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Essay

Cell Polarization Directed by Extracellular Cues in Yeast

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Many cell types are able to generate cellular asymmetry, or polarize, in response to chemical gradients in the environment. The question of how external signals influence cell polarity requires an understanding of how a chemoattractant signaling pathway leads to organization of the cytoskeleton. Studies of polarizing yeast cells and other chemotactic cells indicate that similar molecules and pathways may be involved in these diverse systems. This review will focus on cell polarization in response to extracellular signals during yeast mating. First, the phenomenon of cell polarization during yeast mating will be described. Then the extensive work on cell polarization during yeast budding will be reviewed, and a model for mating cell polarity will be presented. Finally, eucaryotic chemotaxis will be briefly summarized, and the similarities to polarization during yeast mating will be discussed.

CELL POLARIZATION DURING MATING

Cells of the yeast *Saccharomyces cerevisiae* polarize toward an environmental signal during conjugation. When two yeast cells of opposite mating type (**a** and α) come into contact, they grow toward each other in a polarized fashion (Byers and Goetsch, 1975; Tkacz and MacKay, 1979; Field and Schekman, 1980; Hasek *et al.*, 1987). Secretion and new cell-surface growth are concentrated in the direction of the mating partner (Tkacz and MacKay, 1979). The actin and microtubule cytoskeletons also polarize toward the mating partner (Byers, 1981; Ford and Pringle, 1986; Hasek *et al.*, 1987). This response facilitates efficient cell and nuclear fusion, resulting in a diploid **a**/α cell (reviewed in Cross *et al.*, 1988) (see Figure 1A).

Mating yeast cells signal each other with cell type–specific pheromones. These pheromones are secreted peptides and are recognized by cell-surface receptors; **a** cells produce **a**-factor that binds to a receptor on α cells, and α cells produce α-factor that binds to a receptor on **a** cells. Pheromone receptors belong to the large family of G protein-coupled receptors with seven transmembrane domains (reviewed in Marsh *et al.*, 1991; Kurjan, 1992).

The spatial signal emanating from the mating partner that directs localized growth during mating is apparently a high concentration of mating pheromone. Given a choice between mating partners that do or do not produce pheromone, yeast cells mate almost exclusively with the pheromone-producing cells (Jackson and Hartwell, 1990a,b; Jackson *et al.*, 1991). This phenomenon is termed mating partner discrimination and is thought to reflect cell morphogenesis toward a gradient of mating pheromone in the environment. Exogenously added pheromone restores mating to pheromone-deficient mutant strains only very poorly (Kurjan, 1985; Michaelis and Herskowitz, 1988), indicating that the mating pheromone must be presented by a cell in a spatially meaningful manner. Recent work has demonstrated the polarized growth of **a** cells toward a micropipet filled with α-factor (Segall, 1993).

The addition of purified pheromone to cells of the opposite cell type causes a variety of responses, including cell cycle arrest, gene induction, and formation of a polarized cell shape ("shmoo"). The projection of the shmoo (or "shmoo tip") is formed by deposition of new membrane and cell wall material to a localized region of the cell surface (Lipke *et al.*, 1976; Tkacz and MacKay, 1979; Field and Schekman, 1980). Many molecules with roles in mating localize to the shmoo tip, including **a**-agglutinin (Watzele *et al.*, 1988), Fus1p (Trueheart *et al.*, 1987), Spa2p (Gehrung and Snyder, 1990), Ste2p (Jackson *et al.*, 1991), Fus2p (Elion, personal communication), and Ste6p (Kuchler *et al.*, 1993). Most organelles, including the nucleus, accumulate on the side of the cell where the projection forms (Byers and Goetsch, 1975; Tkacz and MacKay, 1979; Hasek *et al.*, 1987; Rose and Fink, 1987; Baba *et al.*, 1989; Gehrung and Snyder, 1990). The cytoskeleton is oriented in a polarized manner in a shmoo (Barnes *et al.*, 1990; Read *et al.*, 1992) (Figure 1B). Actin accumulates at the growing region of the cell cortex, and actin cables align along the growth axis (Ford and Pringle, 1986; Hasek *et al.*, 1987; Gehrung and Snyder, 1990). The spindle pole body (the yeast analogue of the centrosome or microtubule organizing center) orients toward the shmoo tip, and microtubules emanating from it extend into the tip (Byers, 1981; Rose and Fink, 1987; Gehrung and Snyder, 1990; Meluh and Rose, 1990) (see Figure 1B). A ring of 10-nm filaments is observed at the base of the projection (Kim *et al.*, 1991; Ford and Pringle, 1986). Recent studies using several mutant alleles of the actin

