DOI: 10.1534/genetics.105.042796

Multiple Roles of a Heterotrimeric G-Protein γ-Subunit in Governing Growth and Development of *Aspergillus nidulans*

Jeong-Ah Seo,* Kap-Hoon Han*,[1] and Jae-Hyuk Yu*,†,[2]

**Department of Food Microbiology and Toxicology and Food Research Institute,* †*Molecular and Environmental Toxicology Center, University of Wisconsin, Madison, Wisconsin 53706*

Manuscript received March 1, 2005
Accepted for publication April 27, 2005

ABSTRACT

Vegetative growth signaling in the filamentous fungus *Aspergillus nidulans* is primarily mediated by the heterotrimeric G-protein composed of FadA (Gα), SfaD (Gβ), and a presumed Gγ. Analysis of the *A. nidulans* genome identified a single gene named *gpgA* encoding a putative Gγ-subunit. The predicted GpgA protein consists of 90 amino acids showing 72% similarity with yeast Ste18p. Deletion (Δ) of *gpgA* resulted in restricted vegetative growth and lowered asexual sporulation. Moreover, similar to the Δ*sfaD* mutant, the Δ*gpgA* mutant was unable to produce sexual fruiting bodies (cleistothecia) in self-fertilization and was severely impaired with cleistothecial development in outcross, indicating that both SfaD and GpgA are required for fruiting body formation. Developmental and morphological defects caused by deletion of *flbA* encoding an RGS protein negatively controlling FadA-mediated vegetative growth signaling were suppressed by Δ*gpgA*, indicating that GpgA functions in FadA-SfaD-mediated vegetative growth signaling. However, deletion of *gpgA* could not bypass the need for the early developmental activator FluG in asexual sporulation, suggesting that GpgA functions in a separate signaling pathway. We propose that GpgA is the only *A. nidulans* Gγ-subunit and is required for normal vegetative growth as well as proper asexual and sexual developmental progression.

HETEROTRIMERIC G-proteins (G-proteins) consisting of α, β, and γ-subunits relay and propagate signals sensed by membrane-bound G-protein-coupled receptors (GPCRs) to a diverse group of regulatory proteins (effectors). Upon activation by agonists, GPCRs undergo conformational changes that promote the GDP-to-GTP exchange of the Gα-subunit. This exchange provokes the dissociation of GTP-Gα from the Gβγ heterodimer, and GTP-Gα, Gβγ, or both can mediate signals by modulating effectors. The signal is turned off when GTP is hydrolyzed to GDP, resulting in the formation of the inactive heterotrimer Gα-GDP:Gβγ. The rates of GTP hydrolysis by the intrinsic GTPase activity of the Gα-subunit determine the lifetime of the active G-proteins and thereby the intensity of the signal (reviewed in Morris and Malbon 1999).

A G-protein (FadA) in the filamentous fungus *Aspergillus nidulans* was first identified by studying a dominant activating mutation that caused undifferentiated hyphal growth followed by autolysis, *i.e.*, a "fluffy autolytic" phenotype (Yu *et al.* 1996). Genetic studies revealed that activated GTP-FadA (Gα) mediates signaling that promotes vegetative growth and inhibits both asexual and sexual development as well as production of the mycotoxin sterigmatocystin (ST; Yu *et al.* 1996; Hicks *et al.* 1997). This FadA signaling is negatively controlled by a *r*egulator of *G*-protein *s*ignaling (RGS) protein called FlbA, which is proposed to function by enhancing the intrinsic GTPase activity of FadA (Yu *et al.* 1996). Loss of *flbA* function results in fluffy-autolytic phenotypes similar to those caused by constitutively active FadA mutant alleles (Lee and Adams 1994a; Yu *et al.* 1996, 1999; Wieser *et al.* 1997). As if FadA is the primary target of FlbA function, the deletion (Δ) or dominant negative (G203R) FadA mutations suppressed the fluffy-autolytic phenotype caused by Δ*flbA* and restored asexual development (conidiation) and ST production (Yu *et al.* 1996; Hicks *et al.* 1997).

In addition to *fadA*, four other suppressor loci bypassing the requirement of *flbA* in conidiation have been previously isolated (Yu *et al.* 1999). One of the suppressors, *sfaD*, was found to encode a Gβ-subunit with the central conserved Trp-Asp sequence that is referred to as the "WD-40" motif (Rosén *et al.* 1999). SfaD is required for normal vegetative growth and proper downregulation of conidiation, as well as formation of sexual fruiting bodies (Rosén *et al.* 1999). The fact that Δ*sfaD* suppressed the Δ*flbA*-induced fluffy-autolytic phenotype led us to propose that SfaD (with the presumed Gγ-subunit) functions in vegetative growth signaling that is negatively regulated by FlbA. However, elimination of FadA or SfaD could not bypass the need for *fluG* in conidiation, where FluG is proposed to trigger

[1]*Present address:* Department of Biochemistry, Pai-Chai University, Dae-Jeon, Korea 302-735.

[2]*Corresponding author:* Department of Food Microbiology and Toxicology, 1925 Willow Dr., Madison, WI 53706-1187. E-mail: jyu1@wisc.edu

conidiation-specific events and to (indirectly) activate FlbA (Lee and Adams 1994a,b, 1996; Yu *et al.* 1996). Taken together, we proposed that two antagonistic signaling pathways govern growth and asexual development of *A. nidulans* and that FlbA plays a pivotal role in fine-tuning the degree of FadA-SfaD-mediated vegetative growth signaling to allow both asexual and sexual development to occur.

As has been found for all eukaryotes (for review see Morris and Malbon 1999), it is presumed that the Gβ-subunit (SfaD) functions as a heterodimer with the cognate Gγ-subunit in *A. nidulans*. Recently, a putative Gγ-subunit (GNG-1) was identified and shown to form the heterodimer with a Gβ-subunit (GNB-1) in the filamentous fungus *Neurospora crassa* (Krystofova and Borkovich 2005). This GNB-1::GNG-1 heterodimer is found to be necessary for normal female fertility, asexual development, and Gα-protein levels. In this study, we report the identification and characterization of a gene (*gpgA*) encoding a putative Gγ-subunit in *A. nidulans*. Gene disruption, genetic, and expression studies indicate that GpgA is required for normal vegetative growth and developmental progression. We found that deletion of *gpgA* resulted in reduced vegetative growth and highly elevated formation of Hülle cells (sexual-development-specific cells) similar to that caused by Δ*sfaD*. As if GpgA functions in the FadA-SfaD-mediated vegetative growth signaling pathway, deletion of *gpgA* suppressed the fluffy-autolytic phenotype resulting from Δ*flbA* and restored conidiation at the wild-type level. No mutations were identified in the *gpgA* gene region of the three previously isolated, yet unidentified, *flbA* suppressors (*sfaA*, *sfaC*, and *sfaE*; Yu *et al.* 1999), indicating that *gpgA* defines the sixth *flbA*-suppressor locus. As with Δ*sfaD*, deletion of *gpgA* caused severely impaired sexual fruiting body formation. We present a model for the roles of GpgA in controlling vegetative growth and development.

