Gpa2p, a G-protein α-Subunit, Regulates Growth and Pseudoohyphal Development in Saccharomyces cerevisiae via a cAMP-dependent Mechanism*

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The small GTP-binding protein Ras and heterotrimeric G-proteins are key regulators of growth and development in eukaryotic cells. In mammalian cells, Ras functions to regulate the mitogen-activated protein kinase pathway in response to growth factors, whereas many heterotrimeric GTP-binding protein α-subunits modulate cAMP levels through adenylyl cyclase as a consequence of hormonal action. In contrast, in the yeast Saccharomyces cerevisiae, it is the Ras1 and Ras2 proteins that regulate adenylyl cyclase. Of the two yeast G-protein α-subunits (GPA1 and GPA2), only GPA1 has been well studied and shown to negatively regulate the mitogen-activated protein kinase pathway upon pheromone stimulation.

In this report, we show that deletion of the GPA2 gene encoding the other yeast G-protein α-subunit leads to a defect in pseudohyphal development. Also, the GPA2 gene is indispensable for normal growth in the absence of Ras2p. Both of these phenotypes can be rescued by deletion of the PDE2 gene product, which inactivates cAMP by cleavage, suggesting that these phenotypes can be attributed to low levels of intracellular cAMP. In support of this notion, addition of exogenous cAMP to the growth media was also sufficient to rescue the phenotype of a GPA2 deletion strain. Taken together, our results directly demonstrate that a G-protein α-subunit can regulate the growth and pseudohyphal development of S. cerevisiae via a cAMP-dependent mechanism. Heterologous expression of mammalian G-protein α-subunits in these yeast GPA2 deletion strains could provide a valuable tool for the mutational analysis of mammalian G-protein function in an in vivo null setting.

When shifted to nitrogen starvation conditions, diploid cells of the yeast Saccharomyces cerevisiae switch from normal vegetative growth to filamentous growth (1–3). This switch in developmental patterning is regulated by intracellular signaling pathways.

Many signaling molecules have been identified that control filamentous growth. These include the genes encoding the protein kinase Ste20p (a homologue of mammalian p65PAK protein kinase), Ste11p (a MEKK or MAPK kinase kinase), Ste7p (a MEK or MAPK kinase), and the transcription factor Ste12p (4). Furthermore, during nitrogen starvation the Ras2 protein, in addition to activating adenylyl cyclase (5, 6), induces filamentous growth by stimulating the MAPK pathway (7). This pathway also regulates pheromone responsiveness via the evolutionary conserved Cdc42p/Ste20p/MAPK module (7). These observations suggest that in response to a nutritional signal, the MAPK pathway is activated by a cascade of small G-proteins, similar to the activation of the Jun N-terminal kinase pathway in mammalian cells (8).

Although the pheromone response pathway and the pathway regulating filamentous growth share a common MAPK module, the upstream regulators of each pathway seem to be specific to a given pathway. For example, neither the G-protein α-subunit (Gpa1p) nor the Gβγ-protein subunits (Ste4p/Ste18p) of the heterotrimeric G-protein that regulates the pheromone response pathway (9) are involved in pseudohyphal development. Thus, the upstream regulators in the signaling pathway that controls filamentous growth remain unknown.

In the present study, we examine whether the G-protein α-subunit encoded by GPA2 regulates filamentous growth. Using a genetic approach, we show that Gpa2p modulates pseudohyphal development via a cAMP-dependent pathway. In addition, Gpa2p is necessary for normal growth on rich medium in cells that lack a functional Ras2 protein, implicating Gpa2p as an important regulator of normal cell growth.

EXPERIMENTAL PROCEDURES

Strains, Growth Conditions, and Genetic Manipulations—All strains used are congenic to the S2178b genetic background (4, 10). A haploid gpa2 deletion strain was constructed by transforming a disruption cassette in which the GPA2 open reading frame has been replaced by the LEU2 gene. The ras1 and ras2 mutations were constructed by using the disruption cassettes pras1::HIS3 and pras2::URA3, respectively (11). The pde2 deletion strain was constructed by transforming a polymeric chain reaction-based disruption cassette with a marker conferring G418 resistance. For the filamentous growth assay, the strains were made nutritionally prototrophic by transforming the respective vectors of the pRS series of Centromer-based plasmids (12). Standard yeast culture medium was prepared essentially as described (13). Low ammonium medium plates for scoring pseudohyphal growth were prepared as described (1). Standard procedures were used for yeast transformation and genetic manipulations. Addition of cAMP was performed by streaking a 100 µg/ml solution of the sodium salt of cAMP (Sigma) in sodium phosphate buffer (pH 6.5) onto the assay plates to obtain a final concentration of 5 mM. Sodium phosphate buffer was added to the control plates.

