**AFR1 Promotes Polarized Apical Morphogenesis in Saccharomyces cerevisiae**

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The conjugation of the yeast Saccharomyces cerevisiae provides a genetically accessible system for the analysis of hormone-induced cell polarization and morphogenesis. Haploid S. cerevisiae cells of opposite mating type, \( \text{MAT}^a \) and \( \text{MAT}^a \) (24), signal each other with mating pheromones to undergo conjugation (32, 35, 55). \( \text{MAT}^a \) cells secrete \( \alpha \)-factor pheromone that activates a receptor on the surface of \( \text{MAT}^a \) cells; \( \text{MAT}^a \) cells secrete \( \alpha \)-factor pheromone that activates a receptor on the surface of \( \text{MAT}^a \) cells. Pheromone receptor signaling stimulates yeast cells to arrest cell division in \( G_1 \) and then undergo intracellular reorganization to polarize cell growth toward an appropriate partner cell. The cells then polarize growth toward a gradient of pheromone which enables them to sense the spatial position of an appropriate mating partner (27, 51). Polarized morphogenesis enables pairs of mating cells to make contact and fuse to form zygoty.

The mating-pheromone signal pathway is similar to sensory pathways used in multicellular organisms to detect diverse stimuli such as light, odor, taste, and hormones (15, 23). The \( \alpha \)-factor receptor, encoded by \( \text{STE2} \) (6, 42), and the \( \alpha \)-factor receptor, encoded by \( \text{STE3} \) (21, 42), belong to a large family of receptors that are distinguished by possessing seven transmembrane domains. Biochemical analysis of the most thoroughly studied receptors in this family, the \( \beta \)-adrenergic receptor and rhodopsin, indicates that these receptors transduce their signal by stimulating the \( G \) subunit of a heterotrimeric G protein to bind GTP (23). The \( G_\alpha \) subunit then dissociates from the \( G_\beta \gamma \) subunit; either \( G_\alpha \) or \( G_\beta \gamma \) goes on to activate an effector. In S. cerevisiae, \( G_\beta \gamma \) transmits the pheromone signal by activating the subsequent steps in the signaling pathway that include MAP kinase homologs (16). Phosphorylation of a pheromone-responsive transcription factor, \( \text{STE12} \), stimulates transcription of genes that function in mating (54). Pheromone signaling stimulates cell division arrest through inactivation of the CDC28 protein kinase and associated cyclin proteins by transcriptional and posttranscriptional mechanisms (9, 16).

The yeast pheromone receptors play a key role by signaling cell polarization through G protein-dependent and G protein-independent signal pathways (27). Pheromone-induced morphogenesis is mediated by many of the same components that mediate polarized morphogenesis during bud formation (39). Some of these genes encode cytoskeletal elements such as actin, and others encode signal transduction components such as the CDC24 guanine nucleotide exchange factor (56). In some cases, the products of the genes involved in cell polarization show a polarized distribution within the cell themselves. For example, the leading edge of growth in both structures contains actin, SPA2, calmodulin, and CDC42 proteins (2, 5, 53, 57). Pheromone signal transduction components also become polarized, which suggests that this may be part of the signaling mechanism for cell polarization. The \( \alpha \)-factor receptors and the \( \text{STE6} \) protein that promotes \( \alpha \)-factor secretion are localized to the region of pheromone-induced morphogenesis (27, 34). However, it remains to be determined whether polarization of the pheromone signaling components is a cause or a consequence of cell polarization. The mechanisms by which pheromones stimulate polarized growth in S. cerevisiae may be conserved in other organisms. Chemokines such as interleukin-8, which stimulate leukocyte chemotaxis, and cyclic AMP, which stimulates Dictyostelium discoideum chemotaxis, activate G protein-coupled receptors that promote actin reorganization and cell polarization (13, 36).

