemotropic growth site in mating mixtures. Polarity of the Ste2-GFP and Gic2-PBD-RFP reporters is categorized in one of three ways: polarity established at the initial site (PE; dotted arrowheads), polarity switching to the future growth site (PS; solid arrowheads), and polarity fully switched and fixed at the final site (PF; filled arrowheads). (D) Percentages of cells that showed sequential relocalization of the Ste2-GFP and Gic2-PBD-RFP reporters in mating mixtures. PS corresponds to the solid arrowheads in (C) \( (n = 19\) cells). PF corresponds to the filled arrowheads in (C) \( (n = 16\) cells). Two experiments were performed.
The formation of polarized receptor crescents in shmooning yeast was first reported in 1991 (40), and its importance has been debated ever since. Is receptor polarization simply the result of global internalization followed by actin cable–directed delivery of secretory vesicles to the plasma membrane, or does the local concentration of receptor determine the position of this site? In addition to suggesting that the distribution of the receptor and G protein can be polarized by differential internalization, our data indicate that the receptor alone can support a chemotopic response. In mating cells that could not internalize the pheromone receptor, the unphosphorylated receptor crescent was often seen to move from the presumptive default polarity site to an apparent chemotropic growth site in wild-type cells that had not yet changed shape (fig. S2). Together, these data indicate that receptor polarization occurs earlier than directed secretion and contributes to gradient sensing.

In contrast to the robust receptor polarization that we observed in the absence of directed secretion, the receptor cannot polarize if its internalization is blocked by mutation or by treatment with LatA together with pheromone (6). However, when pheromone-induced receptor internalization was allowed to continue for 15 min before actin was depolymerized with LatA, robust receptor crescents subsequently appeared (Fig. 6) (6). We infer that a polarity site is established while the receptor is being internalized, and that the position of this site is most likely determined by the receptor itself. A mutant form of the receptor that cannot be internalized, and thus unable to polarize (Ste27XR), conferred a defect in gradient sensing, as was measured in low-density unilateral mating mixtures (Fig. 4). Moreover, the receptor crescent shifted from the default polarity site to the chemotropic growth site in wild-type cells that had not yet changed shape (fig. S2). Together, these data indicate that receptor polarization occurs earlier than directed secretion and contributes to gradient sensing.
The yeast chemotropism (evant mechanisms have been identified. The binding of free G sites with pheromone gradients are not well understood; however, two rel-
treceptor is critical to establishing the chemotropic growth site. Internalization, and that the localized concentration of unphosphorylated
These data suggest that receptor phosphorylation plays an essential role in the default polarity site to the chemotropic polarity site and were compro-

cable establishment of pheromone-induced cell polarity before the initiation of actin

chemotropic shmooing. More recently, Dyer et al. (35) and Hegemann et al. (31) used live-cell imaging to show that a dynamic polarity complex, which is likely composed of the core constituents Bem1, Cdc24, Cdc42, and Bni1, moves in a random walk around the cortex of vegetative cells. When cells are exposed to pheromone, the movement of the polarity complex is biased toward the incipient shmoo site through the interaction of Far1-Bem1-Cdc24 with free Gβγ (31, 35). In principle, therefore, yeast chemotropism can be explained as follows: A gradient of pheromone induces a gradient of activated receptor on the surface of the cell, which, in turn, is mirrored by a gradient of activated G protein. Free Gβγ then restricts the movement of the polarity patch along the cell cortex on the side of the cell exposed to the highest concentration of pheromone and ultimately stabilizes its position at the incipient growth site. Actin cables are subsequently nucleated at this site.

As the primary determinant of the chemotropic growth site downstream of the receptor, Gβγ must be locally concentrated where the pheromone concentration is greatest. Polarization of Gβγ is required to constrain the polarity patch and nucleate actin cables. There are two challenges to

