Membrane recruitment of the kinase cascade scaffold protein Ste5 by the Gβγ complex underlies activation of the yeast pheromone response pathway

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In the Saccharomyces cerevisiae pheromone response pathway, the Gβγ complex activates downstream responses by an unknown mechanism involving a MAP kinase cascade, the PAK-like kinase Ste20, and a Rho family GTPase, Cdc42. Here we show that Gβγ must remain membrane-associated after release from Ga to activate the downstream pathway. We also show that pheromone stimulates translocation of the kinase cascade scaffold protein Ste5 to the cell surface. This recruitment requires Gβγ function and the Gβγ-binding domain of Ste5, but not the kinases downstream of Gβγ, suggesting that it is mediated by Gβγ itself. Furthermore, this event has functional significance, as artificial targeting of Ste5 to the plasma membrane, but not intracellular membranes, activates the pathway in the absence of pheromone or Gβγ. Remarkably, although independent of Gβγ, activation by membrane-targeted Ste5 requires Ste20, Cdc42, and Cdc24, indicating that their participation in this pathway does not require them to be activated by Gβγ. Thus, membrane recruitment of Ste5 defines a molecular activity for Gβγ. Moreover, our results suggest that this event promotes kinase cascade activation by delivering the Ste5-associated kinases to the cell surface kinase Ste20, whose function may depend on Cdc42 and Cdc24.

[Key Words: Heterotrimeric G protein; MAP kinase; signal transduction; PAK/Ste20 kinase; Rho/Rac/Cdc42 GTPase]

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The mating reaction of the yeast Saccharomyces cerevisiae provides a model signal transduction system in which a G-protein-coupled receptor activates a mitogen-activated protein (MAP) kinase cascade (for review, see Sprague and Thorner 1992; Leberer et al. 1997a). Here, two haploid cells of opposite mating types (α and α) fuse into a single diploid cell in response to secreted pheromones (α-factor and α-factor), which stimulate cell cycle arrest, transcriptional induction of mating-related genes, and morphological changes. These responses are activated through a pathway that begins with a cell surface receptor and its associated heterotrimeric G protein, Gαβγ, which is composed of Gpa1 (Ga), Ste4 (Gb), and Ste18 (Gγ). Downstream of the G protein lies a cascade of protein kinases—Ste11, Ste7, and Fus3—that is related to mammalian MAP kinase cascades, and an associated "kinase scaffold" protein Ste5 (for review, see Herskowitz 1995; Madhani and Fink 1998). Finally, targets of this kinase cascade include Far1, which activates cell cycle arrest, and Ste12, a DNA-binding protein responsible for transcriptional induction.

The mechanism by which the kinase cascade is activated by the heterotrimeric G protein remains poorly understood. It is clear that the free Gβγ complex activates downstream responses, after binding of pheromone to the receptor stimulates its dissociation from Gα, as deletion of the gene (GPA1) encoding Gα mimics pheromone treatment (for review, see Sprague and Thorner 1992). But the identity of the immediate target of Gβγ and the molecular mechanism used by Gβγ to activate the kinase cascade are not clear. Additional participants in this process include Cdc42, a Rho family GTPase; Cdc24, a guanine nucleotide exchange factor for Cdc42; and Ste20, a member of the PAK family of Rac/Cdc42-dependent kinases (for review, see Leberer et al. 1997a; Sells and Chernoff 1997). These proteins are also required for functions other than mating, including cytoskeletal organization (for review, see Chant and Stowers 1995). The simplest model positions these proteins as intermediates in an activation pathway from Gβγ to the kinase cascade (Gβγ → Cdc42 → Cdc42 → Ste20 → Ste11 → etc.), as the Gβ subunit Ste4 binds to the acti-
Role of membrane recruitment of Ste5 by yeast Gβγ

Results

Role of Gγ carboxy-terminal modifications

To address whether carboxy-terminal modifications of the yeast Gγ subunit Ste18 are required for membrane association or for binding a target protein, we asked whether they could be replaced with other membrane-targeting sequences. Four potential membrane targeting domains (MTDs) were tested (Table 1): an amino-terminal myristoylation sequence (Nmyr), the first four amino-terminal transmembrane domains (NTM) from yeast Ste6, a carboxy-terminal prenylation and palmitoylation motif (Cpr), and a single carboxy-terminal transmembrane domain (CTM). Plasmids were constructed that encode fusions of these MTDs to a Ste18 product (Ste18ΔC) lacking its final five carboxy-terminal residues (normally CCTLM). These were placed under control of a galactose-inducible promoter to facilitate assays of Gβγ function in cells deleted for Gα.

When transcriptional induction by Gβγ complexes containing the Ste18ΔC-MTD fusions was assayed in cells that lacked the Gα subunit (gpa1Δ ste18Δ), two of the four MTD fusions, Cpr and CTM, rescued the signaling defect (Fig. 1A, left). When assayed in cells that still express the Gα subunit (GPA1 ste18Δ), the same two carboxy-terminal MTD fusions rescued pheromone-responsive transcriptional induction (Fig. 1A, right), mating ability (Fig. 1B), and growth arrest (Fig. 1C). The

| Table 1. MTD sequences |
|------------------------|
| MTD name | Source genea | Sequenceb | Localizationc |
| Ammon-terminal MTDs | | |
| Nmyr | RSV v-src | MGSSKSK PKDPSN RHR SLEEPPDSTHHGFPA SNT-- (Ste6 amino acid residues 1-236) | N.D. |
| NTV | S.c. STE6 | | N.D. |
| Carboxy-terminal MTDs | | |
| CPM | S.c. SNC2 | --WWKDLKM RMCFLVVIII LLLVIII VPI VVHSV | PM |
| Snc28 | S.c. SNC2 | --WWKDLKM RMG LFLVVIII LLLVIII VPI VVHSV | PM |
| Snc2B | S.c. STRS | --LFLVVIII LLLVIII VPI VVHSV | PM |
| Sso1B | S.c. SSO1 | --WLIVFAVVV VVVVVPAA VVKT | PM |
| Sed9 | S.c. SED9 | --NRWLVAILV I VVVF SVFLVV | ER/Golgi |
| Sec22 | S.c. SEC22 | --SQYAPTVIVAFVFFFLFWI FLK | ER/Golgi(PM) |
| Cpr | S.c. RAS2 | --APGGNT EASKGSGG SGI | PM |
| Cpr-SS | S.c. ras2-SSI | --APGGNT EASKGSGG SSI | cytoplasm |

a(RSV) Roux sarcoma virus; S.c., Saccharomyces cerevisiae.

bBold lowercase type indicates residues changed from native sequence.