MATERIALS AND METHODS

Aspergillus strains, culture conditions, and phenotypic characterization: *A. nidulans* strains used in this study are listed in Table 1. Genetic, culture, and transformation techniques were similar to those described previously (Pontecorvo *et al.* 1953; Käfer 1977; Seo *et al.* 2003). Liquid and solid minimal media with the supplements (simplified as MM) were prepared as described (Käfer 1977). All fungal strains were incubated at 37° and liquid submerged cultures were done at 250 rpm. Measurements of growth rates, dry weight, and sporulation levels were done in triplicate cultures in MM and MM with 0.1% yeast extract (YM). Radial growth rates were determined every 24 hr for 7 days by measuring colony hyphal extension of individual strains point inoculated on solid medium (both MM and YM). Dry weight of each strain was measured as previously described (Rosén *et al.* 1999). The numbers of conidia and Hülle cells (per plate) were determined for 5-day cultures of each strain on solid MM or YM. Briefly, 20 ml of 0.01% Tween 80 was added and conidiophores and Hülle cell aggregates were loosened from the agar surface with a glass rod, thoroughly homogenized by a Dounce homogenizer, and filtered through Miracloth (Calbiochem, La Jolla, CA). Conidia or Hülle cells were counted using a hemocytometer. Genetic crosses were carried out on specialized medium containing 20 mm glycine and 2% glucose (D'Souza *et al.* 2001). From each cross >20 progeny were selected and analyzed for relevant genotypes by PCR using the oligonucleotides described in Table 2. Differences in size and restriction enzyme digestion patterns of the individual amplicons were used to determine genotypes of various mutants described in this study.

Examination of sexual development was carried out in three ways: (1) $\sim 10^6$ conidia of relevant strains were spread onto MM and the plates were sealed with plastic films and incubated at 37° for ~7–10 days in the dark; (2) conidia of appropriate strains were point inoculated at the center of solid MM and incubated at 37° for 2–3 days and then the plates were sealed and further incubated at 37° for 7 days in the dark; and (3) 5×10^5 conidia/ml were inoculated into 100 ml liquid MM in 250-ml flasks and incubated at 37°, 250 rpm for 18 hr. Mycelia were then collected by filtering through Miracloth (Calbiochem) and transferred to solid MM. The plates were sealed and incubated at 37° for 7 days in the dark.

For Northern blot analyses, samples from vegetative growth and postdevelopmental induction cultures were collected as described (Han *et al.* 2004a). Briefly, 5×10^7 conidia of relevant strains were inoculated in 100 ml liquid MM with 0.1% yeast extract in 250-ml flasks and incubated at 37°, 250 rpm. For vegetative growth phases, samples were collected at designated time points of liquid submerged cultures, squeeze dried, and stored at −80° until subjected to total RNA isolation. For sexual and asexual developmental induction, 18-hr vegetatively grown mycelia were transferred to solid MM and the plates were either air exposed for asexual developmental induction or tightly sealed and blocked from light for sexual developmental induction.

Construction of the *gpgA* deletion mutant: The *gpgA* deletion cassette was constructed via multiple cloning processes because of the incomplete development of a PCR-assisted technique at that time (Yu *et al.* 2004). The 5′ (590 bp) and 3′ (597 bp) flanking regions of the *gpgA* open reading frame (ORF) were amplified with primer pairs of OJA22-OJA27 and OJA23-OJA28, respectively, and the amplicons were digested with *Xho*I. These restricted 5′ and 3′ flanking DNA fragments were ligated to give rise to a ~1.2-kb joined fragment (Δ*gpgA*), which was cloned into the pGEM-T Easy vector (Promega, Madison, WI). The *argB*$^+$ marker (~1.8 kb) was generated by cutting the *argB*$^+$ plasmid pJW88 (J. K. Wieser and T. H. Adams, unpublished data) with *Xho*I and ligated with the *Xho*I-cut Δ*gpgA*/pGEM-T Easy plasmid. The final Δ*gpgA* construct (pJAG2) was composed of a 5′ (590-bp) flanking region, *argB*$^+$ (~1.8 kb), and a 3′ flanking region (597 bp) in the pGEM-T Easy vector. This pJAG2 plasmid was directly introduced to *A. nidulans* RMS011 to generate TJAG2.7 (Table 1). The Δ*gpgA* genotype was confirmed by PCR amplification of the *gpgA* coding region with a primer pair of OJA26 and OJA29 followed by restriction enzyme digestion of the amplicons and Southern blot analysis (see Yu *et al.* 2004). Phenotypic alterations caused by Δ*gpgA* were 100% linked with the Δ*gpgA* PCR amplicon size and digestion patterns.

Nucleic acid manipulation: Genomic DNA or total RNA isolation and Northern blot analyses were carried out as previously described (Seo *et al.* 2003; Han *et al.* 2004a). The DNA probes used to examine mRNA levels of *gpgA* were prepared by PCR amplification of the coding region of *gpgA*, using genomic DNA of FGSC4 as a template with OJA24 and OJA25 (Table 2). Genotypes of double-deletion mutants of

TABLE 1

***A. nidulans* strains used in this study**

Strain	Genotype[a]	Source
FGSC4	*veA*+	FGSC[b]
RMS011	*pabaA1*, *yA2*; Δ*argB::trpC*$^+$; *trpC801*	FGSC
RJA56.25	*pabaA1*, *yA2*	Seo *et al.* (2004)
RJY1.12	*pabaA1*, *yA2*, *flbA98*; *methG1*; *sfaA1*	Yu *et al.* (1999)
RJY67.3	*pabaA1*, *yA2*, *flbA98*; *argB2*; *methG1*; *sfaC67*	Yu *et al.* (1999)
RJY83.21	*pabaA1*, *yA2*, *flbA98*; *argB2*; *methG1*; *sfaE83*	Yu *et al.* (1999)
RJA71.4	*pabaA1*, *yA2*; Δ*fadA::argB*$^+$	This study
RJA5.9	*pyrG89*, Δ*flbA::argB*$^+$; *pyroA4*	This study
RJA4.4	*pyrG89*, *yA2*; Δ*fluG::trpC*$^+$	Seo *et al.* (2003)
RJA20.12	*pabaA1*, *yA2*; Δ*fluG::trpC*$^+$	This study
RJA15.48	*biA1*, Δ*flbA::argB*$^+$; Δ*fadA::argB*$^+$	This study
RSRFA.1	*pabaA1*, *biA1*; *methG1*; Δ*fadA::argB*$^+$, Δ*sfaD::argB*$^+$	S. Rosén (unpublished data)
TJAG2.7	*pabaA1*, *yA2*; Δ*argB::trpC*$^+$; Δ*gpgA::argB*$^+$; *trpC801*	This study
RJAG19.6[c]	*pabaA1*, *yA2*; Δ*gpgA::argB*$^+$	This study
RJA55.4	*pabaA1*, *yA2*; Δ*gpgA::argB*$^+$; Δ*sfaD::argB*$^+$	This study
RJA71.57	*pabaA1*, *yA2*, Δ*flbA::argB*$^+$; Δ*gpgA::argB*$^+$	This study
RJA41.18	Δ*fluG::trpC*$^+$; Δ*gpgA::argB*$^+$	This study
RSRB1.15	*biA1*; Δ*sfaD::argB*$^+$	S. Rosén (unpublished data)
RJA55.11	*pabaA1*, *yA2*; Δ*sfaD::argB*$^+$	This study
RSRF1.34	*biA1*, Δ*flbA::argB*$^+$; *methG1*; Δ*sfaD::argB*$^+$	S. Rosén (unpublished data)
RSR61.2	*pabaA1*, *yA2*; Δ*fluG::trpC*$^+$; Δ*sfaD::argB*$^+$	This study

[a] All strains carry the *veA1* mutation except FGSC4.
[b] Fungal Genetics Stock Center.
[c] RJAG19.6, -19.8, and -19.9 are isogenic and were crossed with Δ*sfaD* and Δ*fadA*Δ*flbA*. These strains were examined for growth rates and dry weight.