Qualitative Assays of Filamentous Growth—The growth assay for filament formation and light microscopy of microcolonies were performed as described (1, 14).

Iodine Staining—Strains were grown on rich medium for 2 days at

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** The abbreviations used are: MEK, mitogen-activated/extracellular signal-regulated kinase; MEKK, mitogen-activated/extracellular signal-regulated kinase kinase; MAPK, mitogen-activated protein kinase.

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Interestingly, such a synthetic growth phenotype was not observed for the gpa2, ras2 double mutant. Strains were grown on low ammonia plates and photographed after 3 days.

RESULTS AND DISCUSSION

Previous studies using a yeast strain deleted for GPA2 have been conducted by other investigators (15). However, no discernible phenotype was described, and pseudohyphal development was not examined (15).

To evaluate if the yeast G-protein α-subunit, Gpa2p, regulates the signaling cascade that controls pseudohyphal development, we constructed a diploid strain homozygous for a deletion of the GPA2 open reading frame. In our strain background, pseudohyphal development of the cells deleted for GPA2 was strongly inhibited as compared with a corresponding wild-type strain (Fig. 1, upper left and right panels). Only occasionally could we detect filamentous outgrowth.

Assuming that Gpa2p acts linearly via Ras2p on the MAPK pathway to regulate pseudohyphal development, a ras2 deletion mutant should exhibit the same phenotype as a gpa2Δ,ras2Δ double mutant. Fig. 1, lower left panel shows that a ras2 deletion strain is still capable of forming filaments; however, the cells constituting the filaments are round rather than being elongated. A gpa2Δ,ras2Δ double deletion strain exhibited the additive phenotype of the respective single mutants, i.e. very few filaments with round cells. This result suggests that Gpa2p and Ras2p do not act on the same pathway leading to pseudohyphal development, but rather that Gpa2p activates pseudohyphal development through a pathway parallel to the Ras2p pathway. These experiments were confirmed by testing the transcriptional activation of the Ste12p-dependent FG::LacZ reporter gene (7) (that depends on a functional MAPK pathway), which in a diploid gpa2 deletion strain under starvation condition was not significantly altered (data not shown).

On rich medium, we also observed a very slow growth phenotype for the gpa2Δ,ras2Δ double deletion strain, while each of the single mutants grew as the wild-type (Fig. 2, upper panel). Interestingly, such a synthetic growth phenotype was not observed when a gpa2 deletion was combined with a ras1 deletion (Fig. 2, lower panel). This observation provides further genetic evidence for a differential function of the RAS1 and RAS2 genes.

During normal haploid growth, Ras2p regulates intracellular cAMP levels (5). Therefore, we wondered if Gpa2p may influence cAMP levels in parallel to Ras2p. A combinatorial absence of the two proteins could lower the cAMP levels so drastically that cells would grow only very slowly. This hypothesis was tested by iodine staining of intracellular glycogen, a well-established inverse-proportional in vivo measurement of intracellular cAMP levels (5). Compared with the wild-type strain, both of the single mutants, gpa2Δ and ras2Δ, respectively, showed only slightly darker staining (i.e. reduced intracellular cAMP levels; Fig. 3). For the ras2Δ strain this result was expected and is consistent with previous studies. The double mutant gpa2Δ,ras2Δ, however, showed very dark staining indicating very low intracellular cAMP levels; this observation demonstrates a strong synthetic effect of both mutations.

The correlation between dark iodine staining of the double mutant and low intracellular cAMP levels was independently validated by further deleting the PDE2 gene. This gene encodes a phosphodiesterase that inactivates cAMP by cleavage. The absence of Pde2p leads to an increase in intracellular cAMP levels (16, 17). Indeed, after iodine staining, the triple mutant gpa2Δ,ras2Δ,pde2Δ appeared as the wild-type strain (Fig. 3).

The pde2 deletion within the gpa2Δ,ras2Δ strain also augmented cell growth to wild-type levels (Fig. 4a). These results clearly illustrate the necessary involvement of Gpa2p in positively regulating cAMP levels in the absence of Ras2p.