(N.D.) Not determined; (PM) plasma membrane; (ER) endoplasmic reticulum; see Fig. 5.
which we did not pursue. The CTM sequence was not as effective as the native $\gamma$ carboxyl terminus or the Cpr sequence, as maturing efficiency was lower and halos were turbid in the growth arrest assay (Fig. 1B,C). This may result from conformational constraints on the $\gamma$ carboxyl terminus, as related fusions in which linker sequence was absent and the hydrophobic sequence was placed closer to Ste18 (e.g., the Snc2D MTD; Table 1) was even less effective than the CTM fusion (not shown). Palmitoylation of a cysteine residue within the CTM sequence (Couve et al. 1995) was not required for rescue of Ste18\(\Delta\)C, as a sequence mutated at this position (Snc2B; Table 1) was equally effective (data not shown). The fact that the carboxy-terminal MTDs could functionally replace the carboxyl terminus of the $\gamma$ subunit argues that the normal requirement for this region is to localize G$p\gamma$ to the membrane.

We also tested whether the membrane targeting information for G$p\gamma$ could be provided by G$p\delta$, rather than G$p\gamma$, by fusing the same four MTDs to Ste4 (G$p\delta$). Unfortunately, two of the fusions (NTM and CTM) disrupted Ste4 function (Fig. 1D, left). Of the remaining two MTDs, the same one (Cpr) that rescued G$p\gamma$ function when fused directly to Ste18\(\Delta\)C also rescued the Ste18\(\Delta\)C defect when fused to Ste4 (Fig. 1D, right). Interestingly, the Ste4–Cpr fusion could not signal in the absence of Ste18, but only when Ste18\(\Delta\)C was expressed (Fig. 1E), indicating that G$p\gamma$ is required for aspects of G$p\beta$-$\gamma$ function other than membrane localization; this is consistent with point mutations in the $\gamma$ amino terminus that disrupt G$p\beta$-$\gamma$ signaling (Grishin et al. 1994). These observations, coupled with those above, demonstrate that activation of the downstream signaling pathway by G$p\gamma$ requires it to remain at the membrane after dissociation from G$\alpha$.

Targeting of Ste5 to the membrane activates the pheromone response pathway

To explain why signaling by G$p\gamma$ might require that it remain at the membrane, we considered the possibility that it recruits its target to the membrane. Therefore, we tested whether membrane recruitment of Ste5 might play a role in activation by artificially targeting it to the membrane. For this, the carboxy-terminal MTDs used in the previous section (CTM and Cpr) were fused to full-length Ste5 as well as to a derivative of Ste5 (Ste5\(\Delta\)N) lacking an amino-terminal G$p\beta$-$\gamma$-binding domain (Fig. 2A). Because some fusions might cause growth arrest, they were expressed from a galactose-inducible promoter.

The MTD fusions to Ste5 and Ste5\(\Delta\)N activated the pathway in the absence of pheromone, giving levels of Fus1–LacZ induction similar to pheromone-induced levels in cells expressing wild-type Ste5 (Fig. 2B, left). Fusions with Ste5\(\Delta\)N showed somewhat less induction than those with Ste5, and addition of pheromone did not compensate for this difference. We also created fusions with a sequence (Cpr–SS) in which the Cpr MTD contained an amino-terminal MTD (NTM and Nmyr) were ineffective, either because they did not confer membrane association, or because they interfered with G$p\gamma$ function.

**Figure 1.** Rescue of Ste18\(\Delta\)C signaling defect by fusion with heterologous membrane targeting domains. (A) Transcriptional induction. Fus1–LacZ activation is shown, after galactose induction of Ste18 derivatives for 4 or 18 hr (left) or for 4 hr ± α-factor (ωF; right). Bars, average of four measurements of two transformants (left) or mean ± s.d. for three transformants (right). Strains: PPY 885, PPY 865. Plasmids, from top to bottom: pPP449, pGS18–WT, pGS18–Cpr, pGS18–C–NTM, pGS18–C–Nmyr, pGS18–C–CTM, pGS18–C–Cpr. (B) Mating of strains in A, right. Partner: PPY 262. Bars, mean ± s.d. for three transformants. (C) Growth arrest. Lawns of transformants (as in A, right) were exposed for 4 days at 30°C on –TRP + RAFF + GAL to filter disks containing 25 µl of 500 µM α-factor. (D) Rescue by fusion of prenylation/palmitoylation sequence to Ste4. Patch mating tests of Ste4–MTD fusions for complementation of ste4Δ (PPY 794; left), or ability to rescue mating in a ste18Δ strain (PPY 832) that also expressed a Ste18\(\Delta\)C allele from pBH21–Q98ter (right). Plasmids, from top to bottom: pPP449, pGS4–WT, pGS4–Cpr, pGS4–C–NTM, pGS4–C–Nmyr, pGS4–C–CTM, pGS4–C–Cpr. (E) Signaling by Ste4–Cpr still requires the Ste18 amino terminus. Patch mating of a ste18Δ strain (PPY 832) expressing the indicated plasmid-borne Ste4 and Ste18 alleles: pGS4 (Ste4), pGS4–Cpr (Ste4–Cpr), pBH21–WT (WT), pBH21–Q98ter (Ste18\(\Delta\)C), pRS425 (vector).
hydrophobic palmitoyl and farnesyl groups (Mitchell and Deschenes 1995). These point mutations eliminated the effect of the Cpr fusion (Fig. 2B), arguing that it is the membrane association properties conferred by these modifications that lead to activation by Ste5. In mating assays, unfused Ste5DN was completely defective, but fusion with the MTDs restored mating to levels similar to wild-type Ste5 (Fig. 2B, right). These fusions also caused growth arrest (Fig. 2C) and shmoo formation (not shown), and allowed mating of cells lacking the pheromone receptor Ste2 (Fig. 2D). All phenotypes were eliminated by point mutations in the Cpr MTD (Cpr–SS; Fig. 2B–D), or by deletion of STE11 or STE7 (not shown). In summary, fusion of MTDs to Ste5 or Ste5DN mimics the addition of pheromone, activating responses normally induced by Gβγ, consistent with the requirement for membrane localization of Gβγ documented in the previous section.

To address whether expression levels contributed to these phenotypes, we used glucose to reduce expression of Ste5 derivatives under control of the GAL1 promoter (Fig. 2E). Pheromone-induced Fus1–LacZ levels mediated by the GAL-driven Ste5 construct were most similar to genomic-expressed Ste5 when induced by galactose alone, and were reduced to 30% or 11% of those levels by 0.1% or 0.4% glucose, respectively, indicating that expression levels of Ste5 became limiting. Under these conditions, Ste5–CTM and Ste5DN–CTM still activated Fus1–LacZ in the absence of pheromone, to at least 50% (Ste5–CTM) or 25% (Ste5DN–CTM) of the level mediated by similarly expressed Ste5 in response to pheromone. These data suggest that the activation phenotype does not require overproduction, although the degree of activation may depend on expression levels. Notably, the lowered expression conditions enhanced detection of pheromone responsiveness of Ste5–CTM, hinting at a pheromone-inducible event besides membrane localization that can contribute to Ste5-mediated signaling; this event depends on the amino terminus of Ste5, as Ste5DN–CTM was unresponsive to pheromone at all expression levels. We observed similar results by replacing the GAL1 promoter in these constructs with the native STE5 promoter, although the sequence changes remaining at the carboxyl terminus (for inserting MTD cassettes) caused poor expression of Ste5 (P.M. Pryciak and F.A. Huntress, unpubl.).