Δ*gpgA*, Δ*fadA*, Δ*sfaD*, Δ*flbA*, and Δ*fluG* were confirmed by PCR amplification of the coding region of individual genes. For instance, the primer pair OJA24 and OJA25 were used to differentiate the deletion or wild-type *gpgA* alleles present in the progeny of crosses and the amplicons of Δ*gpgA* and *gpgA*$^+$ were ~2.0 kb and ~0.6 kb, respectively. The PCR products from progeny analyses were confirmed by restriction enzyme digestion of the amplicons.

Microscopy: Colonies were photographed with a SONY digital camera (DSC-F707). Photomicrographs were taken using an Olympus BH2 compound microscope installed with a Kodak MDS290 digital camera.

TABLE 2

Oligonucleotides used in this study

Oligo	Sequence	Position/purpose
OJA22	atc gtc agc cgt tga tga gc	606 bp upstream of the *gpgA* ATG
OJA27	**CCG** **ctc** gag agg tcg cga gcg ga	5′ flanking region of the *gpgA* ORF for *gpgA* deletion with *Xho*I tail
OJA23	gct cgt aga tac tag tgt ag	573 bp downstream of the *gpgA* stop
OJA28	**CCG** **ctc** gag gct gct gta cga tca t	3′ flanking region of *gpgA* ORF for *gpgA* deletion with *Xho*I tail
OJA24	ccg gaa gcg caa tca atc	sequencing the *gpgA* ORF, RT-PCR product and *gpgA* deletion confirmation
OJA25	tag cgg ccg ggg gta tca	Sequencing the *gpgA* ORF, RT-PCR product, and *gpgA* deletion confirmation
OJA26	ctg caa gac gct tga act g	3′ of the *gpgA* ORF
OJA29	cct gca tta gat gct tta	5′ of the *gpgA* ORF
OKH43	gcc aca ttc acg ata gcc	5′ of the *fadA* ORF
OKH44	atg aaa gtc tca acg cca	3′ of the *fadA* ORF
OKH01	gaa aac cac caa cag tgc	5′ of the *sfaD* ORF
OKH02	ttc ttt cca gat gat ccg	3′ of the *sfaD* ORF
OJA133	gag att cga gcc tgt gc	5′ of the *flbA* ORF
OJA134	ctg tca tga acg ttg tg	3′ of the *flbA* ORF
OYG4	tca caa cct cct ctc tca g	5′ of the *fluG* ORF
OYG5	ttg aac ggc tca cgg tac ta	3′ of the *fluG* ORF

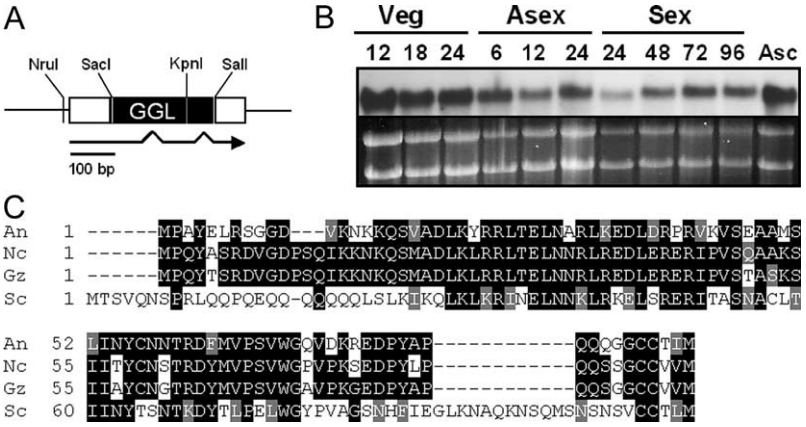

FIGURE 1.—Summary of GpgA structure. (A) A partial restriction map of a *gpgA* region is shown. The GpgA ORF (open box) and introns (discontinuities of the arrow) were determined by RT-PCR followed by sequencing. The solid box presents the *G*-protein *g*amma-*l*ike domain (GGL). (B) The steady-state *gpgA* mRNA levels in various growth and development stages of wild type (FGSC4) are shown. Numbers indicate incubation time (hours) in liquid submerged culture (Veg) or hours after developmental induction for asexual (Asex) or sexual (Sex) sporulation. Asc stands for ascospores (sexual spores). (C) Alignment of *A. nidulans* (An) GpgA with putative Gγ-subunits of *Neurospora crassa* (Nc; GNG-1, NCU00042.1), *Gibberella zeae* (Gz; XP_387411.1), and *Saccharomyces cerevisiae* (Sc; Ste18p, GI:6322545) is shown. Alignment was carried out via ClustalW with a default setting and displayed using BoxShade (identical and similar amino acids are in solid and shaded areas, respectively).

RESULTS

Identification of *gpgA* encoding a putative Gγ-subunit: TBLASTN search of the *A. nidulans* genome (the Broad Institute: http://www.broad.mit.edu/annotation/fungi/aspergillus/index.html/) with the yeast Gγ-subunit Ste18p (WHITEWAY *et al.* 1989) identified a single gene showing 72% similarity with Ste18p. We designated this gene as *gpgA* for a *G-p*rotein *g*amma subunit (mapped on chromosome VI; the Broad Institute). Direct sequencing of a reverse transcription PCR (RT-PCR) amplicon of *gpgA* revealed that it encodes an ORF of 391 bp with two short introns (68 and 48 bp) and the predicted GpgA protein consists of 90 amino acids (Figure 1A). It should be noted that GpgA is slightly different from the hypothetical protein AN2742.2 (XP_406879.1) annotated by the Broad Institute in that AN2742.2 is 95 aa long and its stop codon is located at 51 bp downstream of the GpgA stop and the ORF is intervened by three introns (68, 48, and 36 bp).

The predicted GpgA protein has a typical G-protein gamma-like domain (Figure 1A; analyzed by SMART: http://smart.embl-heidelberg.de) with a highly probable (95% likelihood) coiled-coil domain at ~20–40 aa (http://www.ch.embnet.org/software/COILS_form.html). Furthermore, GpgA has the conserved Ste18p-like Cys-A-A-X box, where A stands for any aliphatic amino acids. As with other fungal Gγ-subunits, the CTI*M* aa sequence at the C-terminal end of GpgA indicates that it is likely to be farnesylated (SINENSKY 2000). Northern blot analysis demonstrated that ~1.7-kb *gpgA* mRNA levels are constantly high throughout the life cycle of *A. nidulans* (Figure 1B). Besides Ste18p, GpgA shows high levels of similarity with a putative Gγ-protein of *N. crassa* (GNG-1; 93 aa; 75% similarity) and with *Gibberella zeae* (XP_387411.1; 93 aa; 75% similarity; Figure 1C). Perhaps not surprisingly, GpgA shows 99% identity and 100% similarity with the corresponding Gγ-subunit of *A. fumigatus* [The Institute for Genomic Research (TIGR): http://www.tigr.org/tdb/e2k1/afu1/].