Several investigators have suggested that the pheromone receptors may directly determine the region of polarized growth (4, 11, 27, 40). Interestingly, truncation of the cytoplasmic C terminus of the \( \alpha \)-factor receptor causes a defect in forming acute projections of pheromone-induced morphogenesis (33). A similar morphogenesis defect was observed in \( afr1 \) mutant cells (31). Genetic evidence indicates that \( \text{AFR1} \) acts in the same pathway as the receptor \( \text{C} \) terminus to promote pheromone-induced morphogenesis. \( \text{AFR1} \) also acts in conjunction with the C terminus of the \( \alpha \)-factor receptor to promote adaptation to \( \alpha \)-factor. To better understand how \( \text{AFR1} \) functions to coordinate signal transduction and morphogene-
sis, the subcellular localization of AFR1 protein and the effects of constitutive AFR1 expression were examined. The results suggest that AFR1 promotes pheromone-induced morphogenesis by interacting with a family of putative filament-forming proteins encoded by CDC3, CDC10, CDC11, and CDC12 that were shown previously to be involved in bud morphogenesis and cytokinesis (18, 20, 22, 29).

MATERIALS AND METHODS

Strains and media. The yeast strains used in this study are described in Table 1. Cells were grown in media described by Sherman (52). Plasmid-containing cells were grown in synthetic medium containing adenine, uracil, and amino acid additives but lacking leucine to select for plasmid maintenance. Plasmids were transformed into yeast strains with lithium acetate (25).

Galactose-regulated expression of AFR1. PCR was used to introduce a SalI site 5' to the coding region of AFR1. A yeast strain (CTY10-5d) containing four lacZ-binding sites inserted upstream of a lacZ reporter gene (obtained from R. Sternglanz) was cotransformed with pLAF and the Gal10 promoter fragment. Transformed colonies were screened for induction of the lacZ reporter gene specifically in combination with a lacE-AFR1 fusion plasmid recovered into E. coli. The identity of the yeast insert was determined by using an oligonucleotide primer corresponding to the Gal4 activation domain to obtain the DNA sequence across the junction.

RESULTS

Pheromone-induced cell polarization in afr1 and ste2-T326 mutants. S. cerevisiae cells that are stimulated with a high dose of pheromone arrest cell division in the unboosted G1 phase and then form acute projections of new cell growth. Previous studies showed that mutation of AFR1 or truncation of the α-factor pheromone receptor (ste2-T326) caused a defect in forming the acute projections (31). Some morphogenesis mutants, such as bent1" mutants, are thought to be defective because they fail to polarize actin (11). Therefore, we investigated the localization of actin in afr1:URA3 and ste2-T326 mutants. Cells were stained with rhodamine-conjugated phalloidin, and the distribution of actin was visualized by fluorescence microscopy. The distribution of actin in 200 cells was analyzed for each cell type. Pheromone-induced wild-type cells showed the expected result (Fig. 1A); at least 97% of the cells showed a highly polarized distribution of actin with cortical dots of actin at the leading edge of growth and actin cables emanating from the tip into the cell body. A polarized distribution of actin was also detected in at least 90% of afr1 and 70% of ste2-T326 mutants after pheromone stimulation (Fig. 1B and C). However, actin was not concentrated into an acute projection in the mutant cells. Thus, AFR1 and the C terminus of the α-factor receptor function to organize actin into a tight cluster but are not essential for polarization.

Chitin localization was also examined, since this cell wall
Component is enriched in the region at the base of pheromone-induced projections (50). This distinct subcellular localization makes chitin a very useful marker for morphological studies. Pheromone-induced cells were stained with Calcofluor and then examined by fluorescence microscopy to visualize chitin. Two hundred cells of each cell type were examined. At least 94% of the wild-type cells showed the expected localization of chitin at the base of the projection but not at the leading edge of growth, where the cortical dots of actin are concentrated (Fig. 2A). In contrast, Calcofluor staining was not uniformly localized in afr1 or ste2-T326 cells and many cells varied in staining intensity (Fig. 2B and C). Only 5% of afr1 and 4% of ste2-T326 mutants showed the normal staining at the base of a projection. Overall, $\geq 96\%$ of wild-type cells showed highly polarized chitin staining whereas only 59% of afr1 and only 15% of ste2-T326 mutants displayed highly polarized staining. Nonpolarized staining patterns were not due to staining of previous bud sites. Mislocalization of chitin in the afr1 and ste2-T326 cells suggests that these cells are defective in localizing other components that are necessary for projection formation.