Pheromone-activated recruitment of GFP–Ste5 to the cell surface

Because artificial targeting of Ste5 to the membrane stimulated the pheromone response pathway, we tested
whether Ste5 normally appears at the membrane, by tagging Ste5 with green fluorescent protein (GFP; Fig. 3). In untreated cells, GFP–Ste5 was present diffusely throughout the cytoplasm, and was enriched in the nuclei (confirmed by costaining with DAPI; not shown) of many cells. Pheromone caused some of the GFP–Ste5 to accumulate at the cell surface, at the tips of pheromone-induced projections (Fig. 3A,B), and often led to decreased nuclear localization. Cell surface localization was visible after brief pheromone treatment in cells with only slight projections, as well as in more typical pear-shaped shmoo, but became more difficult to detect at later times (Fig. 3B), for unclear reasons. Even at the earlier times, cell surface GFP–Ste5 was not visible in all cells, and therefore this localization may be transient or dynamic. Nevertheless, these results support the notion that artificially targeting Ste5 to the membrane mimics normal pathway activation.

In contrast to full-length Ste5, a GFP fusion to Ste5ΔN, which lacks the Gβγ-binding domain and is signaling defective, was not recruited to the cell surface in either ste5Δ or STE5 backgrounds (Fig. 3C); thus, cell surface localization of Ste5 correlates with its signaling competence. We also examined the Ste5-CTM fusion, which localized to a peripheral rim around the cell indicative of plasma membrane (Fig. 3D), even in a ste11Δ strain where constitutive signaling was blocked. Interestingly, targeting Ste5 to the membrane made nuclear Ste5 undetectable, and yet the pathway became activated, as evident from the projections formed (Fig. 3D). Although we cannot exclude the possibility that a small fraction did transit to the nucleus, this may indicate that nuclear localization is not required for signaling by Ste5. In contrast, localization of Ste5 to the plasma membrane is strongly correlated with pathway activity.

Recruitment of GFP–Ste5 to the cell surface by pheromone requires Gβγ activity but not kinase cascade activity.

To address how pheromone stimulates translocation of GFP–Ste5 to the cell surface, we used mutant strains lacking components of the pathway (Fig. 4A). In ste4Δ mutants, no change in GFP–Ste5 localization was detected with pheromone addition, indicating that G-protein function was required. In contrast, despite the block to downstream signaling, pheromone could still induce cell surface localization of GFP–Ste5 in the absence of the kinases Ste20, Ste11, and Ste7, although more strikingly in ste20Δ than ste11Δ and ste7Δ mutants. Because these mutants did not form projections, cell surface GFP–Ste5 was distributed more broadly than in signaling-competent cells (like the ste5Δ mutant, which was complemented by the GFP–Ste5 fusion), although it was still asymmetric in a manner possibly oriented toward an incipient bud site—where projections emanate in a uniform field of pheromone (Madden and Snyder 1992). Because translocation of GFP–Ste5 was observed in ste20Δ, ste11Δ, and ste7Δ mutants but not in ste4Δ, it requires G-protein function but not the ability of Gβγ to activate the kinases or to stimulate cell cycle arrest or transcription.

In a complementary approach, we also examined GFP–Ste5 localization after activation at different steps in the pheromone response pathway (Fig. 4B). Like pheromone, activation at the Ste4 step (using Gal–Ste4) caused recruitment of GFP–Ste5 into projection tips in the absence of pheromone. In contrast, activation at the Ste5 and Ste11 steps (using Ste5–CTM and Gal–Ste11Δ, respectively) did not cause GFP–Ste5 translocation (Fig. 4B). In comparison, another protein, GFP–Ste20, did localize to projection tips induced by Ste5–CTM, and therefore its localization does not require pheromone or activated Gβγ; this is consistent with the fact that GFP–Ste20, unlike GFP–Ste5, is at the cell surface before pheromone exposure (Peter et al. 1996; Leberer et al. 1997b). Our results indicate that kinase cascade activity and projection formation are neither necessary nor sufficient for translocation of GFP–Ste5 to the cell surface, whereas Gβγ activity is both necessary and sufficient. This suggests that GFP–Ste5 is brought to the membrane...
Pathway activation requires delivery of Ste5 to the plasma membrane

To gain insight into why bringing Ste5 to the membrane activates the pheromone response pathway, we tested whether any cellular membrane, or only the plasma membrane, would produce this effect. Because transmembrane domains can target proteins to distinct membrane compartments (Rayner and Pelham 1997), we fused GFP–Ste5ΔN to carboxy-terminal transmembrane domains from four yeast proteins—Snc2, Sso1, Sed5, and Sec22 (Table 1)—that function at different stages of the secretory pathway (Ferro-Novick and Jahn 1994). We then followed both the intracellular localization of the fusions and their signaling phenotypes (Fig. 5).

The Snc2 and Sso1 MTDs directed the fusions primarily to the plasma membrane, with more of the Sso1 fusion retained in a punctate location suggestive of Golgi. The Sed5 and Sec22 MTD fusions gave localizations reminiscent of endoplasmic reticulum and Golgi (see Rayner and Pelham 1997), although a small fraction of the Sec22 fusion was detectable at the plasma membrane (arrowheads). Fus1–LacZ assays showed that pathway activation by these fusions correlated with their delivery to the plasma membrane; signaling was activated strongly by the Snc2 and Sso1 MTD fusions, not at all by the Sed5 MTD fusion, and weakly by the Sec22 MTD fusion. A similar correlation was seen comparing fusions to the Cpr and Cpr–SS sequences. Note that for none of these Ste5ΔN fusions could pheromone increase pathway activity, regardless of the level activated by the fusion itself, and thus removal of the Ste5 amino terminus from the MTD fusion uncouples kinase cascade activity from pheromone input. These experiments show that pathway activation specifically requires delivery of Ste5 to the plasma membrane, and other cellular membranes will not suffice. Furthermore, they argue that the MTD sequences cause activation by their effects on subcellular location, rather than by some other, unintentional consequence, such as oligomerization (see Inouye et al. 1997; Feng et al. 1998).