GpgA is required for normal conidiation: To characterize functions of this putative Gγ-subunit, we generated the Δ*gpgA* mutant by replacing its ORF with the *argB*$^{+}$ marker. The Δ*gpgA* mutant (multiple strains tested: Table 1, RJAG19.6, -19.8, and -19.9) exhibited not only reduced vegetative growth (Figures 2 and 3, see below) but also delayed-conidiation phenotypes. The Δ*gpgA* mutant displayed a nonconidial fluffy phenotype for 2–3 days of growth (see Figure 2) before the production of conidiophores from the center of the colony, resulting in a slightly reduced number of conidia (per colony) in the Δ*gpgA* mutant (Figure 3). On the contrary, levels of Hülle cell production in the Δ*gpgA* mutant were much higher (~2.26×10^6/ml) than those in wild type (WT) or other strains (Figures 2 and 3).

To further understand effects of deletion of *gpgA* on conidiation, we examined the ability of the selected mutants to elaborate conidiophores in liquid submerged culture. We found that, while the Δ*sfaD* and Δ*gpgA*Δ*sfaD* mutants produced conidiophores within 22 hr of liquid submerged culture, neither the Δ*gpgA* mutant nor WT produced conidiophores (Figure 4). Taken together, these results suggest that SfaD (Gβ) plays a crucial role in negative regulation of conidiation under submerged culture conditions (ROSÉN *et al.* 1999; HAN *et al.* 2004b) and GpgA may not be involved in this controlling process (see DISCUSSION).

Deletion of *gpgA* causes severe impairment in fruiting body formation: Our previous study showed that mutational inactivation of SfaD had profound negative effects on sexual fruiting body (cleistothecium) formation (ROSÉN *et al.* 1999). To examine the possible role of GpgA in sexual development, we determined levels of Hülle cells and cleistothecia production in the Δ*gpgA* mutant. Despite elevated Hülle cell formation, the Δ*gpgA* mutant was unable to produce cleistothecia under

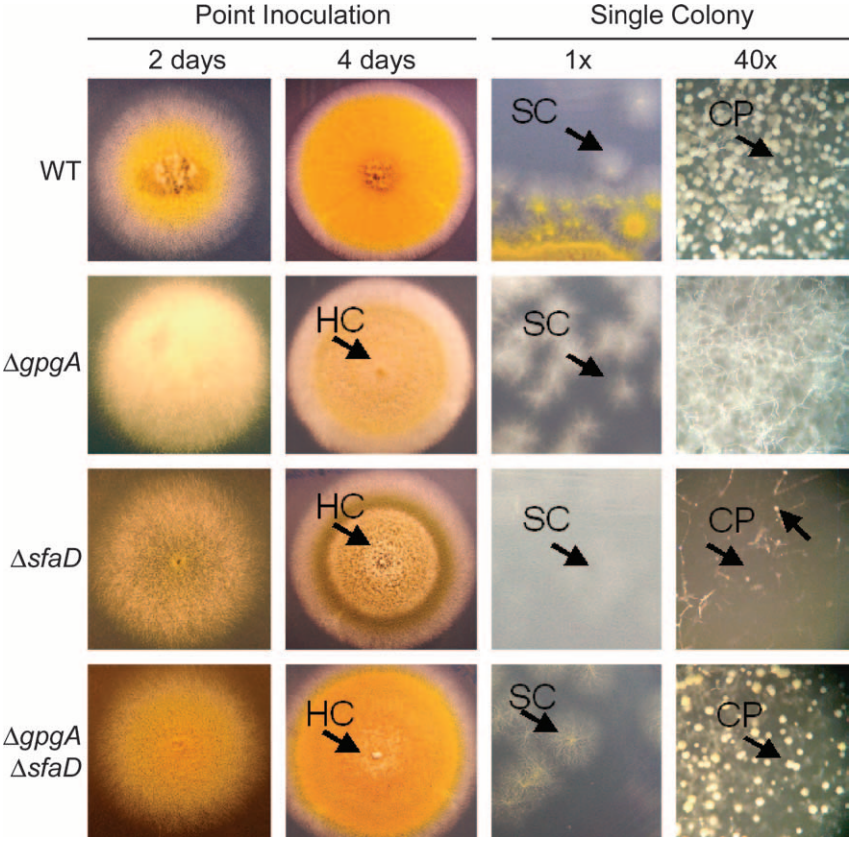

FIGURE 2.—Phenotypes of the mutant colonies. Relevant mutant and wild-type (WT) strains were point inoculated or streaked onto solid medium. The Δ*gpgA* mutant (RJAG19.9) exhibited a fluffy nondevelopmental phenotype for 2–3 days and then produced both conidiophores (CP) and Hülle cells (HC). The Δ*gpgA* mutant produced fewer asexual spores but more Hülle cells than the Δ*sfaD* (RSRB1.15) or Δ*gpgA*Δ*sfaD* (RJA55.4) mutants. However, compared to WT, the Δ*gpgA*, Δ*sfaD*, and Δ*gpgA*Δ*sfaD* mutants produced higher numbers of Hülle cells (HC). As evident in the close-up view of a single colony (SC) originated from a single conidium, the Δ*gpgA* mutant colonies do not produce conidiophores at this time (48 hr).

self-fertilizing (homothallic) conditions. These phenotypes are almost identical to those caused by Δ*sfaD* (ROSÉN *et al.* 1999). Furthermore, deletion of *sfaD* or *gpgA*, but not *fadA*, resulted in severely impaired cleistothecia formation in outcrosses with WT or other mutants. This trait made genetic analyses of *gpgA* (and *sfaD*) extremely difficult. In most cases, while heterokaryotic mycelia could be formed between two strains, no cleistothecia were developed. Due to this (semi-) dominant nature of Δ*gpgA* or Δ*sfaD*, the Δ*gpgA*Δ*sfaD*, Δ*gpgA*Δ*fadA*, and Δ*gpgA*Δ*flbA* mutants were generated by repeated (approximately five to eight times) meiotic crosses of various (~8–22 different) combinations of multiple Δ*gpgA*, Δ*sfaD*, and Δ*flbA*Δ*fadA* mutant strains (only representative strains are shown in Table 1). From these crosses only a few cleistothecia were produced and isolated. Collectively, as with SfaD (and possibly FadA), GpgA is required for normal sexual fruiting body formation.

GpgA is required for normal vegetative growth: Our hypothesis was that a cognate Gγ-subunit forms the heterodimer with SfaD and functions in vegetative growth signaling. The role of GpgA in vegetative growth signaling was examined by determining growth rates of the Δ*gpgA* (RJAG19.9), Δ*sfaD* (RSRB1.15), Δ*fadA* (RJA71.4), and Δ*gpgA*Δ*sfaD* (RJA55.4) mutants (see Table 1). Although Δ*gpgA* did not clearly affect radial growth rates on solid medium, Δ*gpgA* and Δ*gpgA*Δ*sfaD* caused significantly reduced vegetative growth in liquid submerged culture, where hyphal branching and extension were reduced. Again, these phenotypes were almost identical to those caused by Δ*sfaD*. Dry weights of the Δ*gpgA* (RJAG19.9) and Δ*gpgA*Δ*sfaD* (RJA55.4) mutants grown in liquid MM were only 34 and 27% of that of WT, respectively (Figure 3). These are comparable with dry weights of the Δ*fadA* and Δ*sfaD* mutants grown in liquid MM, *i.e.*, ~10–35% of that of WT.