Immunolocalization of AFR1 protein in α-factor-induced cells. The relationship between actin organization and AFR1 was analyzed further by immunofluorescence since previous studies showed that actin-binding proteins such as fimbrin and tropomyosin colocalize with actin in vivo (1, 38). Anti-AFR1 antibodies were raised in rabbits (see Materials and Methods), and then the specificity of affinity-purified anti-AFR1 antibodies was tested by Western immunoblot analysis (Fig. 3). A prominent band of approximately 90 kDa was detected in α-factor-induced AFR1 cells. This band was not detected in extracts from uninduced cells or from cells that lack the AFR1 gene (afr1Δ). α-Factor induction of AFR1 was expected since an AFR1-lacZ fusion gene was previously shown to be regulated by α-factor (31). A protein product of similar gel mobility was detected in cells that were engineered to express AFR1 in response to galactose instead of α-factor. The apparent molecular mass of the AFR1 protein was about 18 kDa larger than the predicted molecular mass of 72 kDa determined from the DNA sequence. The slightly anomalous gel mobility is probably due to the structure of the AFR1 protein and not to post-translational modification, since E. coli-produced AFR1 protein showed similar gel mobility (data not shown). These results confirm that the antibodies are specific for the AFR1 protein.

Affinity-purified anti-AFR1 antibodies were used to examine the localization of AFR1 protein in a MATa tetraploid strain. The increased size of yeast strains with higher ploidy facilitates immunofluorescence analysis (47). Cells incubated without α-factor showed only a low level of background staining (Fig. 4A). α-Factor-induced cells showed staining that was detected primarily at the base of the projection (Fig. 4C and D). More than 90% of the cells were stained by the anti-AFR1 antibody, and nearly all of the cells containing projections showed at least some staining at the base. The AFR1 staining was distinct from actin staining, which was found primarily at the leading edge of the projection (Fig. 4B). Thus, AFR1 does not colocalize with the majority of actin in the cell. AFR1 may be
present in a ring around the base of the projection, since AFR1 staining appears to be concentrated at the edge of the projections (Fig. 4D). AFR1 staining could be detected in the central portion by switching to a new focal plane or by comparison of different photographic prints (Fig. 4D). Similar results were observed for the analysis of chitin, which forms a ring structure in the cell wall around the base of the projection (Fig. 2A).

**Morphological effects of constitutive AFR1 expression.**

AFR1 expression is normally regulated by pheromone, so the effects of AFR1 on cell morphogenesis were examined by engineering constitutive expression of AFR1 in the absence of pheromone. Inducible AFR1 expression was engineered by fusing the coding region of AFR1 to the galactose-inducible GAL10 promoter as described in the Materials and Methods. The GAL10-AFR1 gene was inserted into a YEp plasmid vector and then introduced into a MATa haploid strain for analysis. Galactose induction of AFR1 resulted in about 50% of cells forming elongated buds (Fig. 5C). The elongated buds were caused by AFR1 expression, since they were not seen in uninduced cultures (Fig. 5A) or in control cells that were induced with galactose to express lacZ instead of AFR1 (Fig. 5B).