...
explaining how Gβγ polarizes before polarity patch stabilization and actin cable nucleation occur. First, physiological gradients are shallow. It has been estimated that a 1% difference in receptor occupancy across the 5-μm length of a yeast cell in a pheromone gradient is sufficient to elicit robust orientation of cell growth toward the pheromone source (40), and microfluidic studies suggest an even greater acuity (36). Thus, although there is a slight excess of free Gβγ on the side of the cell exposed to the highest concentration of pheromone, there is almost as much on the opposite side. Second, because activated receptors are targeted for phosphorylation and internalization, the density of receptor and G protein at the plasma membrane is expected to be least where the pheromone concentration is greatest, as is the initial rate of heterotrimeric G protein activation. We postulate that free Gβγ protects proximal receptors from Yck-dependent phosphorylation and internalization, which in turn preserves heterotrimeric G proteins at the plasma membrane (6). Thus, the slightly greater burst of Gβγ released from Go on the up-gradient side of the cell triggers a feedback loop that amplifies signaling at the incipient shmoo site. As yet, it remains to be determined whether the lipid-bound, unphosphorylated receptor activates the G protein more effectively than does the lipid-bound phosphorylated receptor and, if so, how it does so. If confirmed, however, a confined G protein activation center would add another powerful feedback mechanism to our proposed networks. In this scenario, activated receptor would generate activated Go and free Gβγ, which would increase local density of activated, unphosphorylated receptor and G protein, which would further increase the local density of activated Go and free Gβγ, and so on. As discussed earlier, the rapid localization of Gβγ toward the source of the gradient is essential for positioning the chemotropic growth site.

Together, our data suggest that polarity is first established in response to a pheromone gradient by differential phosphorylation and internalization of the receptor, which depends, in turn, on anisotropic protection of the receptor by its G protein. At least two interconnected positive feedback loops are at the heart of the localized amplification mechanism underlying the yeast chemotropic response. Similar processes may contribute to directional sensing in other systems. For example, a role for GPCR phosphorylation other than desensitization has been reported in Dictyostelium chemotaxis (44).

MATERIALS AND METHODS

Molecular and microbiological techniques

Standard methods were used for microbial culture and molecular manipulation, which were performed as described previously (45–47).

Yeast strain construction

The yeast strains used in this study are listed in table S1. Unless otherwise noted, they were derived by transformation from strain 15Dau (MATa bar1Δ ade1 his2 leu2-3, -112 trp1 ura3Δ), which is congenic with strain BF264-15D (48). To generate strains that could be heavy-labeled for mass spectrometric analysis, ARG5,6 was knocked out in strains 15Dau and RYD114 by transplacement with a fragment containing KanMX4 G418, PCR-amplified from pFA6-kanMX4 (49), and flanked by ends homologous to ARG5,6 with the oligomers 5′-TCAATGAGGGATCTAAATTTGACATGTAGCCTTCTGCTCTGACG-3′ and 5′-TCAGGAGATTATGATGGTATGGCGTAGTCGGTCGACG-3′. The output was selected with medium containing geneticin (G418, 200 mg/liter). LYS1 was then replaced with a TEF promoter–pap-TEF terminator fragment amplified from the pAG32 plasmid (EUROSCARF) and flanked by ends homologous to LYS1 with the oligomers 5′-GCTCGCCGTCATATTACAATCAGAGCTG-3′ and 5′-GTACCAGAACGTTAGGTATGGTATGGACACGATTCCATATTATGTCGTTTGCAGC-3′. Integrants were selected with medium containing geneticin (G418, 200 mg/liter). LYS1 was then replaced with a TEF promoter–hpl-TEF terminator fragment amplified from the pAG32 plasmid (EUROSCARF) and flanked by ends homologous to LYS1 with the oligomers 5′-GCTCGCCGTCATATTACAATCAGAGCTG-3′ and 5′-GTACCAGAACGTTAGGTATGGTATGGACACGATTCCATATTATGTCGTTTGCAGC-3′. Integrants were selected on medium containing hygromycin B. To enable exclusive expression of 6×His-Ste18 and 3×HA-Ste18, native STE18 (Gr) was replaced with a fragment containing URA3 as described previously (50). Strain AY109 was generated by integrating ste4ΔSTE4Δ4 into strain YDB111 (22) in situ with Msc1-cut Yplac128/ste4Δ4 into strain ARS5 (pAIB130). Strain AY197 was generated by integrating hpl-1 ΔHLP1921 (51) into RYD114 (21). Strain AY301 was generated by integrating Apad-digested Yplac211-GIC2-PBD-RFP (52) into DMY169 (6). Strains XWY005, XWY008, and XWY018 were generated by integrating Clai-digested DLB3850, DLB3874, and DLB3851, respectively, into strain DSY257. The fluorescent-marked MATa mating tester strain, XWY027, was generated by integrating Bsu361-digested pRS406-GFP-BUD1 at the BUD1 locus of DSY129. All integrations intended to cause gene deletion, replacement, or in situ tagging were checked by genomic sequencing.