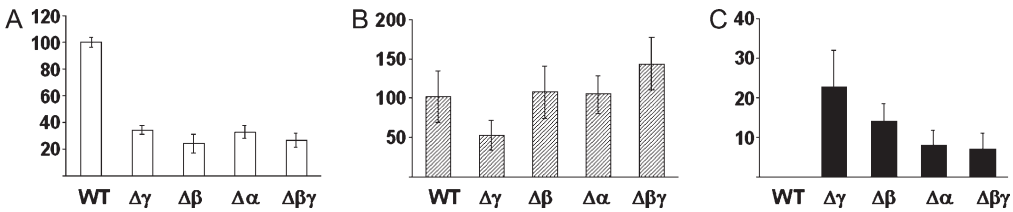

FIGURE 3.—Effects of G-protein mutations on vegetative growth and development. (A) Dry weights (percentage) of WT and G-protein mutant strains grown in liquid MM for 24 hr as well as numbers of (B) conidia ($\times 10^6$/plate) and (C) Hülle cells ($\times 10^5$/plate) produced by the colonies of designated strains grown on solid MM for 5 days are presented (average of triplicate cultures/measurements with standard error bars). Strains shown are WT (RJA56.25), Δγ (Δ*gpgA*; RJAG19.9), Δβ (Δ*sfaD*; RSRB1.15), Δα (Δ*fadA*; RJA71.4), and Δβγ (Δ*sfaD*Δ*gpgA*; RJA55.4).

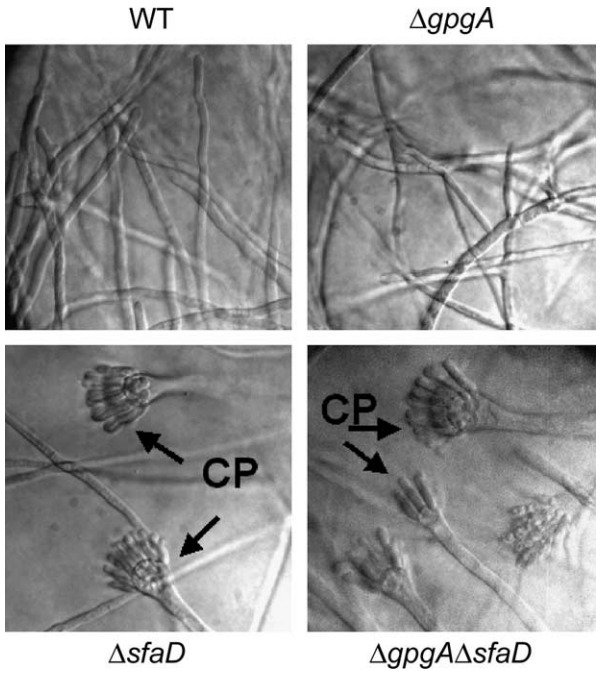

Figure 4.—Submerged conidiophore development caused by Δ*sfaD* but not by Δ*gpgA*. Conidiophore (CP) formation of WT and designated mutant strains grown in liquid submerged culture was observed from ~17 to 36 hr at 1-hr intervals. Whereas Δ*gpgA* (RJAG19.9) or WT (RJA56.25) strains did not produce conidiophores even at 36 hr, Δ*sfaD* (RSRB1.15) and Δ*gpgA*Δ*sfaD* (RJA55.4) strains began to elaborate conidiophores (CP) as early as 18 hr. The photographs were taken at 22 hr of growth in YM.

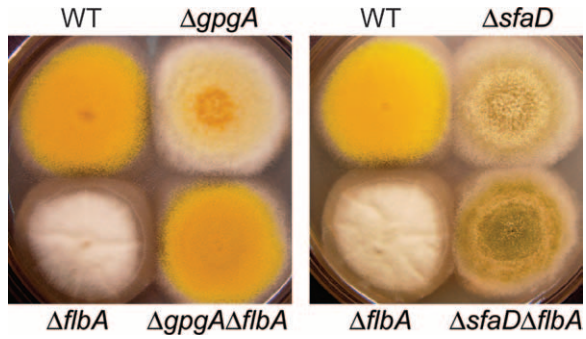

Figure 5.—Deletion of *gpgA* restores conidiation in the Δ*flbA* mutant. WT (RJA56.25), Δ*gpgA* (RJAG19.9), Δ*sfaD* (RSRB1.15), Δ*flbA* (RJA5.9), Δ*gpgA*Δ*flbA* (RJA71.57), and Δ*sfaD*Δ*flbA* (RSRF1.34) strains were point inoculated on solid MM and incubated at 37° for 3 days. Note that the Δ*gpgA*Δ*flbA* and Δ*sfaD*Δ*flbA* mutant colonies restored conidiation and no longer exhibited fluffy-autolytic phenotypes.

Collectively, these results suggest that GpgA likely functions in FadA/SfaD-mediated vegetative growth signaling.

Deletion of *gpgA* bypasses the need for *flbA* in conidiation: To further provide genetic evidence of the involvement of GpgA in FadA/SfaD-mediated vegetative growth signaling, we generated the Δ*gpgA*Δ*flbA* double mutant. It should be noted that deletion of *fadA* and/or *sfaD* could suppress the fluffy-autolytic phenotype caused by Δ*flbA* (Yu *et al.* 1996; Rosén *et al.* 1999). Theoretically, if GpgA is the Gγ-subunit forming a heterodimer with SfaD and if SfaD-GpgA interaction is required for vegetative growth signaling, then the absence of GpgA function should suppress uncontrolled activation of vegetative growth caused by Δ*flbA*. We found that, as with Δ*fadA* or Δ*sfaD*, deletion of *gpgA* suppressed fluffy-autolytic and developmental defect phenotypes of the Δ*flbA* mutant in that the Δ*gpgA*Δ*flbA* mutant recovered conidiation at the WT level (Figure 5). These results indicate that GpgA functions in vegetative growth signaling controlled by FlbA.

The *gpgA* gene defines the sixth *flbA* suppressor: Previously, five *flbA* loss-of-function suppressor loci (~*sfaA*–*sfaE*) were isolated (Yu *et al.* 1999). Among these, *sfaB* and *sfaD* defined FadA and a Gβ-subunit, respectively (Rosén *et al.* 1999; Yu *et al.* 1999). The fact that deletion of *gpgA* eliminated the need for *flbA* in conidiation led us to test whether *gpgA* could identify one of the *sfaA1*, *sfaC67*, or *sfaE83* mutations. A region of the *gpgA* gene including its promoter (~1 kb upstream of the ATG), ORF, and terminator (~0.5 kb) was PCR amplified using the individual suppressor mutant genomic DNA as template and the resulting amplicons were directly sequenced. No mutations were identified in those amplicons, suggesting that the *gpgA* gene defines the sixth suppressor of *flbA* loss-of-function. This result confirms the prediction that the previous *flbA* suppressor screenings did not reach saturation (Yu *et al.* 1999). The small size of the *gpgA* ORF (391 bp) probably reduced the probability of introducing mutation(s) in the *gpgA* region via random chemical mutagenesis carried out in the previous study.

FluG is required for conidiation in the absence of *gpgA*: FluG is an early developmental regulator that is required for activation of downstream conidiation-specific events (reviewed in Adams *et al.* 1998). Previously, we showed that, while the deletion or dominant interfering mutation (G203R) of *fadA* mutation or Δ*sfaD* restored conidiation in the Δ*flbA* mutant, these mutations could not eliminate the need for FluG in conidiation (Yu *et al.* 1996; Rosén *et al.* 1999). To test the requirement of FluG in conidiation in the absence of GpgA functions, we constructed the Δ*gpgA*Δ*fluG* mutant and found that deletion of *gpgA* could not suppress conidiation defects caused by Δ*fluG* (Figure 6), supporting that the FadA/SfaD/GpgA vegetative growth signaling pathway and the asexual developmental cascade activated by FluG are separate and independent (see model in Figure 7). Moreover, the facts that Δ*fluG* eliminated Hülle cell production in the Δ*sfaD* and Δ*gpgA* mutants indicate that enhanced vegetative growth by Δ*fluG* might be sufficient to block inappropriate Hülle cell production caused by the absence of Gβγ functions.