Signaling activity of membrane-targeted Ste5 requires Ste20, Cdc42, and Cdc24

One explanation for why targeting Ste5 to the plasma membrane activates the pathway is that it may bring the Ste5-associated kinases into proximity of a membrane-associated activator. Therefore, we examined the dependence of this effect on Gβγ and Ste20, both of which localize at or near the plasma membrane (Hirschman et al. 1996; Peter et al. 1996; Leberer et al. 1997b) and are predicted to function upstream of Ste5 (Leberer et al. 1992; Hasson et al. 1994). Remarkably, Fus1–LacZ induction by Ste5–CTM and Ste5ΔN–CTM was independent of the Gβ subunit Ste4 (and the Gγ subunit Ste18; by Gβγ itself, and defines a molecular activity of the free Gβγ complex that can be monitored independent of kinase cascade activation.
Figure 5. Pathway activation by Ste5ΔN fusions requires delivery to the plasma membrane. Localization (left) and Fus1-LacZ induction (right) for carboxy-terminal MTD fusions to GFP-Ste5ΔN. Snc2D, Sso1B, and Cpr MTD fusions show peripheral rim localization indicative of plasma membrane. Intracellular circular localization observed with Sed5 and Sec22 MTD fusions are reminiscent of endoplasmic reticulum, and punctate spots seen with Sso1B, Sed5, and Sec22 are reminiscent of Golgi localization. With the Sec22 MTD fusion, faint plasma membrane localization was also detectable (arrowheads). All images show cells untreated with pheromone. Strain: PY858. Plasmids, from top to bottom: pGFP-G5ssΔN, pGFP-G5ssΔN–Snc2D, pGFP-G5ssΔN–Sso1B, pGFP-G5ssΔN–Sed5, pGFP-G5ssΔN–Sec22, pGFP-G5ssΔN–Cpr, pGFP-G5ssΔN–Cpr-SS. Bars, mean ± S.D. for four transformants.

not shown), but was completely dependent on Ste20 (Fig. 6A). In comparison, activation of the pathway further downstream using an activated Ste11 allele (Ste11ΔN) or overproduced Ste12 (2 µm Gal–Ste12) was insensitive to the presence of both Ste4 and Ste20. Activation of growth arrest by Ste5–CTM and Ste5ΔN–CTM was also independent of Ste4 yet dependent on Ste20 (Fig. 6B), as were activation of mating and projection formation (not shown). Because these phenotypes required Ste20 but not pheromone or Gbγ, Ste20 can participate in this pathway without its kinase activity being activated by Gbγ. We saw little evidence that this role of Ste20 is substantially potentiated by Gbγ or pheromone, as pheromone stimulated minor (Ste5 fusions) or no (Ste5ΔN fusions) increase in signaling when Ste5 was targeted to the membrane (Fig. 6A; see also Figs. 2B,E, and 5).

Because Cdc24 and Cdc42 are also required for activation of the kinase cascade by Gbγ (Simon et al. 1995; Zhao et al. 1995), and Cdc24 is membrane-associated (Ziman et al. 1993), we asked whether they were required for activation by membrane-targeted Ste5 derivatives. Their roles were tested using temperature-sensitive mutant alleles, and signaling was analyzed after shift to the restrictive temperature. As seen for response to pheromone, Cdc24 and Cdc42 were required for pathway activation by Ste5–CTM or Ste5ΔN–CTM that occurred in the absence of pheromone (Fig. 6C). In contrast, their inactivation did not interfere with Fus1-LacZ induction by Ste11ΔN or 2 µm Gal–Ste12, ensuring that the mutant cells were competent to induce transcription, and suggesting that Cdc24 and Cdc42 are required for events upstream of Ste11. Therefore, similar to our observations with Ste20, participation of Cdc24 and Cdc42 in the pheromone response pathway does not require that they have their activities increased in magnitude by pheromone or Gbγ.

To further address the requirement for Ste20 at the cell surface and the role of Cdc42, we examined the mutant Ste20S879A/S880A/P883A, which lacks its Cdc42-binding site and is depleted from the cell surface (Peter et al. 1996; Leberer et al. 1997b). This mutant showed a partial defect in supporting activation by membrane-targeted Ste5 derivatives, as Fus1–LacZ levels were 13–14% (Ste5ΔN–CTM) or 31–38% (Ste5–CTM) of that promoted by Ste20WT (Table 2); this could be alleviated somewhat by addition of pheromone, but only with Ste5–CTM and not Ste5ΔN–CTM. When we assayed the Ste20S879A/S880A/P883A mutant for response to pheromone in a strain (ste20Δ) that expressed native Ste5, we observed a stronger defect (55% of wild-type Fus1–LacZ induction) than reported previously, which ranged from 80% (Peter et al. 1996) to 100% of wild type (Leberer et al. 1997b). It is unclear why we uncovered a stronger defect, but our observations were highly reproducible (see Table 2 footnotes). Our findings suggest a detectable, but subtle, role for the Cdc42-binding domain of Ste20. Because this domain is required for cell surface localization of Ste20, these results are consistent with the idea that the functional effect of targeting Ste5 to the membrane is to increase its proximity to Ste20.

Finally, we also analyzed another Ste20 mutant, Ste20S879A/S880A/P883A, which contains mutations in a recently identified Gbγ-binding domain (Leeuw et al. 1998). This mutant is defective at response to pheromone, suggesting a critical role for a Gbγ–Ste20 interaction (Leeuw et al. 1998). In contrast, our observations using membrane-targeted Ste5 derivatives show that Ste20 can participate in this pathway in the absence of Gbγ. Therefore, we tested the Ste20S879A/S880A/P883A mutant for its ability to support pathway activation by Ste5–CTM and Ste5ΔN–CTM, and found that it shows a strong defect in this ability (Table 2). Results were similar in cells (ste4Δ ste5Δ ste20Δ) that lack the Gbγ subunit.
Ste4, emphasizing that the defect is distinct from effects on binding Gβγ. Therefore, the Ste20<sup>3879A/3880A/3883A</sup> mutant is impaired for a signaling function that is independent of pheromone and interaction with Gβγ, and its pheromone-response defect cannot be attributed to disruption of Gβγ–Ste20 binding.

**Discussion**

Our observations establish five points pertaining to the mechanism by which the yeast Gβγ complex activates the signaling pathway downstream of it. First, Gβγ must remain at the membrane after dissociation from Gα to activate the pathway, as deletion of the CaaX motif in Gγ results in a signaling defect that can be rescued by fusing heterologous membrane targeting sequences to either Gβ or Gγ. Second, signaling by Gβγ can be mimicked by artificial placement of Ste5 at the plasma membrane, suggesting the functional role of Gβγ membrane localization may be to recruit Ste5. Third, a portion of the cellular population of Ste5 is recruited to the cell surface on exposure to pheromone, and this recruitment is most likely mediated by Gβγ itself. Fourth, the effect of Ste5 membrane localization is critically dependent on the cell surface-associated kinase Ste20, suggesting that membrane localization of Ste5 causes activation of its associated kinases by increasing their proximity to Ste20. Fifth, the participation of Ste20, Cdc42, and Cdc24 in the pheromone response pathway does not require their previous activation by pheromone or Gβγ, raising the possibility that Gβγ does not activate them per se but instead promotes their action on new substrates (Fig. 7A). A model that incorporates these points is presented in Figure 7B. In addition, our findings provide two ways to separate the contributions of different proteins to pathway activity: Cell surface recruitment of GFP–Ste5 provides an assay for Gβγ function independent of Ste20 and other kinases, and activation by mem-