In a previous study, it was observed that α-factor stimulation of AFR1 expression acted in conjunction with the C terminus of the α-factor receptor to promote projection formation (31). In contrast, galactose induction of AFR1 promoted the formation of elongated buds in a ste2-T326 receptor truncation mutant (Fig. 5D). The α-factor receptor itself was not required, since the elongated buds were induced in a ste2::URA3 strain that lacks the receptor gene (Fig. 5E). In fact, none of the haploid-specific mating genes were required since galactose induced the formation of elongated buds in a MATa/MATa diploid strain (Fig. 5F). These results indicate that in the absence of α-factor-induced cell division arrest, the AFR1 protein alters bud morphogenesis and causes the formation of elongated buds.

**Galactose-induced AFR1 protein is concentrated at the bud neck.**

Immunofluorescence was used to examine the cellular localization of AFR1 protein at various times after induction of the GAL10-AFR1 gene. Prior to the addition of galactose, the cells showed only a low level of diffuse background staining (Fig. 6A). After 1 h of induction, only about 10% of the cells showed fluorescent staining but, interestingly, the AFR1 staining was concentrated at the neck of the bud (Fig. 6B). By 4 h of induction, at least 80% of the cells showed AFR1 staining throughout the cell (Fig. 6C). However, there was still a concentrated region of staining at the bud neck in the mother cell.

**FIG. 4. Immunolocalization of AFR1 in α-factor-induced cells.** MATa strain JK7434-2 was incubated in the absence or presence of α-factor (10^-7 M) for 120 min. Immunofluorescent staining with affinity-purified anti-AFR1 antibody is shown for cells incubated in the absence (A) or presence (C and D) of α-factor. The upper and lower sections of panel D show a light and dark print of the same cells. Staining with anti-actin monoclonal antibody C4 is shown for cells incubated in the presence of α-factor (B). Fluorescein-conjugated secondary antibodies were used to detect staining with the primary antibody. The cells were prepared for immunofluorescence analysis as described in Materials and Methods.

**FIG. 5. Morphological effects of galactose-induced AFR1 expression.** Cells carrying a GAL10-AFR1 plasmid (pJK47) or a control plasmid which contained a GAL10-lacZ gene were grown to mid-logarithmic phase, galactose was added to a 1% final concentration to induce gene expression, and the incubation was continued for 4 h at 30°C. (A) Wild-type cells carrying the GAL10-AFR1 plasmid in the absence of galactose. (B) Wild-type cells carrying a GAL10-lacZ plasmid in the presence of galactose. (C to F) Wild-type (C), ste2-T326 (D), ste2::URA3 (E), and MATa/MATa (F) cells carrying the GAL10-AFR1 plasmid induced with galactose. The wild-type (DJ147-1-2), ste2-T326 (JKY7441-4-4), ste2::URA3 (QCY1-9), and MATa/MATa (JKY35) strains are described in Table 1.
in at least 50% of the cells. Thus, AFR1 staining in projections and buds is similar in that staining is not detected at the leading edge of growth but is instead found at the base of the morphogenic structure.

The localization of AFR1 at the bud neck is interesting because the CDC3, CDC10, CDC11, and CDC12 cell division cycle proteins are also located at the bud neck (18, 20, 29). Therefore, the phenotypic effects of constitutive AFR1 expression were compared with the effects of mutations in CDC3, CDC10, CDC11, or CDC12. Temperature-sensitive mutations in CDC3, CDC10, CDC11, or CDC12 show essentially the same phenotype (22), so only the results for a cdc3-1ts strain are presented. Galactose induction of AFR1 or shifting the cdc3ts strain to the nonpermissive temperature both caused the formation of elongated buds (Fig. 7). At later times, some cells started a second bud or a branch off of an existing bud. Examination of the number of nuclei present in each cell by DAPI staining (Fig. 7A and C) showed that nearly all of the large-budded cells were multinucleate. This contrasts with α-factor-induced cells, which arrest in the G₁ phase with a single nucleus. In addition, Calcofluor staining detected the deposition of chitin throughout the cell wall (Fig. 7B and D) in all of the large-budded cells. These similarities in phenotype suggest that the galactose induction of AFR1 antagonizes the function of CDC3, CDC10, CDC11, and CDC12.