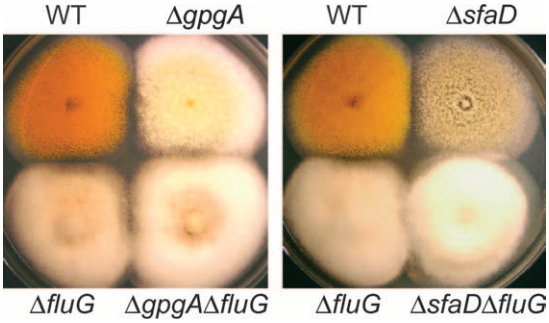

FIGURE 6.—Deletion of *gpgA* cannot bypass the need for *fluG* in conidiation. Designated strains were point inoculated on solid YM and incubated at 37° for 3 days. The Δ*gpgA*Δ*fluG* (RJA41.18) and Δ*sfaD*Δ*fluG* (RSR61.2) mutants were unable to produce conidiophores and formed fluffy colonies almost identical to those of the Δ*fluG* (RJA20.12) mutant.

DISCUSSION

With the available *A. nidulans* genome, we have identified and characterized a putative G-protein γ-subunit in *A. nidulans*. Although the Gγ-subunits have been known to be more diverse than the Gβ-subunits (MORRIS and MALBON 1999), GpgA, Ste18p, and other putative fungal Gγ-subunits including recently reported GNG-1 in *N. crassa* (KRYSTOFOVA and BORKOVICH 2005) share high levels of similarity (Figure 1C). Furthermore, GpgA contains a coiled-coil domain at the N-terminal region, which is shown to be necessary for the interaction of a Gγ with the cognate Gβ to form a heterodimer (for review see CABRERA-VERA *et al.* 2003). After the thorough analyses of the *A. nidulans* genome, we have tentatively concluded that only one each of Gβ- and Gγ-subunit exists in *A. nidulans*. No genes encoding the products similar to the yeast Gpb1/Gpb2 proteins (HARASHIMA and HEITMAN 2002) have been identified.

One of the important findings of this study is that elimination of *gpgA* function could bypass the requirement of *flbA*, but not *fluG*, in conidiation. These results support the idea that GpgA functions in the vegetative growth signaling pathway that is independent and parallel to the FluG-activated asexual sporulation branch (see Figure 7; YU *et al.* 1996; ROSÉN *et al.* 1999). The facts that mutational inactivation of *fadA*, *sfaD*, or *gpgA* all resulted in reduced vegetative growth as well as suppression of fluffy-autolytic phenotypes caused by Δ*flbA* strongly support the hypothesis that FadA, SfaD, and GpgA constitute a functional heterotrimeric unit, of which the primary role is to mediate vegetative growth signaling (Figure 7). However, as shown in the previous study, constitutive activation of FadA alone in the absence of SfaD function was sufficient to cause proliferation of undifferentiated hyphae (ROSÉN *et al.* 1999). This indicates that FadA might be the primary component responsible for vegetative proliferation.

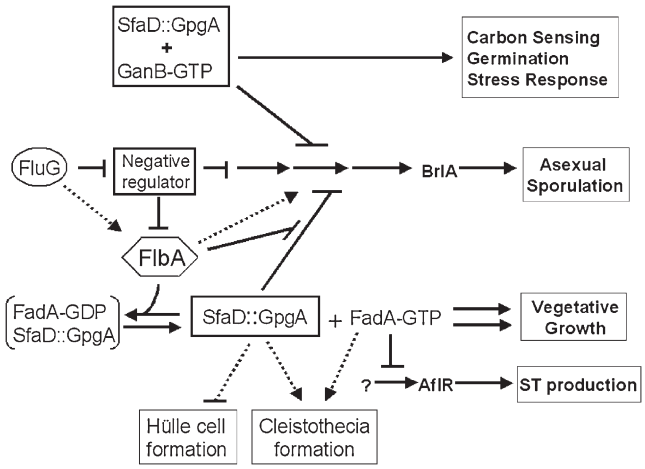

FIGURE 7.—Genetic model for growth and developmental control in *A. nidulans*. We propose that the heterotrimer composed of FadA and SfaD::GpgA functions in vegetative growth and fertilization signaling in *A. nidulans* (YU *et al.* 1996; ROSÉN *et al.* 1999). Moreover, a recent study by LAFON *et al.* (2005; see accompanying article in this issue) revealed that GanB and SfaD::GpgA constitute a functional heterotrimer that functions in conidial germination and sensing external carbon sources. In this model, it is speculated that during the vegetative growth phase FadA and SfaD::GpgA primarily mediate signaling for proliferation. Activation of asexual/sexual development requires at least partial inhibition of this vegetative growth signaling. FlbA is an RGS protein that attenuates vegetative growth signaling by increasing the intrinsic GTPase activity of FadA (LEE and ADAMS 1994a; YU *et al.* 1996). FluG activates asexual development by removing repressive effects imposed by multiple negative regulators (SEO *et al.* 2003), resulting in activation of a key transcription factor BrlA (see ADAMS *et al.* 1998). Genetic data suggest that GanB (CHANG *et al.* 2004) and SfaD (YU *et al.* 1996; ROSÉN *et al.* 1999) function in negative regulation of conidiation under submerged culture conditions. It is further speculated that FadA and SfaD::GpgA may also function in signaling for sexual fruiting body development. A possible negative role of SfaD and/or GpgA in Hülle cell formation is indicated.

If constitutively active FadA alone is sufficient to confer uncontrolled activation of vegetative signaling, how can deletion of *sfaD* or *gpgA* suppress Δ*flbA*? This question might be answered by understanding the proposed roles of Gγ- or Gβ-subunits in G-protein signaling. In general, G-protein γ-subunits are found to play the following roles: (1) transducing signals by forming a heterodimer with Gβ (WHITEWAY *et al.* 1989; GARRITSEN *et al.* 1993), (2) promoting G-protein activation by binding and modulating efficiency of receptor-G-protein coupling (LAMBRIGHT *et al.* 1994; YASUDA *et al.* 1996; RONDARD *et al.* 2001; CHINAULT and BLUMER 2003), (3) granting selective and discrete coupling of GPCRs with Gβ (KISSELEV *et al.* 1995, 1999), and (4) promoting activation of the Gβγ-effectors that cocluster with receptors (CHINAULT and BLUMER 2003). Similarly, Gβ-subunits were found to play an important role in providing a GPCR binding site for the G protein, which is critical for G-protein activation (TAYLOR *et al.*

1996; Hamm 1998; Kisselev *et al.* 1999; Chinault and Blumer 2003). It can be speculated that GpgA and SfaD may be necessary for the proper coupling of GPCR and G-protein, thereby activating the G protein. If this were the case, Δ*sfaD* would suppress Δ*flbA* but not the constitutively active *fadA* alleles, *e.g.*, G42R, Q204L, or R178C (Rosén *et al.* 1999; Yu *et al.* 1999), because dominant-activating FadA mutant alleles are locked in the GTP-bound (active) form and the interaction of the FadA-SfaD-GpgA heterotrimer with GPCR may not be needed to reactivate FadA.