**Figure 6.** Critical role of Ste20, Cdc42, and Cdc24 in pathway activation by membrane-targeted Ste5 derivatives. (A) Analysis of requirement for Ste4 and Ste20. Fus1–LacZ induction in mutant strains expressing galactose-inducible Ste5, Ste11, or Ste12 derivatives. Strains: PPY 858, PPY 886, PPY 860. Plasmids, from top to bottom: pH–G55, pH–G55–CTM, pH–G55ΔN–CTM, pRD–STE11–H3, pNC252. Bars, mean ±s.d. for four transformants. (B) Growth arrest. Streaked transformants (as in A) grown for 5 days at 30°C on a −HIS +RAFF +GAL plate are shown. (C) Dependence on Cdc24 and Cdc42. Fus1–LacZ induction in wild-type vs. cdc mutant strains was compared after pathway activation by pheromone-dependent or -independent methods. (Left) ste5Δ CDC24 (PPY 655) or ste5A cdc24-1 (data combined for PPY 697 and PPY 698) strains harbored, from top to bottom: pL–G55, pL–G55–CTM, pL–G55ΔN–CTM, pRD–STE11–H3, pNC252. Bars, mean ±s.d. for four transformants. (Right) A bar1 cdc42-1 strain (PPY 911) harbored either pRS514–CDC42–WT (CDC42) or pRS314 (cdc42-1) plus, from top to bottom: pRS315, pL–G55–CTM, pL–G55ΔN–CTM, pNC252. Results using the Ste11ΔN allele in this strain are absent because they gave highly variable results, for unknown reasons, and therefore were inconclusive. Bars, mean ±s.d. for four transformants.
brane-targeted Ste5 provides an assay for Ste20 function independent of Gβγ activity.

A role for Gβγ membrane localization: recruitment of Ste5

Our findings offer a simple explanation for why Gβγ must remain at the membrane to activate the kinase cascade. Because recruitment of Ste5 to the membrane is a critical step in activation by Gβγ. Importantly, our ability to detect this recruitment in the absence of Ste20, Ste11, or Ste7 represents an assay for an immediate function of the free Gβγ complex, which does not require activity through the kinase cascade or measurable mating, cell-cycle arrest, or transcriptional induction. Although recent studies found that Ste4 (Gβγ) and Ste5 co-immunoprecipitated independent of pheromone (Leeuw et al. 1998), other studies found that coimmunoprecipitation of these proteins could be stimulated by pheromone (Feng et al. 1997). We envision membrane recruitment of Ste5 as an initial step in kinase cascade activation. The likely immediate result is phosphorylation of Ste11 by Ste20, followed by phosphorylation of Ste7, then Fus3 (for review, see Hernandez 1995; Leberer et al. 1997a; see Fig. 7B); note, however, that we do not yet know whether the kinases Ste11, Ste7, and Fus3 accompany Ste5 to the membrane. Because some responsiveness to pheromone was retained by MTD fusions to Ste5 but not Ste5N, binding of Ste5 to Gβγ may trigger additional effects on Ste5, such as a conformational change or increased association with Ste20 (see model, Fig. 7B). Because activation by Ste5N–CTM was weaker than by Ste5–CTM even in the absence of Ste4, there may be roles for the Ste5 amino terminus that are not mediated by Gβγ. Other steps in activation may involve oligomerization of Ste5 (Yablonski et al. 1996; Inouye et al. 1997). Indeed, fusion of Ste5 with a domain thought to self-dimerize activated the pathway (Inouye et al. 1997), although as available evidence indicates that oligomerization of Ste5 is independent of pheromone (Yablonski et al. 1996; Feng et al. 1998), it may normally facilitate signaling without being induced by pheromone.

Kinase cascade activation

It is tempting to speculate that Ste5 carries signal from the cell surface to the nucleus, where transcription is induced, as it can be observed at both locations. However, nuclear Ste5 was observed in nonsignaling cells (Fig. 4), and Ste5 targeted to the plasma membrane was depleted from the nucleus and yet remained competent to signal, suggesting that nuclear localization of Ste5 may not be required for signaling. Instead of promoting
Role of membrane recruitment of Ste5 by yeast Gßγ

Figure 7. Model for pheromone response pathway activation by Gßγ. (A) General models. Cdc24, Cdc42, and Ste20 are required for Gßγ to activate the downstream kinase cascade (Ste11, Ste7, and Fus3, shown associated with the scaffold protein Ste5). A priori, Gßγ could either activate these proteins (left) or promote the action of already active proteins on the kinase cascade (right). Our observations favor the scheme on the right. Whether Cdc24/Cdc42 act by way of Ste20 is still controversial (see text). (B) Detailed model for molecular activity of Gßγ. Asterisks indicate active proteins, and Cdc24, Cdc42, and Ste20 are suggested to be active before exposure to pheromone. The model proposes that on exposure of cells to pheromone, liberated Gßγ recruits Ste5 and its associated kinases to the membrane, and thus into proximity of active Cdc24, Cdc42, and Ste20, resulting in activation of the kinase cascade by Ste20. The model also incorporates the recently described interaction of Gßγ with Ste20 (Leeuw et al. 1998), which is shown as contributing to the formation of a Ste5-Gßγ-Ste20 complex. Possible subsequent Ste5 fates are indicated below. For simplicity, we show all three kinases—Ste11, Ste7, and Fus3—accompanying Ste5 to the cell surface, but it is possible that only a subset do so.

Classical model for pheromone response pathway activation by Gßγ. (A) General models. Cdc24, Cdc42, and Ste20 are required for Gßγ to activate the downstream kinase cascade (Ste11, Ste7, and Fus3, shown associated with the scaffold protein Ste5). A priori, Gßγ could either activate these proteins (left) or promote the action of already active proteins on the kinase cascade (right). Our observations favor the scheme on the right. Whether Cdc24/Cdc42 act by way of Ste20 is still controversial (see text). (B) Detailed model for molecular activity of Gßγ. Asterisks indicate active proteins, and Cdc24, Cdc42, and Ste20 are suggested to be active before exposure to pheromone. The model proposes that on exposure of cells to pheromone, liberated Gßγ recruits Ste5 and its associated kinases to the membrane, and thus into proximity of active Cdc24, Cdc42, and Ste20, resulting in activation of the kinase cascade by Ste20. The model also incorporates the recently described interaction of Gßγ with Ste20 (Leeuw et al. 1998), which is shown as contributing to the formation of a Ste5-Gßγ-Ste20 complex. Possible subsequent Ste5 fates are indicated below. For simplicity, we show all three kinases—Ste11, Ste7, and Fus3—accompanying Ste5 to the cell surface, but it is possible that only a subset do so.