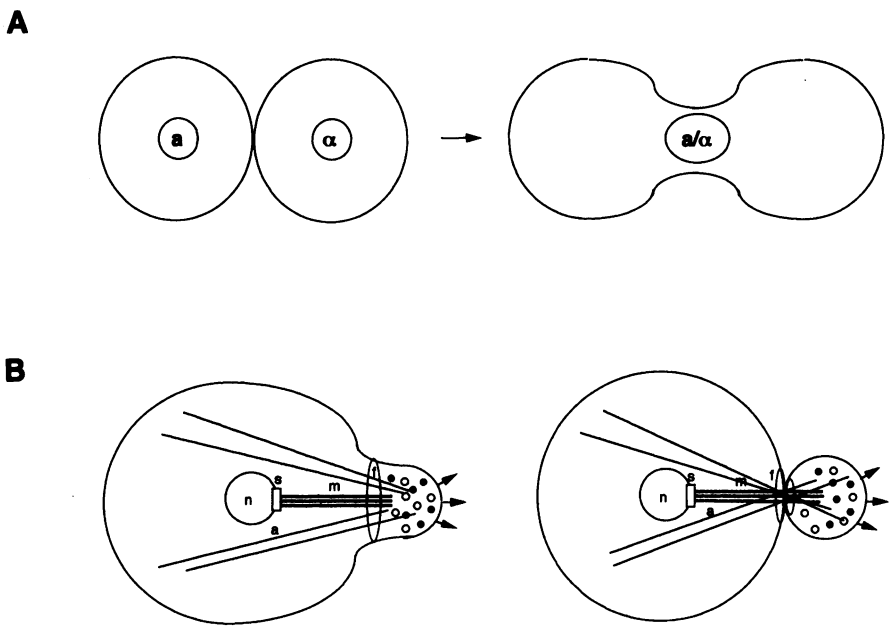


Figure 1. Cell polarity in yeast. (A) Yeast mating. Two haploid yeast cells of mating type **a** and α grow toward one another and fuse at the site of cell contact to form a diploid **a**/cell. The small circles represent nuclei. (B) Polarized components in a yeast cell. Left, pheromone-treated (shmooing) cell; Right, vegetative (budding) cell. All major cytoskeletal elements and organelles are organized around the site of cell-surface growth in the two types of polarized cells. The spindle pole body (s) on the nucleus (n) is positioned on the side of the nucleus near either the shmoo tip or the bud. Extranuclear microtubules (m) radiate from the spindle pole body into the shmoo tip as they do into the bud. Cortical actin patches (O) are concentrated in both the shmoo tip and the bud. Actin cables (a) orient toward the shmoo tip or the bud. A ring of 10-nm filaments (f) is found at the shmoo neck as well as at the mother-bud neck. Secretory vesicles (●) are concentrated in the shmoo tip and the bud. The insertion of new cell-surface material (arrows) occurs at both the shmoo tip and the bud tip.

and tubulin genes show that actin but not tubulin is required for shmoo tip growth (Read *et al.*, 1992). Similarly, studies on mating cells indicate that microtubules are not required for morphogenesis or cell fusion, but that they are required for nuclear migration and fusion (Delgado and Conde, 1984; Hasek *et al.*, 1987; Huffaker *et al.*, 1988).

A signal transduction pathway is activated when **a**- or α-factor bind to their respective receptors (Figure 2) (see Marsh *et al.*, 1991; Kurjan, 1992 for review). Signal transduction downstream of the receptors involves a common set of molecules in both **a** and α cells, including a heterotrimeric G protein, $G_{\alpha\beta\gamma}$. Stimulation of the receptor causes Gα to switch from the GDP-bound state