Previous studies showed that, in addition to their role as a heterodimer, individual Gβ- or Gγ-subunits might play distinct roles in activation of G-proteins and/or downstream effectors (Landry and Hoffman 2001). In this study, we found that deletion of *sfaD* or *gpgA* resulted in certain dissimilar phenotypes. For instance, unlike Δ*sfaD*, deletion of *gpgA* resulted in fluffy-reduced conidiation phenotypes during the early phase of growth and did not cause conidiophore formation in liquid submerged culture. However, the Δ*sfaD*Δ*gpgA* double mutant exhibited phenotypes that are identical to those of the Δ*sfaD* mutant; *i.e.*, Δ*sfaD* is epistatic to Δ*gpgA*. These results suggest that, even in the absence of GpgA, SfaD may be able to (partially) activate downstream effectors that directly/indirectly control inappropriate conidiation in liquid submerged culture. Taken together, hyperactive conidiation by $fadA^{G203R}$ (dominant negative allele), Δ*sfaD*, and the absence of GanB (another Gα) functions suggest that GanB and SfaD play a role in negative regulation of conidiation under submerged culture conditions (Rosén *et al.* 1999; Chang *et al.* 2004; Han *et al.* 2004b).

Sexual development in *A. nidulans* involves the development of two distinct structures: Hülle cells and cleistothecia (reviewed in Champe *et al.* 1994; Braus *et al.* 2002). It is important to note that, although Hülle cells are associated with the sexual reproductive cycle, production of Hülle cells and development of cleistothecia are distinct processes. One striking common phenotype of the *fadA*, *sfaD*, or *gpgA* deletion mutant is the lack (or defect) of cleistothecia formation in self-fertilization (homothallic conditions) but highly elevated production of Hülle cells (Rosén *et al.* 1999). However, the requirement of these genes in cleistothecia development in outcrosses (heterothallic conditions) is different in that deletion of *sfaD* or *gpgA* (but not *fadA*) resulted in severe impairment in cleistothecia development (but not heterokaryon formation) in outcrosses in a somewhat dominant manner. While sexual development is a highly delicate process, which requires a number of genes and fulfillment of various factors, thus far, no *A. nidulans* genes have been found to specifically affect cleistothecia development in outcrosses without altering the ability to form heterokaryons. The requirement of SfaD and GpgA in normal fruiting body formation in both self-fertilization and outcrosses suggests that, similar to yeast mating, the SfaD-GpgA heterodimer may play a vital role in relaying the signals for fertilization. This is somewhat consistent with the findings that the Gβ-subunit is necessary for sexual development and the pheromone-response mating systems in *N. crassa* (Yang *et al.* 2002) and *Ustilago maydis* (Müller *et al.* 2004). In addition, a recent study showed that GNG-1 (Gγ) is necessary for normal female fertility in *N. crassa* (Krystofova and Borkovich 2005). Deletion of *ganA* or *ganB* encoding additional Gα-subunits in *A. nidulans* is found to cause no effects in sexual development (Chang *et al.* 2004; K.-Y. Jahng, personal communication).

In our previous studies, we identified nine putative GPCRs in *A. nidulans* and showed that GprA and GprB are required for cleistothecia formation in self-fertilization, but not in outcrosses (Han *et al.* 2004a; Seo *et al.* 2004). In these studies, we also demonstrated that GprA and GprB are not the GPCRs for FadA-mediated vegetative growth signaling. If SfaD-GpgA and FadA function in both vegetative growth and cleistothecia development signaling, how can the fungal cells determine their fate? This can be explained by differential expression of multiple GPCRs and variation of coclustering effectors. We found that, while mRNA levels of all G-protein subunits are relatively constant throughout the life cycle, those of GPCRs vary (Figure 1, our unpublished data; Han *et al.* 2004a; Seo *et al.* 2004). Particularly, *gprA* and *gprB* were specifically expressed at 48 hr postsexual developmental induction (Seo *et al.* 2004). If the expression and activities of GPCRs are tightly controlled by growth and developmental phases, and if distinct effectors cocluster with a specific GPCR, then discriminated activation of separate signaling cascades by the same G-protein can be achieved. For instance, during the vegetative growth phase certain (yet unidentified) GPCR(s) may be abundantly expressed and sensitized that can activate FadA-SfaD::GpgA-mediated hyphal growth signaling that is, in part, amplified by PKA (Shimizu and Keller 2001). When environmental and internal conditions are met, GprA and GprB are expressed, and sensitization of these GPCRs would exert activation of the FadA-SfaD::GpgA heterotrimer and the subsequent signaling cascade for cleistothecia development. This branch may be composed of SteC (MAPKKK) and SteA (a Ste12 homolog) in *A. nidulans* (Vallim *et al.* 2000; Wei *et al.* 2003). Further genetic and biochemical studies must be carried out to understand the molecular mechanisms underlying signal transduction from GPCRs to G-proteins to downstream effectors that selectively determine cellular responses.

The authors express special thanks to the Broad Institute and The Institute for Genome Research for the genome databases and to Ann Larson, Amy Wong, and Ellin Doyle for critically reviewing this article. This work was supported by the Food Research Institute and the Graduate School of the University of Wisconsin-Madison and by National Science Foundation grant MCB-0421863 to J.H.Y.

LITERATURE CITED

Adams, T. H., J. K. Wieser and J.-H. Yu, 1998 Asexual sporulation in *Aspergillus nidulans*. Microbiol. Mol. Biol. Rev. **12:** 3827–3833.

Braus, G. H., S. Krappmann and S. E. Eckert, 2002 Sexual development in Ascomycetes fruit body formation of *Aspergillus nidulans*, pp. 215–244 in *Molecular Biology of Fungal Development*, edited by H. D. Osiewacz. Marcel Dekker, New York.

Cabrera-Vera, T. M., J. Vanhauwe, T. O. Thomas, M. Medkova, A. Preininger *et al.*, 2003 Insights into G protein structure, function, and regulation. Endocr. Rev. **24:** 765–781.

Champe, S. P., D. L. Nagle and L. N. Yager, 1994 Sexual sporulation. Prog. Ind. Microbiol. **29:** 429–454.

Chang, M.-H., K.-S. Chae, D.-M. Han and K.-Y. Jahng, 2004 The GanB Gα-protein negatively regulates asexual sporulation and plays a positive role in conidial germination in *Aspergillus nidulans*. Genetics **167:** 1305–1315.

Chinault, S.-L., and K. J. Blumer, 2003 The c-terminal tail preceding the C*AAX* box of a yeast G protein γ subunit is dispensable for receptor-mediated G protein activation in vivo. J. Biol. Chem. **278:** 20638–20644.

D'Souza, C. A., B. N. Lee and T. H. Adams, 2001 Characterization of the role of the FluG protein in asexual development of *Aspergillus nidulans*. Genetics **158:** 1027–1036.

Garritsen, A., P. J. van Galen and W. F. Simonds, 1993 The N-terminal coiled-coil domain of β is essential for gamma association: a model for G-protein βγ subunit interaction. Proc. Natl. Acad. Sci. USA **90:** 7706–7710.

Hamm, H. E., 1998 The many faces of G protein signaling. J. Biol. Chem. **273:** 669–672.

Han, K.-H., J.-A. Seo and J.-H. Yu, 2004a A putative G protein-coupled receptor negatively controls sexual development in *Aspergillus nidulans*. Mol. Microbiol. **51:** 1333–1345.