Genes & Development 2693
than their activity levels; (3) pheromone induces only mating genes and not filamentation genes that are induced when Ste20, Ste11, and Ste7 are activated by other methods (Madhani and Fink 1998), perhaps because Gβγ does not activate Ste20 but merely directs it to act on those kinase molecules that are associated with Ste5.

Recently it was found that Gβγ can bind Ste20, and that defects in this binding correlate with impaired pheromone response (Leeuw et al. 1998). In our experiments with membrane-targeted Ste5, however, Ste20 can function in the absence of Gβγ, and the Gβγ binding-deficient mutant Ste20(S879A/S880A/P883A) cannot support pathway activation even by Gβγ-independent means. Nevertheless, a role for a Gβγ–Ste20 interaction remains supported by their pheromone-inducible communoprecipitation as well as by signaling-defective Ste4 mutants that disrupt binding to Ste20 (Leeuw et al. 1998). Therefore, to unite these various observations, we suggest that Gβγ normally performs two functions: It recruits Ste5 to the membrane and brings Ste20 close to the recruited Ste5 (see Fig. 7B), thereby nucleating a complex that promotes phosphorylation of Ste11 by Ste20. A requirement for Gβγ–Ste20 binding may be bypassed in our experiments either because the lifetime of Ste5 at the cell surface is sufficiently increased that Ste20 can find Ste5 without help from Gβγ, or because expression levels of our membrane-targeted Ste5 derivatives are sufficiently high to present all cell surface Ste20 molecules with a nearby Ste5 molecule. Consistent with this latter suggestion, when membrane-targeted Ste5 was expressed at limiting levels (Fig. 2E), pathway activation became submaximal and responsive to further input by pheromone. In either scenario, the role of the Gβγ–Ste20 interaction would not be to stimulate the kinase activity of Ste20, but to nucleate a complex including both Ste20 and Ste5. This view is suggested by our observation that Ste20 can support pathway activation by membrane-targeted Ste5 in the absence of Gβγ.

The precise role for Cdc24 and Cdc42 in pheromone response remains unclear. Some evidence suggests that they are required to generate active Ste20, as the requirement for Cdc24 can be alleviated by activated Ste20 (Zhao et al. 1995) and Ste20 kinase activity can be stimulated by GTP-loaded Cdc24 (Simon et al. 1995). Other evidence argues that the role of Ste20 is independent of Cdc24, as Ste20 mutants lacking a Cdc24-binding site still transmit pheromone response (Peterson et al. 1996; Liber et al. 1997a). Our results support the role of Cdc24 and Cdc42 being related to Ste20 function, as they are required for pathway activation by means that require Ste20 (i.e., pheromone or membrane-targeted Ste5) and not by those that do not (i.e., Ste11N or Ste12 overproduction). In addition, we uncovered noticeable defects in the Ste20 mutant lacking its Cdc42-binding site. It is possible that Cdc24 and Cdc42 are required less for the kinase activity of Ste20 than for its cell surface localization, and that this role is obscured in some experiments because of the ability of Ste20 to associate with other cell surface molecules such as Gβγ, Ste5, actin, and Bem1 (Leeuw et al. 1995, 1998).
30°C to an OD660 of 0.3–0.5, then GAL (2%) was added ±10 µM cultures, inoculated from RAFF stocks, were grown overnight at Fus1–LacZ assays were performed on 1-ml culture and units alone or with 0.1% or 0.4% GLU ± 10 µM 2E, cells were grown in RAFF, then treated with GAL either unless indicated otherwise, W303 and 381G strains under these conditions; not shown). a-factor (determined by dose-response assays to be saturating for GAL1p–ste5), and pGS5 (DAPY 832a Strain Genotype

| Strain     | Genotype                                      |
|------------|-----------------------------------------------|
| PPY 409a   | MATa ade2 his2 leu2 trp1 ura3 can1 ste2a::URA3 |
| PPY 496a   | MATa ade2 his3 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste20-1::TRP1 |
| PPY 640a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 |
| PPY 794a   | MATa ade2 his2 leu2 trp1 ura3 can1 ste4a::ura3::OA |
| PPY 832a   | MATa ade2 his2 leu2 trp1 ura3 can1 ste18::URA3 |
| PPY 858a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste5::ADE2 |
| PPY 860a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste5::ADE2 ste20-1::TRP1 |
| PPY 865a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste18::URA3 |
| PPY 866a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste5a::Ade2 ste20-1::TRP1 |
| PPY 886a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste5::ADE2 ste4a::ura3::OA |
| PPY 889a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste4::ADE2 |
| PPY 890a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste11::ADE2 |
| PPY 891a   | MATa ade2 his2 leu2 trp1 ura3 can1 FUS1::FUS1-lacZ::LEU2 ste7::ADE2 |
| PPY 262a   | MATa cry1 leu2 lys2 trp1 ura3 SU-P4-3 FUS1::FUS1-lacZ::URA3 |
| PPY 655a   | MATa cry1 ade2 ade3 his4 leu2 lys2 trp1 ura3 SU-P4-3 FUS1::FUS1-lacZ::LYS2 ste5a::LYS2 |
| PPY 697a   | MATa cry1 ade2 ade3 his4 leu2 lys2 trp1 ura3 SU-P4-3 FUS1::FUS1-lacZ::LYS2 ste5a::LYS2 cdcd2-1 |
| PPY 698a   | MATa cry1 ade2 ade3 his4 leu2 lys2 trp1 ura3 SU-P4-3 FUS1::FUS1-lacZ::LYS2 ste5a::LYS2 cdcd2-1 |
| PPY 885a   | MATa cry1 ade2 his4 leu2 lys2 trp1 ura3 SU-P4-3 FUS1::FUS1-lacZ::LYS2 ste18::LEU2 gpa1::::URA3 |
| PPY 198a   | MATa his7 lys9 trp1 ura3 can1 cy2 |
| PPY 911a   | MATa bar1 ade2 his2 leu2 trp1 ura3 cdcd4-1 FUS1::FUS1-lacZ::HIS2 |
| PT2a       | MATa hom3 ilv1 can1 |

Strain backgrounds: *W303,* 3381G, a3364A, 15Dau, other. (GAL1p–STE5), and pGS5A (GAL1p–ste5A), or the CEN LEU2 plasmids pL–G55 (GAL1p–STE5) and pL–G55A (GAL1p–ste5A).

MTD sequences (Nmyr, NTM, CTR, Cpr, Cpr–SS, Nco2B, Nco2C, S6S, Sec22) were amplified by PCR. Amino-terminal MTDs were inserted as BamH1–Ncol fragments, and carboxy-terminal MTDs as Mlu1–Apal fragments, using primer-introduced sites. In this way, pGS5A, pGS18A–C, pGS5A, pG55A, pL–G55A, and pL–G55A served as recipients to create the following: pG54–NTM, Nmyr, CTR, and Cpr; pGS18C–NTR, Nmyr, CTR, S6S, and S6S, and Cpr; pGS5–CTR, and Cpr, and Cpr–SS; pGS55–CTR, CTR, and Cpr–SS; pL–G55–CTR, and pL–G55–CTR. The Sac–Apl fragments from pG55A, pGS55–CTR, and pGS55–CTR were transferred into pRS413 to create the HIS3-marked derivatives pH–G55, pH–G55–CTR, pH–G55–CTR, and pH–G55–CTR, respectively. GFP with signal-enhancing mutations S65A, V68L, N–Snc2D, –Sso1B, –Sed5, –Sec22, –Cpr, and –Cpr–SS.