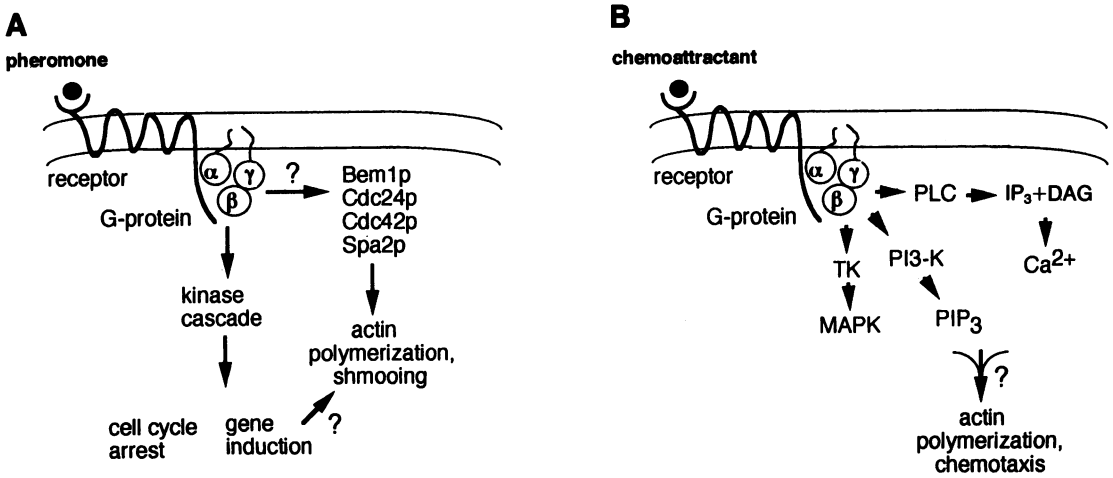


Figure 2. Components of a possible link between a chemoattractant and the cytoskeleton. (A) Shmooing in yeast. Stimulated receptors activate a G protein that triggers a cascade of protein kinases, resulting in several mating responses. The arrow from the G-protein to morphology changes is speculative and indicates the position at which the localized signal for cytoskeletal organization may branch off from the rest of the pathway. This signal may induce the activation or localization of a group of proteins including Bem1p, Cdc24p, and Cdc42p, which organize the actin cytoskeleton. Some components required for morphological response may need to be induced by the pheromone response pathway, hence an arrow from gene induction to shmooing. (B) Chemotaxis in neutrophils or *Dictyostelium*. Similarly, chemoattractants such as cAMP (in *Dictyostelium*) or N-formylated peptides (in neutrophils) stimulate a G protein-coupled receptor and lead to actin polymerization. Several signaling pathways are activated by chemoattractants (see text), but how they lead to cytoskeletal reorganization is not known. PLC, phospholipase C; DAG, diacylglycerol; IP_3, inositol triphosphate; Ca^{2+}, calcium; PI 3-K, phosphatidylinositol 3-kinase; PIP_3, phosphatidylinositol (3,4,5) triphosphate; TK, tyrosine kinase; MAPK, mitogen-activated protein kinase.

to the GTP-bound state, which leads to release of $G\beta\gamma$. Free $G\beta\gamma$ then initiates the pheromone response. The direct target of $G\beta\gamma$ is not known, but it activates a cascade of protein kinases (reviewed in Neiman, 1993). The molecular details of how the pheromone-induced signal transduction pathway leads to some pheromone responses such as transcriptional induction and cell-cycle arrest are beginning to be understood (Peter *et al.*, 1993), but the connection between the pheromone response pathway and molecules that act to polarize the cell is much less clear.

CELL POLARIZATION DURING BUDDING

During vegetative division, yeast cells grow in a polarized manner by budding from distinct sites on the cell surface (see Chant and Pringle, 1991; Drubin, 1991; Chant, 1994 and references therein). Unlike sites of growth during mating, sites of growth during budding are determined by the genetic makeup of a cell and are not influenced by the presence of other cells. Otherwise, the directed growth that occurs during budding has strong similarities to the growth that occurs during mating. As the bud forms on the surface, cell-surface growth and secretion are concentrated to a small patch on the yeast cell surface. Many of the same intracellular components are organized in a polarized manner in both budding and mating (Figure 1B); these include actin, microtubules, the spindle pole body, and the 10-nm neck filaments. During budding, as during mating, actin filaments but not microtubules are required for localized cell surface growth.