To evaluate whether the absence of Pde2p would also facilitate the diploid gpa2Δ,ras2Δ double mutant to form filaments under nitrogen starvation conditions, we next constructed a diploid homozygous triple mutant, gpa2Δ,ras2Δ,pde2Δ. Fig. 4b shows the dramatic effect of a pde2 deletion within the gpa2Δ,ras2Δ double deletion strain. As predicted, pseudohyphal development was completely restored, resulting in filaments similar to wild-type filaments. Importantly, a pde2 deletion also reversed the effect of the gpa2Δ and ras2Δ single mutants with respect to pseudohyphal development (data not shown).

Taken together, the above observations indicate that both phenotypes of Gpa2p-deficient yeast strains, (i) a defect in pseudohyphal development in diploid cells and (ii) a strong growth defect in ras2 deleted cells, are due to alterations in the regulation of cAMP levels.

Several genetic and biochemical studies in S. cerevisiae have demonstrated that externally added cAMP is taken up by yeast cells and can induce cAMP-dependent signals (19, 20). Fig. 5 shows that upon addition of exogenous cAMP to the growth medium the gpa2Δ mutant strain behaved as the wild-type strain, i.e. dramatically rescuing the defect in pseudohyphal development. Note that both the gpa2Δ mutant and the wild-type strain are auxotrophic for histidine, and therefore all colonies on low nitrogen plates represent gpa2Δ,ras2Δ double mutants and big colonies are wild-type or single mutants as assessed by the presence of nutritional markers.
type strain formed filaments in the presence of cAMP (Fig. 5, lower panels), while only the wild-type strain showed filamentous growth without added cAMP (Fig. 5, upper panels). These results provide additional evidence that Gpa2p normally modulates pseudohyphal development by positively regulating cAMP levels within the yeast cell.

CONCLUSIONS

The present genetic evidence suggests that Gpa2p plays a role in the normal regulation of cAMP levels in yeast. We demonstrate that this regulation is important for the induction of pseudohyphae during nitrogen starvation conditions. However, on rich medium (YPD), Gpa2p is only necessary for normal cAMP levels and, thus, not sufficient to ensure wild-type-like growth. These observations argue that Gpa2p is responsible for inducing cAMP levels in a ras2 deletion strain to a considerable amount, and not only Ras1p. In addition, under low nitrogen conditions, cAMP levels regulated by Ras2p are not sufficient to induce filament formation. The presence of Gpa2p is necessary, in addition to Ras2p, to induce this dimorphic switch.

These observations suggest a new pathway in yeast by which intracellular levels of cAMP are positively regulated by a G-protein α-subunit. This pathway may be analogous to the known Gαs-mediated pathway in mammalian cells. Whether Gpa2p regulates cAMP levels through a direct or indirect interaction with adenylyl cyclase remains to be elucidated. In this regard, heterologous expression of mammalian G-protein α-subunits in these yeast GPA2 deletion strains will provide an invaluable tool for the mutational analysis of mammalian G-protein function in a unique in vivo null setting.

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FIG. 3. Iodine staining of wild-type and various mutant strains. A haploid wild-type, a gpa2Δ, a ras2Δ, a gpa2Δras2Δ, and a gpa2Δras2Δ,pde2Δ strain were grown on rich medium plates for 2 days before staining with iodine and photographed.

FIG. 4. The absence of Pde2p suppresses the phenotypes associated with a gpa2 deletion mutation. a, suppression of the synthetic growth phenotype of a gpa2Δras2Δ double mutant by introducing a pde2 deletion mutation. Designated strains were grown on rich medium for 3 days before photographic image was taken. b, suppression of the filamentous growth defect of a gpa2Δras2Δ strain by introducing a pde2 deletion mutation. The assay was performed as described in the legend to Fig. 1.

FIG. 5. Externally added cAMP rescues the gpa2 mutant phenotype. a, gpa2 mutant strain and a wild-type strain were tested for filament formation on medium containing 5 mM cAMP (lower panels) or without the addition of cAMP (upper panels). The assay was performed as described in the legend to Fig. 1. Note that both the gpa2Δ mutant and the wild-type strain formed filaments in the presence of cAMP, while only the wild-type strain showed filamentous growth without added cAMP. Addition of cAMP was performed by streaking a 100 mg/ml solution of the sodium salt of cAMP (Sigma) in sodium phosphate buffer (pH 6.5) onto the assay plates to obtain a final concentration of 5 mM (19, 20). Sodium phosphate buffer was added to the control plates.
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