β-Galactosidase assays

Fus1–LacZ assays were performed on 1-ml culture and units calculated as described (Pryciak and Hartwell 1996). Fresh Raff cultures, inoculated from Raff stocks, were grown overnight at 30°C to an OD650 of 0.3–0.5, then GAL (2%) was added ±10 µM β-factor (determined by dose-response assays to be saturating for W303 and 381G strains under these conditions; not shown). Unless indicated otherwise, β-galactosidase activity was then measured after 4 hr of incubation at 30°C. For assays in Figure 2E, cells were grown in Raff, then treated with GAL either alone or with 0.1% or 0.4% GLU ± 10 µM β-factor. For bar1 cdcd2-1 strains, transformants were grown at 28°C in Raff, preincubated by 90 min at 37.5°C, then induced for 5 hr at 37.5°C with GAL ±10 µM β-factor. For bar1 cdcd2-1 strains, cotransformants were grown at 28°C in Raff containing 0.1% GLU (growth was poor in Raff), preincubated for 2 hr at 38°C, then induced for 4 hr with GAL ±0.1 µM β-factor.

Mating assays

For quantitative mating (Schrick et al. 1997), overnight Raff cultures were used, 1 × 10⁴ a transformants were mixed with 2 × 10³ α partner cells, collected onto sterile filters, and the filters placed on SC + Raff + GAL plates for 18 hr at 30°C. Dilutions of harvested cells were plated on media selective for either diploids or total plasmid-containing cells. Mating efficiency was the percentage of total plasmid-containing (a + α) a cells that were diploids. For patch mating, a transformants were patched onto lawns of PT2a on SC + Raff + GAL plates, incubated at 30°C overnight, and replicated to plates selective for diploids.

Microscopy

Fresh colonies (2- to 3-day-old) from selective glucose plates were suspended in selective Raff + GAL liquid and incubated at 30°C; when included, α-factor (10 µM) was added 2 hr later. Live cells were generally examined after 4 hr in Raff + GAL by spotting onto poly-l-lysine coated slides, using a Nicon E600 epifluorescence microscope with a 100× Plan Fluor oil immersion objective. Images were collected using a cooled, black and white, CCD camera (DAGE-MTI).

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Pryciak and Huntress

(GM 57769). P.M.P. dedicates this study to the memory of Maria Pryciak.

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Note added in proof

A recent study (L.J.W.M. Oehlen and F.R. Cross. 1998. The role of Cdc42 in signal transduction and mating of the budding yeast Saccharomyces cerevisiae. J. Biol. Chem. 273: 8556–8559) suggests that decreased pheromone-induced transcription in cdc24 and cdc24 mutant cells can be attributed largely to inhibitory Cln1/2-Cdc28 kinase activity that accumulates during cell cycle arrest.

References

Adams, A.E., D.I. Johnson, R.M. Longnecker, B.F. Sloat, and J.R. Pringle. 1990. CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast Saccharomyces cerevisiae. J. Cell Biol. 111: 131–142.

Akada, R., L. Kallal, D.I. Johnson, and J. Kurjan. 1996. Genetic relationships between the G protein βγ-complex, Ste6p, Ste20p and Cdc42p: Investigation of effector roles in the yeast pheromone response pathway. Genetics 143: 103–117.

Carraway, K.L. and C.A. Carraway. 1995. Signaling, mitogenesis and the cytoskeleton: Where the action is. Bioessays 17: 171–175.

Chant, J. and L. Stowers. 1995. GTPase cascades choreographing cellular behavior: Movement, morphogenesis, and more. Cell 81: 1–4.

Choi, K.Y., B. Satterberg, D.M. Lyons, and E.A. Elion. 1994. Ste5 tetters multiple protein kinases in the MAP kinase cascade required for mating in S. cerevisiae. Cell 78: 499–512.

Cormack, B.P., R.H. Valdivia, and S. Falkow. 1996. FACS-optimized mutants of the green fluorescent protein (GFP). Gene 173: 33–38.

Couve, A., V. Protopopov, and J.E. Gerst. 1995. Yeast synaptobrevin homologs are modified posttranslationally by the addition of palmitate. Proc. Natl. Acad. Sci. 92: 5987–5991.

Cvrckova, F., C. DeVirgilio, E. Mancer, J.R. Pringle, and K. Nasmyth. 1995. Ste20-like protein kinases are required for normal localization of cell growth and for cytokinesis in budding yeast. Genes & Dev. 9: 1817–1830.

Feng, Y., L.Y. Song, E. Kincaid, S.K. Mahanty, and E.A. Elion. 1998. Functional binding between Gβγ and the LIM domain of Ste9 is required to activate the MEKK Ste11. Curr. Biol. 8: 267–278.

Ferro-Novick, S. and R. Jahn. 1994. Vesicle fusion from yeast to man. Nature 370: 191–193.

Grishin, A.V., J.L. Weiner, and K.J. Blumer. 1994. Biochemical and genetic analysis of dominant-negative mutations affecting a yeast G-protein γ subunit. Mol. Cell. Biol. 14: 4571–4578.

Hasson, M.S., D. Blinder, J. Thorner, and D.D. Jenness. 1994. Mutational activation of the STE5 gene product bypasses the requirement for G protein beta and gamma subunits in the yeast pheromone response pathway. Mol. Cell. Biol. 14: 1054–1065.

Herskovitz, I. 1995. MAP kinase pathways in yeast. For mating and more. Cell 80: 187–197.

Hirschman, J., G. DeZutter, W. Simonds, and D.D. Jenness. 1996. The Gβγ complex of the yeast pheromone response pathway: Subcellular fractionation and protein–protein interactions. J. Biol. Chem. 272: 240–248.

Inouye, C., N. Dhillon, and J. Thorner. 1997. Ste5 RING-H2 domain: Role in Ste4-promoted oligomerization for yeast pheromone signaling. Science 278: 103–106.

Leberer, E., D. Dignard, D. Harcus, D.Y. Thomas, and M. Whitley. 1992. The protein kinase homologue Ste20p is required to link the yeast pheromone response G-protein βγ subunits to downstream signalling components. EMBO J. 11: 4815–4824.

Leberer, E., D.Y. Thomas, and M. Whitley. 1997a. Pheromone signaling and polarized morphogenesis in yeast. Curr. Opin. Genet. Dev. 7: 59–66.