A large amount of work on budding has revealed two classes of genes that regulate budding. Mutations that cause improper positioning of a bud but no growth defects have identified five bud-site selection genes (*BUD1–5*) (Chant and Herskowitz, 1991; Chant *et al.*, 1991). Their role is apparently to define the site for bud emergence. A second group of genes, known as polarity establishment genes, (which includes *CDC24*, *CDC42*, *CDC43*, and *BEM1*) is necessary for the restriction of cell-surface growth to a specific site (Sloat *et al.*, 1981; Adams *et al.*, 1990; Chant *et al.*, 1991; Chenevert *et al.*, 1992, 1994). Mutants defective in the polarity establishment genes are unable to localize growth to form a bud and instead enlarge in a uniform manner and are slow growing or inviable (Sloat and Pringle, 1978; Field and Schekman, 1980; Sloat *et al.*, 1981; Adams *et al.*, 1990; Johnson and Pringle, 1990; Chant *et al.*, 1991; Bender and Pringle, 1991; Chenevert *et al.*, 1992). Thus the polarity-establishment gene products appear to function as central organizers of cell polarity. Consistent with this possibility, both Bem1p (Corrado, 1992) and Cdc42p (Ziman *et al.*, 1993) localize to the site of bud emergence.

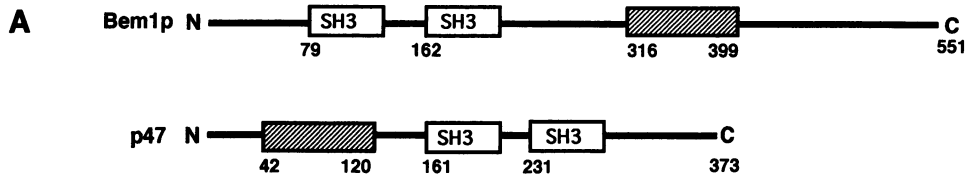
The sequences of the polarity-establishment genes are suggestive of their possible functions. *CDC42* encodes a ras-like GTP-binding protein (Johnson and Pringle, 1990) for which CDC24 protein is a proposed guanine nucleotide exchange factor (Hart *et al.*, 1991). *CDC43* encodes a subunit of the geranylgeranyl transferase essential for the modification of Cdc42p (Finegold *et al.*, 1991). In mammalian cells, small GTP-binding proteins modulate actin assembly in response to growth factors (reviewed in Hall, 1992); rac induces the accumulation of actin filaments in the plasma membrane, forming membrane ruffles (Ridley *et al.*, 1992), and rho promotes the formation of focal adhesions and stress fibers (Ridley and Hall, 1992). Cdc42p is a member of the rho subfamily of GTP-binding proteins, and it may function in a similar way to promote the localized organization of actin in yeast. *BEM1* encodes a protein that contains two SH3 domains (Chenevert *et al.*, 1992); SH3 domains are proposed to promote association with the actin cytoskeleton (Drubin *et al.*, 1990; Bar-Sagi *et al.*, 1993).

A further connection between small GTP-binding proteins and yeast cell polarity genes is suggested by a similarity between *BEM1* and a component of the rac-containing NADPH oxidase in neutrophils. The NADPH oxidase is a superoxide-generating enzyme system that consists of a membrane-bound cytochrome and three cytosolic proteins, rac, p47, and p67 (Abo *et al.*, 1991; Knaus *et al.*, 1991; reviewed in Bokoch and Knaus, 1994). These three proteins become localized to the plasma membrane upon neutrophil activation to form the assembled oxidase. Like Bem1p, p47 contains two SH3 domains, and the two proteins share an additional region of homology (29% identity, 57% similarity over 84 amino acids) outside of the SH3 domains (Neiman, personal communication) (Figure 3). An intriguing possibility is that this sequence represents a recognition domain for Cdc42p (rac). Alternatively, it might mediate interaction with p67, or it may be involved in membrane localization.

The phenotypes conferred by mutations in the bud-site selection genes or the polarity-establishment genes and genetic interactions among the two groups of genes have led to a model for bud formation (Chant and Herskowitz, 1991; Chant, 1994) (Table 1). The *BUD* gene products are proposed to recognize a cell-surface landmark and guide the positioning of the polarity-establishment gene products, which in turn organize the cytoskeleton and initiate bud growth at the proper site. The nature of the cell-surface landmark recognized in a budding cell is not known, but a putative candidate is the ring of 10-nm filaments present at the mother-bud neck (Chant and Herskowitz, 1991; Chant, 1994).

A MODEL FOR MATING CELL POLARITY

The same set of organizational molecules used in budding, which includes at least Cdc24p, Cdc42p, and Bem1p, appears to direct polarized growth in mating.