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Plasmid construction
The plasmids used in this study are listed in table S2. YCplac22/GAL1p>STE18 was constructed by PCR-amplifying STE18 from pGEX-KG-ST18 (MCB35) with the oligomers 5'-TGCTATCCACGTCGACATGATCCATGACCTCGCCAGTACCGTTACATACGACGCTAGGGCTACTGCAATGGCTCTACCCTACGAGCTGACGCTACTGCAATGGCTCTACCCTACGAGCTGACGCTACTGCAATGGCTCTACCAAGGTGATGCTCCAGCTGAGGGACCAGCATAGCTCAACGGTATACATATATGGTCTACCCAAAGTATGTTGTTCAAA

Gβ-Yck1 split-Venus BiFC assay
Because constitutive overexpression of Gβ causes permanent cell cycle arrest, the Gβ-Yck1 BiFC assay was performed in a strain that enabled conditional expression of STE7. MATA STE7a-KanMX YCplac22/GAL1p>STE7 cells transformed with pRS416/ADH1-STE4-VF2 and pRS415/ADH1-STE4-VF2 (Aiy273), pRS416/ADH1-STE4-VF2 (Aiy276), or pRS415/ADH1-STE4-VF2 and pRS416/ADH1-VF1 (Aiy275) were grown to mid-log phase in selective medium containing 2% sucrose and were induced with 2% galactose for 1 hour. Cells were harvested at 2300g at room temperature, washed once with ice-cold deionized water, and frozen in dry-ice ethanol. Cell pellets were lysed at 4°C with 0.5-mm silica beads in 1× tris-buffered saline (TBS) buffer containing protease inhibitors (150 mM NaCl, 10 mM tris-HCl (pH 8), 100 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM aprotinin, 2 mM pepstatin, and 2 mM leupeptin). Crude lysates were centrifuged at 16,100g at 4°C for 20 min, and protein concentrations were determined with the Pierce 660nm Assay kit (Thermo Fisher). Each cell lysate (7.3 mg) was mixed with 30 µl of anti-HA agarose beads (Thermo Fisher), adjusted to a total volume of 1.25 ml with 1× TBS buffer containing protease inhibitors, and incubated for 2 hours at 4°C with end-over-end rotation. The beads were washed three times with 1× TBS buffer [150 mM NaCl, 10 mM tris-HCl (pH 8), and 1% Tween 20]. To obtain cell lysates containing myc-Yck1, strain NYW073 was cultured and processed as described earlier except that it was induced with galactose for 3 hours. Low (L; 225 µg) and high (H; 900 µg) amounts of total protein from the NYW073 lysate were added to the Gβγ or Gβγ affinity beads. Volumes were adjusted to 1.25 ml with 1× TBS buffer containing protease inhibitors, and the slurries were incubated at 4°C for 1 hour with end-over-end rotation. The beads were washed three times with 1× TBS-T buffer and then were reduced in 1× SDS sample buffer. The supernatant was split into two fractions for Western blotting and mass spectrometric analyses. For Western blotting analysis, myc-Yck1 was detected with a horseradish peroxidase (HRP)–conjugated mouse anti–c-myc antibody (1:900, Thermo Fisher); 3×HA-Gγ was detected with a primary mouse anti-HA antibody (1:900, Covance) and an HRP-conjugated secondary goat anti-mouse immunoglobulin G antibody (1:400,000, Jackson ImmunoResearch Inc.). Densitometric analysis of scanned Western blotting films was performed with the ImageJ gel analysis tool, which provided AI values for each band. The relative amount of myc-Yck1 binding to Gβγ as compared to Gβγ was determined by subtracting the corresponding negative control signal (L or H) and then taking the ratio of the resulting SAN values. Normalization to HA-Gγ was not necessary because the signal detected with the anti-HA antibody did not vary from lane to lane. For the mass spectrometric analysis, 225 µg of total protein from NYW073 lysates was incubated with the affinity beads. Eluted proteins were alkylated and resolved by SDS-PAGE. The ~55- to 70-kD (for myc-Yck1 and Yck2) and 45- to 55-kD (myc-Yck1 cleavage products; Gβγ) regions of the gel were then excised, the proteins were in-gel digested with sequencing-grade modified trypsin (Promega), and the resulting tryptic fragments were analyzed by nanoscale liquid chromatography combined with mass spectrometry (LC-MS/MS), as previously described (55, 56).