Leberer, E., C. Wu, T. Leeuw, A. Fourost-Lieuvin, J.E. Segall, and D.Y. Thomas. 1997b. Functional characterization of the Cdc42p binding domain of yeast Ste20p protein kinase. EMBO J. 16: 83–97.

Leeuw, T., A. Fourost-Lieuvin, C. Wu, J. Chenevert, K. Clark, M. Whitley, D.Y. Thomas, and E. Leberer. 1995. Pheromone response in yeast: Association of Bem1p with proteins of the MAP kinase cascade and actin. Science 270: 1210–1213.

Leeuw, T., C. Wu, J.D. Schrag, M. Whitley, D.Y. Thomas, and E. Leberer. 1998. Interaction of a G-protein β-subunit with a conserved sequence in Ste20/PAK family protein kinases. Nature 391: 191–195.

Lilly, P.J. and P.N. Devreotes. 1995. Chemotactant and GTPγS-mediated stimulation of adenyl cyclase in Dictyostelium requires translocation of CRAC to membranes. J. Cell Biol. 129: 1659–1665.

Madden, K. and M. Snyder. 1992. Specification of sites for polarized growth in Saccharomyces cerevisiae and the influence of external factors on site selection. Mol. Biol. Cell 3: 1025–1035.

Madhani, H.D. and G.R. Fink. 1998. The riddle of MAP kinase signaling specificity. Trends Genet. 14: 151–155.

Mitchell, D.A. and R.J. Deschenes. 1995. Characterization of protein prenylation in Saccharomyces cerevisiae. Methods Enzymol. 250: 68–78.

Neiman, A.M. and I. Herskowitz. 1994. Reconstitution of a yeast protein kinase cascade in vitro: Activation of the yeast MEK homologue STE7 by STE11. Proc. Natl. Acad. Sci. 91: 3398–3402.

Nern, A. and R.A. Arkowitz. 1998. A GTP-exchange factor required for cell orientation. Nature 391: 195–198.

Peter, M., A.M. Neiman, H.-O. Park, M. van Lohuizen, and I. Herskowitz. 1996. Functional analysis of the interaction between the small GTP binding protein CDC42 and the Ste20 protein kinase in yeast. EMBO J. 15: 7046–7059.

Pitcher, J.A., J. Inglese, J.B. Higgins, J.L. Arriza, P.J. Casey, C. Kim, J.L. Benovic, M.M. Kwatra, M.G. Caron, and R.J. Lefkowitz. 1992. The role of G-protein βγ subunits of G proteins in targeting the β-adrenergic receptor kinase to membrane-bound receptors. Science 257: 1264–1267.

Pryciak, P.M. and L.H. Hartwell. 1996. AKR1 encodes a candidate effector of the Gβγ complex in the Saccharomyces cerevisiae pheromone response pathway and contributes to control of both cell shape and signal transduction. Mol. Cell. Biol. 16: 2614–2626.

Raymer, J.C. and H.R.B. Pelham. 1997. Transmembrane domain-dependent sorting of proteins to the ER and plasma membrane in yeast. EMBO J. 16: 1832–1841.

Schrick, K., B. Garvik, and L.H. Hartwell. 1997. Mating in Saccharomyces cerevisiae: The role of the pheromone signal transduction pathway in the chemotropic response to phero-
Role of membrane recruitment of Ste5 by yeast Gβγ

mone. Genetics 147: 19–32.
Sellis, M.A. and J. Chernoff. 1997. Emerging from the Pak: The p21-activated protein kinase family. Trends Cell Biol. 7: 162–167.
Sherman, F. 1991. Getting started with yeast. Methods Enzymol. 194: 3–21.
Sikorski, R.S. and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122: 19–27.
Simon, M.-N., C. DeVirgilio, B. Souza, J.R. Pringle, A. Abo, and S.I. Reed. 1995. Role for the Rho-family GTPase Cdc42 in yeast mating-pheromone signal pathway. Nature 376: 702–705.
Sprague, G.F. and J.W. Thorner. 1992. Pheromone response and signal transduction during the mating process of Saccharomyces cerevisiae. In The molecular and cellular biology of the yeast Saccharomyces, Vol. 2 (ed. E.W. Jones, J.R. Pringle, and J.R. Broach), pp. 657–744. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Tedford, K., S. Kim, D. Sa, K. Stevens, and M. Tyers. 1997. Regulation of the mating pheromone and invasive growth responses in yeast by two MAP kinase substrates. Curr. Biol. 7: 228–238.
Whiteway, M.S. and D.Y. Thomas. 1994. Site-directed mutations altering the CAAX box of Ste18, the yeast pheromone-responsive pathway Gγ subunit. Genetics 137: 967–976.
Whiteway, M., L. Hougan, and D.Y. Thomas. 1990. Overexpression of the STE4 gene leads to mating response in haploid Saccharomyces cerevisiae. Mol. Cell. Biol. 10: 217–222.
Whiteway, M.S., C. Wu, T. Leeuw, K. Clark, A. Fourest-Lieuvin, D.Y. Thomas, and E. Leberer. 1995. Association of the yeast pheromone response G protein βγ subunits with the MAP kinase scaffold Ste5p. Science 269: 1572–1575.
Wu, C., M. Whiteway, D.Y. Thomas, and E. Leberer. 1995. Molecular characterization of Ste20p, a potential mitogen-activated protein or extracellular signal-regulated kinase kinase (MEK) kinase kinase from Saccharomyces cerevisiae. J. Biol. Chem. 270: 15984–15992.
Yablonski, D., I. Marbach, and A. Levitzki. 1996. Dimerization of Ste5, a mitogen-activated protein kinase cascade scaffold protein, is required for signal transduction. Proc. Natl. Acad. Sci. 93: 13864–13869.
Zhang, F.L. and P.J. Casey. 1996. Protein prenylation: Molecular mechanisms and functional consequences. Annu. Rev. Biochem. 65: 241–269.
Zhao, Z.-S., T. Leung, E. Manser, and L. Lim. 1995. Pheromone signalling in Saccharomyces cerevisiae requires the small GTP-binding protein Cdc42p and its activator CDC24. Mol. Cell. Biol. 15: 5246–5257.
Ziman, M., J.M. O’Brien, L.A. Ouellette, W.R. Church, and D.I. Johnson. 1991. Mutational analysis of CDC42Sc, a Saccharomyces cerevisiae gene that encodes a putative GTP-binding protein involved in the control of cell polarity. Mol. Cell. Biol. 11: 3537–3544.
Ziman, M., D. Preuss, J. Mulholland, J.M. O’Brien, D. Botstein, and D.I. Johnson. 1993. Subcellular localization of Cdc42p, a Saccharomyces cerevisiae GTP-binding protein involved in the control of cell polarity. Mol. Biol. Cell 4: 1307–1316.
Membrane recruitment of the kinase cascade scaffold protein Ste5 by the G\(\beta\gamma\) complex underlies activation of the yeast pheromone response pathway

Peter M. Pryciak and Frederick A. Huntress

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