B

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Bem1p RYYQDFYDLQVQLLDAFPAEAGKLRDAGGQWSKRIMPYIPGPVPYVTNSITKKRKEDLNIYVADLVNLPDYISRSEMVHSLFVV
      R + ++Y+++  L ++FP+EAG + ++  +   RI+P++P+P  +  + +   R   L  Y + L+ LP  ISR   +  +F V
 p47  RRFTEIYEFHKTLKEMFPIEAGAI-NP--E--NRIIPHLPAPKWFDGQRAAENRQGTLTEYCSTLMSLPTKISRCPHLLDFFKV
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Figure 3. Homology between yeast Bem1p and human p47. (A) Schematics of Bem1p and p47. The numbers refer to amino acid positions in the proteins. The hatched region indicates the area of homology between the two proteins in addition to the SH3 domains. (B) Sequence of homologous region. Identical residues are noted: +, conservative change.

Temperature-sensitive *cdc24* mutants exhibit defects in mating (Reid and Hartwell, 1977) and shmooing (Field and Schekman, 1980; Chenevert *et al.*, 1994) at the nonpermissive temperature. Special alleles of *BEM1* exist that are defective in mating and shmooing but not budding (Chenevert *et al.*, 1992). An additional protein, Spa2p, is required for shmoo tip formation but not bud formation (Gehrung and Snyder, 1990). Like Spa2p, Cdc42p (Ziman *et al.*, 1993) and Bem1p (Chenevert, unpublished data) localize to the shmoo tip in pheromone-treated cells. The creation of cell polarity during mating or shmoo formation could occur via a pathway analogous to that of budding; proteins involved in site selection may generate an intracellular spatial signal that is recognized by the polarity establishment proteins that in turn locally organize the cytoskeleton (Table 1).

The proteins used for site selection during mating are apparently distinct from those used for site selection during budding; yeast strains mutant in any one of the *BUD* genes do not exhibit defects in mating (Chant and Herskowitz, 1991; Chant, personal communication; Chenevert, unpublished data) or mating partner discrimination (Dorer and Hartwell, personal communication). Mutants defective specifically in selection of the mating site and no other aspects of mating (analogous to bud site selection mutants) are not known, but their identification should be facilitated by a new assay for orientation of cells to an external source of pheromone (Segall, 1993).

The molecules responsible for defining the site for cell polarization during mating must retain directional information from the pheromone gradient. This requirement could be fulfilled by a connection from the pheromone receptor or its G protein to molecules that organize the cytoskeleton (Figure 2). It is possible that a conformational change or a clustering in the intracellular domains of pheromone receptors caused by pheromone binding could directly recruit one or more polarity-establishment proteins. Cells containing a mutant form of the α-pheromone receptor that lacks the intracellular C-terminus produce uniformly enlarged cells rather than polarized shmoos (Konopka *et al.*, 1988). Alternatively, information could be passed from receptors to the cytoskeleton via the G protein. Consistent with this possibility, the pheromone receptor, the G protein, and the Sst2 protein, but none of the other genes in the conventional signal transduction pathway are required for mating partner discrimination (Jackson and Hartwell, 1990b; Jackson *et al.*, 1991; Schrick and Hartwell, personal communication). If the discrimination assay truly measures the ability of a cell to organize the cytoskeleton toward a mating partner, then these

Table 1. Molecules involved in the flow of information from the membrane to the cytoskeleton during formation of two types of polarized yeast cells

	Mating	Budding
Landmark	extracellular, pheromone gradient	intracellular, neck filaments?
↓		
Site selection	pheromone receptor, G protein	BUD gene products
↓		
Polarity establishment	Bem1p, Cdc24p, Cdc42p, Spa2p	Bem1p, Cdc24p, Cdc42p, Cdc43p
↓		
Cytoskeleton	actin, microtubules, secretory apparatus	actin, microtubules, secretory apparatus

In both mating and budding cells, site selection proteins are proposed to recognize a cell-surface landmark and guide the positioning of the polarity-establishment gene products, which in turn organize the cytoskeleton. Mating and budding differ primarily in the mechanism of site selection.

results suggest a functional branchpoint from the G protein to polarity-establishment proteins (see Figure 2). The role of Sst2p in morphogenesis is unclear, because yeast strains mutant in *SST2*, which are also supersensitive to pheromone, are able to orient to the pheromone source at low pheromone concentrations (Segall, 1993). Communication between the G protein and the polarity-establishment proteins may involve Ste20p, a component of the kinase signaling cascade that has recently been found to bind to Cdc42p (Manser *et al.*, 1994). Testing mutant strains defective in each gene in the pathway for the ability to polarize toward a gradient of pheromone using the shmoo orientation assay (Segall, 1993) will give an indication of which signaling components are required for cell polarity.