Han, K.-H., J.-A. Seo and J.-H. Yu, 2004b Regulators of G-protein signaling in *Aspergillus nidulans*: RgsA downregulates stress response and stimulate asexual sporulation through attenuation of GanB (Gα) signaling. Mol. Microbiol. **53:** 529–540.

Harashima, T., and J. Heitman, 2002 The Gα protein Gpa2 controls yeast differentiation by interacting with kelch repeat proteins that mimic Gβ subunits. Mol. Cell **10:** 163–173.

Hicks, J. K., J.-H. Yu, N. P. Keller and T. H. Adams, 1997 *Aspergillus* sporulation and mycotoxin production both require inactivation of the FadA Gα protein-dependent signaling pathway. EMBO J. **16:** 4916–4923.

Käfer, E., 1977 Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. **19:** 33–131.

Kisselev, O., A. Pronin, M. Ermolaeva and N. Gautam, 1995 Receptor-G protein coupling is established by a potential conformational switch in the βγ complex. Proc. Natl. Acad. Sci. USA **92:** 9102–9106.

Kisselev, O. G., C. K. Meyer, M. Heck, O. P. Ernst and K. P. Hofmann, 1999 Signal transfer from rhodopsin to the G-protein: evidence for a two-site sequential fit mechanism. Proc. Natl. Acad. Sci. USA **96:** 4898–4903.

Krystofova, S., and K. A. Borkovich, 2005 The heterotrimeric G-protein subunits GNG-1 and GNB-1 form a Gβγ dimer required for normal female fertility, asexual development, and Gα protein levels in *Neurospora crassa*. Eukaryot. Cell **4:** 365–378.

Lafon, A., J.-A. Seo, K.-H. Han, J.-H. Yu and C. d'Enfert, 2005 The heterotrimeric G-protein GanB(α)-SfaD(β)-GpgA(γ) is a carbon source sensor involved in early cAMP-dependent germination in *Aspergillus nidulans*. Genetics **171:** 71–80.

Lambright, D. G., J. P. Noel, H. E. Hamm and P. B. Sigler, 1994 Structural determinants for activation of the alpha-subunit of a heterotrimeric G protein. Nature **69:** 621–628.

Landry, S., and C. S. Hoffman, 2001 The git5 Gβ and git11 Gγ form an atypical Gβγ dimer acting in the fission yeast glucose/cAMP pathway. Genetics **157:** 1159–1168.

Lee, B. N., and T. H. Adams, 1994a Overexpression of *flbA*, an early regulator of *Aspergillus* asexual sporulation leads to activation of *brlA* and premature initiation of development. Mol. Microbiol. **14:** 323–334.

Lee, B. N., and T. H. Adams, 1994b The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal. Genes Dev. **8:** 641–651.

Lee, B. N., and T. H. Adams, 1996 *fluG* and *flbA* function interdependently to initiate conidiophore development in *Aspergillus nidulans* through *brlA*β activation. EMBO J. **15:** 299–309.

Morris, A. J., and C. C. Malbon, 1999 Physiological regulation of G protein-linked signaling. Physiol. Rev. **79:** 1373–1430.

Müller, P., A. Leibbrandt, H. Teunissen, S. Cubasch, C. Aichinger *et al.*, 2004 The Gβ-subunit-encoding gene *bpp1* controls cyclic-AMP signaling in *Ustilago maydis*. Eukaryot. Cell **3:** 806–814.

Pontecorvo, G., J. A. Roper, L. M. Hemmons, K. D. Macdonald and A. W. J. Bufton, 1953 The genetics of *Aspergillus nidulans*. Adv. Genet. **5:** 141–238.

Rondard, P., T. Iiri, S. Srinivasan, E. Meng, T. Fujita *et al.*, 2001 Mutant G protein α subunit activated by Gβγ: A model for receptor activation? Proc. Natl. Acad. Sci. USA **98:** 6150–6155.

Rosén, S., J.-H. Yu and T. H. Adams, 1999 The *Aspergillus nidulans sfaD* gene encodes a G protein β subunit that is required for normal growth and repression of sporulation. EMBO J. **18:** 5592–5600.

Seo, J.-A., Y. Guan and J.-H. Yu, 2003 Suppressor mutations bypass the requirement of *fluG* for asexual sporulation and sterigmatocystin production in *Aspergillus nidulans*. Genetics **165:** 1083–1093.

Seo, J.-A., K.-H. Han and J.-H. Yu, 2004 The *gprA* and *gprB* genes encode putative G protein-coupled receptors required for self-fertilization in *Aspergillus nidulans*. Mol. Microbiol. **53:** 1611–1623.

Sinensky, M., 2000 Recent advances in the study of prenylated proteins. Biochim. Biophys. Acta **1484:** 93–106.

Shimizu, K., and N. P. Keller, 2001 Genetic involvement of a cAMP-dependent protein kinase in a G protein signaling pathway regulating morphological and chemical transitions in *Aspergillus nidulans*. Genetics **157:** 591–600.

Taylor, J. M., G. G. Jacob-Mosier, R. G. Lawton, M. Vandort and R. R. Neubig, 1996 Receptor and membrane interaction sites on Gβ. A receptor-derived peptide binds to the carboxyl terminus. J. Biol. Chem. **271:** 3336–3339.

Vallim, M. A., K. Y. Miller and B. L. Miller, 2000 *Aspergillus* SteA (Sterile 12-like) is a homeodomain-C_2/H_2-Zn^{+2} finger transcription factor required for sexual reproduction. Mol. Microbiol. **36:** 290–301.

Wei, H., N. Requena and R. Fischer, 2003 The MAPKK kinase SteC regulates conidiophore morphology and is essential for heterokaryon formation and sexual development in the homothallic fungus *Aspergillus nidulans*. Mol. Microbiol. **47:** 1577–1588.

Whiteway, M., L. Hougan, D. Dignard, D. Y. Thomas, L. Bell *et al.*, 1989 The STE4 and STE18 genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. Cell **56:** 467–477.

Wieser, J., J. H. Yu and T. H. Adams, 1997 Dominant mutations affecting both sporulation and sterigmatocystin biosynthesis in *Aspergillus nidulans*. Curr. Genet. **32:** 218–224.

Yang, Q., S. I. Poole and K. A. Borkovich, 2002 A G-protein β subunit required for sexual and vegetative development and maintenance of normal G α protein levels in *Neurospora crassa*. Eukaryot. Cell **1:** 378–390.

Yasuda, H., M. A. Lindorfer, K. A. Woodfork, J. E. Fletcher and J. C. Garrison, 1996 Role of the prenyl group on the G protein γ subunit in coupling trimeric G proteins to A1 adenosine receptors. J. Biol. Chem. **271:** 18588–18595.

Yu, J.-H., J. Wieser and T. H. Adams, 1996 The *Aspergillus* FlbA RGS domain protein antagonizes G-protein signaling to block proliferation and allow development. EMBO J. **15:** 5184–5190.

Yu, J.-H., S. Rosèn and T. H. Adams, 1999 Extragenic suppressors of loss-of-function mutations in the *Aspergillus* FlbA regulator of G-protein signaling domain protein. Genetics **151:** 97–105.

Yu, J.-H., Z. Hamari, K.-H. Han, J.-A. Seo, Y. Reyes-Domínguez *et al.*, 2004 Double-joint PCR: a PCR-based molecular tool for gene manipulation in filamentous fungi. Fungal Gen. Biol. **41:** 973–981.

Communicating editor: M. Sachs