AFR1 interacts with CDC12 in the two-hybrid assay. The similar localization of AFR1 and the CDC3, CDC10, CDC11, and CDC12 proteins suggested that they might interact in vivo. This possibility was investigated by a modification of the two-hybrid protein assay developed by Fields and Song (17). In this assay, protein-protein interaction is detected between one protein fused to a DNA-binding domain (LexA) and another protein fused to a transcriptional activation domain (GAL4). Interaction of the hybrid proteins in vivo induces the expression of a lacZ reporter gene. Cells carrying a lexA-AFR1 gene were transformed with a plasmid library of yeast sequences fused to the GAL4 transcriptional activation domain and were screened for plasmids that activate β-galactosidase production. DNA sequence analysis demonstrated that the GAL4 activation domain was fused in frame with the CDC12 gene (Genbank accession number L16551) in two of the six library plasmids that were identified. Both of the CDC12 isolates contained the same 5’ fusion junction which occurred 18 codons before the putative initiator methionine. The other four library plasmids did not correspond to previously identified genes. The interaction between LexA-AFR1 and GAL4-CDC12 was specific (Fig. 8). The lexA-AFR1 plasmid did not activate the reporter gene in combination with the GAL4 activation domain vector; the lexA vector or a lexA-lamin fusion was not able to activate the reporter in combination with a GAL4-CDC12 plasmid. These results indicate that AFR1 and CDC12 form a complex that could be due to direct protein

FIG. 6. Immunolocalization of AFR1 in galactose-induced cells. Wild-type cells (DJ147-1-2) carrying a GAL10-AFR1 plasmid (pJK47) were induced by addition of galactose for 0 min (A), 1 h (B), or 4 h (C). The distribution of AFR1 protein was detected by staining with affinity-purified rabbit anti-AFR1 antibody and with a fluorescein-conjugated goat anti-rabbit immunoglobulin G secondary antibody. Samples were prepared for immunofluorescence analysis as described in Materials and Methods.

FIG. 7. Phenotypic similarities between cells induced with galactose to express AFR1 and a cdc3 mutant. (A and B) Wild-type cells (DJ147-2-1) carrying GAL10-AFR1 plasmid pJK47 were induced with galactose for 18 h. (C and D) cdc3ts mutant cells (H3C1A5) were induced by a shift to the nonpermissive temperature (34°C) for 4 h. The cells were examined by fluorescence microscopy after staining with DAPI to detect DNA and staining with Calcofluor to detect cell wall chitin. A light-microscopic image corresponding to the cells shown in each fluorescent micrograph is shown to the right of each panel.
interactions or could be facilitated by a third protein that acts as a bridge. The ability of AFR1 to interact with CDC3, CDC10, and CDC11 in this assay is under investigation.

**DISCUSSION**

Vegetatively growing yeast cells polarize new growth to create a bud at a predicted site relative to the previous bud site (10). Pheromone stimulation arrests cell division in the unbudded G1 phase and then polarizes new growth toward a gradient of pheromone (51). This presumably enables yeast cells to effectively discriminate between mating partners (26). The morphological effects of \( a \)-factor vary in a dose-dependent manner (41). \( MATa \) cells treated with a low dose of \( a \)-factor (\( \sim 10^{-9} \) M) form elongated cells. At higher doses of \( a \)-factor (\( \geq 10^{-8} \) M), polarized growth forms an acute projection. \( afr1 \Delta \) mutants and cells producing truncated \( a \)-factor receptors (ste2-T326) are defective in forming the acute projections normally seen at high concentrations of \( a \)-factor. Other mutants that are strongly defective in pheromone-induced morphogenesis, such as \( cdc24 \) and \( bem1 \), show a defect in cell polarization which is highlighted by a failure to polarize actin (11). In contrast, \( afr1:URA3 \) cells and \( ste2-T326 \) mutants showed only a slight defect in actin polarization. Furthermore, \( afr1:URA3 \) and \( ste2-T326 \) mutants mate efficiently, which also suggests that they are capable of polarized growth (31). Similar results were reported for \( spa2 \) mutants, which are defective in forming acute projections in response to \( a \)-factor and also show only a slight defect in actin polarization and mating (19). These results indicate that AFR1 and the C terminus of the \( a \)-factor receptor act to restrict morphogenesis to a narrow region of the cell but are not essential for cell polarization.