CHEMOTAXIS

The best-studied eucaryotic models of polarized movement toward a stimulus are chemotaxis in neutrophils and *Dictyostelium* (reviewed in Devreotes and Zigmond, 1988; Downey, 1994), and many similarities with yeast mating are evident. Chemoattractants such as N-formylated peptides (in neutrophils) or cAMP (in *Dictyostelium*) bind to surface receptors that contain seven transmembrane domains. In both neutrophils and *Dictyostelium*, the chemotaxis signal is transmitted through a receptor-coupled heterotrimeric G protein, although which subunit of the G protein mediates the chemotactic response is not yet clear. Within seconds after chemoattractant addition, rapid polymerization of actin occurs in the lamellipodia of neutrophils and amoebae. Actin polymerization is an essential response and is clearly a result of a G protein-mediated signal, but the relevant targets of the G protein and the second messengers that promote actin polymerization are not known. In both cell types, exposure to chemoattractants triggers a cascade of molecular events and the production of several second messengers (Figure 2B). Phospholipase C is activated to generate inositol triphosphate (IP_3), causing release of intracellular calcium stores and diacylglycerol, which leads to activation of protein kinase C. These responses are mediated by G proteins. In *Dictyostelium*, the addition of IP_3 or Ca^{2+} causes rapid actin polymerization in permeabilized amoebae. In neutrophils, gradients of diacylglycerol stimulate chemotaxis, but the elevation of cytoplasmic calcium does not appear to be required.

Recent work on additional signaling pathways in neutrophils has brought to light other potential regulators of cytoskeletal organization. The addition of formyl peptide to neutrophils activates phosphoinositide (PI) 3-kinase (Okada *et al.*, 1994; Thelen *et al.*, 1994). PI 3-kinase generates phosphatidylinositol-3,4,5-triphosphate (PIP_3), which is implicated in the control of actin assembly (Eberle *et al.*, 1990; Norgauer *et al.*, 1992; Wymann and Arcaro, 1994). Stimulation of neutrophils also induces tyrosine phosphorylation (Gaudry *et al.*, 1992; Rollet *et al.*, 1994) and the activation of mitogen-activated protein kinases (Grinstein *et al.*, 1993; Torres *et al.*, 1993). In other cell types it has been shown that tyrosine kinases activate ras (Li *et al.*, 1993; McCormick, 1993; Schlessinger, 1993) and that small GTP-binding proteins modulate actin assembly (Hall, 1992); it is likely that these highly conserved pathways function in neutrophils as well. The precise roles each of these signaling pathways may play in connecting chemoattractant receptors to rearrangements of the cytoskeleton are not yet understood. The products of multiple pathways, and crosstalk between them, may be involved.

In yeast, calcium is a potential mediator of cytoskeletal organization. Addition of pheromone causes a rapid influx of Ca^{2+} from the media (Ohsumi and Anraku, 1985; Tachikawa *et al.*, 1987; Iida *et al.*, 1990). Calmodulin localizes to sites of cell surface growth in both budding and pheromone-treated cells (Brockerhoff and Davis, 1992). A localized elevation in intracellular Ca^{2+} levels could cause organization of the cytoskeleton in the proper place by activating polarity-establishment gene products. Sequence analysis and the isolation of calcium-sensitive alleles suggest that Cdc24 protein may bind calcium (Ohya *et al.*, 1986; Miyamoto *et al.*, 1987). A yeast phospholipase C has recently been identified (Payne and Fitzgerald-Hayes, 1993), but mutants in the phospholipase C gene were not reported to display any defects in mating or shmooing.

In summary, pheromone-induced cell polarization in yeast exhibits great similarity to chemotaxis in other eucaryotes in that binding of chemoattractant to a cell-surface receptor activates a G protein and results in actin reorganization. Much is known about components of the pheromone signaling pathway and molecules that establish cell polarity in response to this pathway in yeast. The identity of the localized signal responsible for initiating cytoskeletal organization is not known in any system, but studies of yeast mating should contribute to an understanding of how cells polarize in response to extracellular signals.

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