Analysis of Gβ phosphopeptides
NYW052 cells were grown to mid-log phase in selective synthetic 2% sucrose medium containing natural arginine and lysine (normal Arg/Lys) and induced with 2% galactose for 5.5 hours (light culture). A parallel culture of NYW052 cells was labeled in medium containing heavy Arg/Yys (20 mg/liter; l-lysine:2HCl, U-13C6 and l-arginine:HC1, U-13C6, U-15N2; Cambridge Isotope Laboratories) and treated with 150 nM α-factor for 1 hour after galactose induction. The light and heavy cultures were prepared for mass spectrometric analysis in parallel. Cells were harvested at 2300g at room temperature, washed once with ice-cold deionized
water, and frozen in dry-ice ethanol. Cell pellets were lysed at 4°C with 0.5-mm silica beads in 1× TBS buffer containing protease and phosphatase inhibitors [150 mM NaCl, 10 mM tris-HCl (pH 8), 100 mM PMSF, 2 mM aprotonin, 2 mM pepstatin, 2 mM leupeptin, 1 mM sodium orthovanadate, 50 mM NaF, 10 mM sodium pyrophosphate, and 10 mM β-glycerol phosphate]. Crude lysates were centrifuged at 16,100g at 4°C for 20 min, and protein concentrations were determined with the Pierce 660nm Assay kit. Total protein (25 mg) from each lysate was mixed with 100 μl of Ni-NTA beads (Qiagen). Volumes were adjusted to 1.25 ml with 1× TBS buffer, containing protease and phosphatase inhibitors, and the slurries were incubated at 4°C with end-over-end rotation for 16 hours. The beads were washed 10 times with 1× TBS-T buffer [150 mM NaCl, 10 mM tris (pH 8), and 0.1% Tween 20] and then reduced in 1× SDS sample buffer. Equal volumes of supernatant from heavy and light cultures were mixed. The proteins were alkylated and separated by SDS-PAGE. The ~45-to 50-kD region of the gel was excised and subjected to in-gel digestion with sequencing grade—modified trypsin. Peptides were concentrated under vacuum and enriched for phosphopeptides with a TiO2 phosphopeptide enrichment and clean-up kit (Pierce Biotechnology). Phosphopeptides were concentrated under vacuum and analyzed by nanoscale LC-MS/MS as previously described (53–57). A peptide search engine using a probabilistic scoring model (Andromeda) was used to analyze the resulting spectra and obtain assignments for sites of phosphorylation (58).

**Analysis of the localization of Sst2-GFP, Ste2XR-mCherry, and Ste2-GFP in G1-synchronized cells**

G1-synchronized cells were purified by elutriation as previously described (6). Cells used to study the localization of Ste2-GFP and Ste2XR-mCherry (strains AIY100, AIY101, and AIY221) were spun down and resuspended in selective synthetic medium containing 2% galactose, cultured for 1 hour at 30°C, and then treated with 1.2 μM α-factor and 200 μM LatA. Cells used to study the localization of Ste2-GFP (strains DMY169 and AIY197) were spun down and resuspended in synthetic medium containing 1.5% sucrose and 0.5% dextrose and treated with 9 nM α-factor at time zero and with 200 μM LatA 15 min later. All cultures were maintained at 30°C. Images were acquired at 15-min intervals with an Axioskop 2 microscope (Carl Zeiss) with a 100× oil immersion objective and a digital AxioCam camera. Laser excitation was at 488 nm. Images were processed with Zeiss AxioVision software (Carl Zeiss).