**AFR1 expression promotes apical morphogenesis.** Increased AFR1 expression correlates with the formation of longer projections: AFR1 is stimulated in a dose-dependent manner by pheromone, and projections are observed only at high doses of pheromone (31). Furthermore, an increased gene dosage of AFR1 causes cells to form longer projections in response to \( a \)-factor. Therefore, we placed the AFR1 gene under the control of a galactose-inducible promoter to investigate the effects of AFR1 on morphogenesis in the absence of \( a \)-factor. The results showed remarkable similarity to the effects of AFR1 expression in pheromone-stimulated cells. Galactose-induced cells formed elongated buds, which indicates that morphogenesis was restricted to a narrow apical region (Fig. 5). In contrast, wild-type cells form round buds because they switch from polarized apical growth to isotropic growth (2, 37).

AFR1 expression affects the type of cell growth that occurs but not the rate of growth. The longer projections and buds take more time to form than do typical projections or buds. The longer projections and buds probably occur because cells maintain polarized apical growth at the same site for a longer period. Thus, AFR1 expression locks cells into apical morphogenesis and prevents cells from switching to a new morphogenesis site. AFR1 may act in a positive manner to stabilize an existing site of morphogenesis or in a negative manner to prevent the formation of a new site.

**Immunolocalization of AFR1.** The subcellular distribution of AFR1 protein was investigated in order to determine whether AFR1 might coincide with the localization of other proteins that function in projection formation, including actin, SPA2, and the \( a \)-factor receptors. Actin plays an essential role in polarized yeast morphogenesis by guiding secretory vesicles to dock at a specific site (45). Since \( afb1 \Delta \) mutants were defective in restricting actin to an acute projection, it seemed possible that AFR1 acts to bind and organize actin. The function of SPA2 protein is unknown, but it is required for projection formation and is also localized at the tips of projections (19, 53). The C terminus of the \( a \)-factor receptors, which are detected throughout the projections (27), act in the same genetic pathway as AFR1 to promote projection formation. Surprisingly, AFR1 was detected primarily at the base of the projection and did not colocalize with the majority of actin at the apex (Fig. 3). AFR1 protein could interact with the actin cables that radiate into the cell body, but actin cables are apparently not required for \( a \)-factor-induced morphogenesis (48). The distribution of AFR1 also differed from the reported location of SPA2 and the \( a \)-factor receptors. However, it is interesting that the location of AFR1 partially overlaps that of the receptors.

The localization of AFR1 to the base of projections is interesting because it coincides with the reported localization of the bud morphogenesis proteins CDC3 and CDC11 (18, 29). The role of CDC3 and CDC11 in projection formation is unknown, but they act together with CDC10 and CDC12 in bud morphogenesis (22). The CDC3, CDC10, CDC11, and CDC12 proteins localize to the neck of the bud, where they appear to form a ring structure (18, 20, 29). Interestingly, AFR1 was detected primarily at the bud neck in cells that were induced with galactose to express AFR1 (Fig. 6). AFR1 and the CDC proteins could use independent signals to localize to the same sites. However, since AFR1 interacts with CDC12 in the two-hybrid protein assay (Fig. 8), it seems possible that the localization of AFR1 is determined by binding to CDC12. The CDC3, CDC10, CDC11, and CDC12 proteins may also be responsible for the proper localization of other components. They are required for deposition of a ring of chitin in the cell wall at the bud neck and may be needed for the proper localization of components needed for cytokinesis. The cell wall at the base of projections is also enriched in chitin. Interestingly, this chitin is mislocalized in \( afr1:URA3 \) and \( ste2-T326 \) mutants (Fig. 2). This suggests that the pheromone receptors, AFR1, and the CDC3, CDC10, CDC11, and CDC12 proteins may mediate the proper localization of other components to the projections.