**Time-lapse analysis of Sst2-GFP and Ste2-GFP localization in mating mixtures**

Wild-type MATa cells and experimental MATa cells (AiY301) were grown to mid-log phase in synthetic 2% dextrose medium, mixed at a ratio of 1:1, and spread at a density of 14,000 cells/mm² on agarose pads made from synthetic dextrose medium. Mating mixtures were maintained at 30°C with a DeltaVision environment control chamber. Images were acquired from 20 fields at 15-min intervals with a DeltaVision Elite microscope (GE Healthcare Biosciences) with a 60× oil immersion objective and a front-illuminated sCMOS camera. To follow localization of Ste2-GFP and Gic2-PBD-RFP reporters, fluorescent images were acquired in 3 z sections over 4.8 min with laser excitations of 461 to 489 nm and 529 to 556 nm, respectively. The exposures were the same for all images. Images were processed with ImageJ software. Processing was the same for all Ste2-GFP images in a given series, and all Gic2-PBD-RFP in a given series.

**Receptor labeling and internalization assays**

The surface-expressed α-factor receptor Ste2 was labeled with Alexa Fluor 594–conjugated α-factor according to the method of Toshima et al. (60). To determine how the density and distribution of the receptor changed over time in response to pheromone, DMY169 cells were G1-synchronized by elutriation and treated with 6 nM α-factor at 30°C in YEPD. Aliquots were taken at 5-min intervals, treated with 10 mM sodium azide, and incubated with the labeled α-factor (a gift from D. King, University of California, Berkeley). The Ste2Δ strain (EDY208) was used as a negative control for receptor labeling. Images were acquired with an Andor Revolution WD spinning disc laser confocal system with a motorized Olympus IX81 microscope, a Yokogawa CSU-W1 spinning disc unit, a Prior motorized stage, and a Neo sCMOS camera controlled by Andor iQ2 software. A UPLSAPO 60× silicon immersion objective (NA, 1.3) was used, and nine z sections were acquired over 4.8 μm with a laser excitation of 561 nm; one center slice was imaged with DIC. Images were sum-projected with ImageJ. To assay the relative rate of receptor internalization as a function of position on the plasma membrane, MATa cells (RYD126) were grown to mid-log phase in YEPD medium, labeled with Alexa Fluor 594–conjugated α-factor, and imaged at 1-min intervals at room temperature as described earlier. Nine z sections were acquired over 5.6 μm with a laser excitation of 561 nm, and one center slice was imaged with DIC. Images were sum-projected and membrane fluorescence was obtained with the segmented line tool of ImageJ.
Relative rates of signal decay for labeled receptors at the plasma membrane

The variation in fluorescence intensity measured by ImageJ from pixel to pixel along the plasma membrane was smoothed using a moving average function with the sliding window equal to 1/10th of the membrane perimeter. For position \( x \) at time \( t \) in cell \( c \) with a window size \( w \), the fluorescence intensity was calculated by the following formula:

\[
\text{Fl}_h(x, t, c) = \text{avg}\left[ \text{Fl}_h\left(x - \frac{w}{2}, t, c\right), \ldots, \text{Fl}_h\left(x + \frac{w}{2}, t, c\right) \right]
\]

in which \( \text{Fl}_h \) denotes the new value after filtering, and \( \text{Fl}_o \) represents the original raw value. The background was removed by subtracting the minimum value from each data set:

\[
\text{FI}(x, t, c) = \text{Fl}_h(x, t, c) - \min_x \text{Fl}_h(x, t, c)
\]

Assuming that the kinetics of internalization resembled a first-order chemical reaction, we calculated the relative fluorescence intensity of time point \( t \) compared to time point 0 to determine how membrane receptor density changed with time using the following formula:

\[
\text{FI}(x, t, c) = \frac{\text{FI}(x, 0, c)}{\text{FI}(x, 0, c)}
\]

Finally, we used the data set for each cell to determine whether a discrete region of the plasma membrane exhibited a statistically significantly slower rate of signal loss than did the remainder of the plasma membrane. This was determined by fitting the 10 values for each pixel (time points 0 to 9) to the exponential decay formula:

\[
\text{FI}(x, t, c) = A \exp(-\lambda t)
\]

in which the decay rate \( \lambda \) and the initial quantity \( A \) are the two parameters to be fitted. A putative area of receptor protection was defined as the points that were in the 30th percentile for decay rate (that is, the slowest 30%). We calculated the average Fl of the “protected region” and compared it to the average of the rest of the cell. In some cases, the protected region was fragmented. The regions were connected if the distance between them was less than 1/10 of the perimeter of the cell and the average decay rate of region after connecting was still above the threshold.

Computational model of pheromone-induced receptor polarization

To model the plasma membrane of a yeast cell, we used a sphere, the surface of which was partitioned into patches by uniformly spaced latitudes and m longitudes (fig. S7). The center of each patch was used to represent its position. The surface distances between neighboring patches are given by Eqs. 1 and 2 (table S3). The \( j \)th patch in the \( k \)th band can be denoted by an index pair \((i,j)\). The position of the \((i,j)\) patch \((x_{ij}, y_{ij}, z_{ij})\) is given by Eq. 3 (table S3). In each patch, we simulated the reaction network model with the pheromone concentration appropriate to its position. Because the pheromone gradient is aligned along the x axis and the pheromone concentration is assumed to change linearly with \( x \), only \( x \) is needed to determine the local pheromone concentration of the \((i,j)\) patch (Eq. 4). All proteins were assumed to diffuse laterally because only the plasma membrane was modeled in this analysis. If the effect of surface curvature is ignored, the diffusion of each molecular species can be obtained from Eqs. 5 to 7 (table S3). To model pheromone-induced receptor polarization that occurs before direct secretion and to evaluate how our postulated feedback mechanisms affect the establishment of receptor polarity, we first created a core network that includes only the most basic and best-characterized components. Network 1 comprises the receptor-pheromone interaction, G protein cycle, and Yck-dependent internalization of the receptor and G protein (Fig. 4B). Network 2 adds the Gip3p-Yck interaction (Fig. 4C), and network 3 adds the Go-Fus3 interaction (Fig. 4D). The mathematical representation of these networks is detailed in tables S4 to S6. A common set of coupled partial differential equations (PDEs) was derived from their corresponding reaction formulae. Each PDE describes how the concentration of a given molecule changes over time and space. Differences in the topology of the three networks were accounted for by varying the initial values of the relevant parameters (color-coded in table S4).

SUPPLEMENTARY MATERIALS

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Fig. S1. Examples of mass spectra used to map the sites of pheromone-induced Gβγ phosphorylation.

Fig. S2. Dynamic localization of the pheromone receptor as cells orient toward mating partners.

Fig. S3. Exogenous pheromone induces the formation of angled zygotes.

Fig. S4. Time-lapse images of elongating and turning MATa STE27XR 6SD and MATa STE27XR 6SD cells in mating mixtures.

Fig. S5. Surface distribution of the pheromone receptor as assayed by Alexa Fluor 594–conjugated u-factor binding.

Fig. S6. Receptor internalization assay.

Fig. S7. Spatial model of the computational yeast cell.

Fig. S8. Computational model of receptor dynamics.

Fig. S9. Computational model of G protein dynamics.

Fig. S10. Computational model of Yck dynamics.

Table S1. Yeast strains used in this study.

Table S2. Plasmids used in this study.

Table S3. Equations used for the spatial model of the yeast cell.

Table S4. Definitions and parameters.

Table S5. Reaction formulae.

Table S6. Partial differential equations.

References (61–65)

REFERENCES AND NOTES


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Gβ promotes pheromone receptor polarization and yeast chemotropism by inhibiting receptor phosphorylation

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Protected pheromone receptors signal

In budding yeast, opposite mating types signal to each other by secreting distinct pheromones, which bind to and activate receptors on the surface of compatible cells. The stimulated cell produces a mating projection (shmoo), which is enriched in pheromone receptors, that grows along the pheromone gradient to fuse with the shmoo of a mating partner. Ismael et al. imaged pheromone-treated yeast cells and found that the G protein β subunit, which is activated by ligand-bound pheromone receptors, competed with a kinase for access to receptors at the site closest to the highest amount of pheromone. In this way, the phosphorylation and internalization of receptors occurring in the rest of the cell was inhibited at this local site, which enabled the receptor to persist at the cell surface and stimulate growth of the shmoo.