**Mechanism of AFR1 action.** \( a \)-Factor arrests cell division in the unbudded phase, and then AFR1 acts in a morphogenesis pathway involving the C terminus of the \( a \)-factor receptor to promote projection formation. Surprisingly, neither the receptor nor any other haploid cell-specific genes were required to observe the elongated buds that were caused by galactose induction of AFR1 (Fig. 5). These results indicate that the galactose-induced AFR1 protein manifests its effect by interacting with components of bud morphogenesis. Several lines of evidence suggest that galactose-induced AFR1 acts in the same
pathway as the CDC3, CDC10, CDC11, and CDC12 bud morphogenesis genes. The phenotype of cells induced with galactose to express AFR1 shows striking similarity to the temperature-sensitive phenotype of cdc3, cdc10, cdc11, and cdc12 mutants. All of these cells form elongated buds that fail to separte, mislocalize chitin, and become multinucleate (Fig. 7). In addition, AFR1 and the CDC3, CDC10, and CDC12 proteins localize to the bud neck in the mother cell. CDC3, CDC10, CDC11, and CDC12 encode a family of homologous proteins that are thought to form a ring of 10-nm filaments in the bud neck (7, 18, 20, 29). This filamentous ring is absent in cdc3, cdc10, cdc11, and cdc12 mutants (8). Thus, AFR1 may negatively regulate the formation of the filamentous ring or its subsequent function. However, it is premature to conclude that the normal function of AFR1 is to negatively regulate the CDC3, CDC10, CDC11, and CDC12 proteins, since galactose-induced AFR1 expression is a nonphysiological perturbation.

The results presented in this paper indicate that AFR1 acts in conjunction with the CDC3, CDC10, CDC11, and CDC12 proteins to form projections in pheromone-stimulated cells. The filamentous ring has not yet been observed in pheromone-induced projections, but CDC3 and CDC11 have been detected by immunolocalization in the same region of projections as the AFR1 protein (18, 29). During budding, CDC3, CDC10, CDC11, and CDC12 are thought to act by facilitating the proper localization of components required for cytokinesis. Perhaps the action of these putative filament proteins is modified by AFR1 to promote proper localization of components required for pheromone-induced morphogenesis. It has been proposed that the α-factor-stimulated receptors promote the assembly of an organizing center that is analogous to the bud site (11, 27, 40). This organizing center could include the CDC3, CDC10, CDC11, and CDC12 proteins as well as other components that function in bud and projection formation.

A Drosophila homolog of CDC3, CDC10, CDC11, and CDC12 called peanut may also function to localize cellular components (43). peanut shows functional similarity to CDC3, CDC10, CDC11, and CDC12 since it was required for cytokinesis, and the peanut protein was localized to cleavage furrows during cytokinesis. Interestingly, peanut was isolated as an enhancer of a defect in photoreceptor cell development and the peanut protein was detected at the apical surfaces of developing photoreceptors in the eye imaginal disc. Neufeld and Rubin (43) speculate that peanut may act to localize molecules, such as signal transduction components, to these domains. It is also interesting that the surface expression of a lymphocyte homing receptor was decreased in mouse cells that express low levels of Diff6, a murine homolog of the yeast filament proteins (44). Additional homologs of the yeast filament proteins have also been found in mice and fungi (14, 28). It will be interesting to determine if homologs of AFR1 participate in the developmentally regulated localization of signal transduction components in other organisms.

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REFERENCES