Extragenic Suppressors of Loss-of-Function Mutations in the Aspergillus FlbA Regulator of G-Protein Signaling Domain Protein

Jae-Hyuk Yu,[1] Stefan Rosén and Thomas H. Adams[1]

Department of Biology, Texas A&M University, College Station, Texas 77843

Manuscript received March 31, 1998
Accepted for publication September 25, 1998

ABSTRACT

We showed previously that two genes, *flbA* and *fadA*, have a major role in determining the balance between growth, sporulation, and mycotoxin (sterigmatocystin; ST) production by the filamentous fungus *Aspergillus nidulans*. *fadA* encodes the α subunit for a heterotrimeric G-protein, and continuous activation of FadA blocks sporulation and ST production while stimulating growth. *flbA* encodes an *A. nidulans* regulator of G-protein signaling (RGS) domain protein that antagonizes FadA-mediated signaling to allow development. To better understand FlbA function and other aspects of FadA-mediated growth control, we have isolated and characterized mutations in four previously undefined genes designated as *sfaA*, *sfaC*, *sfaD*, and *sfaE* (**s**uppressors of *flbA*), and a new allele of *fadA* (*fadA*^{R205H}), all of which suppress a *flbA* loss-of-function mutation (*flbA98*). These suppressors overcome *flbA* losses of function in both sporulation and ST biosynthesis. *fadA*^{R205H}, *sfaC67*, *sfaD82*, and *sfaE83* mutations are dominant to wild type whereas *sfaA1* is semidominant. *sfaA1* also differs from other suppressor mutations in that it cannot suppress a *flbA* deletion mutation (and is therefore allele specific) whereas all the dominant suppressors can bypass complete loss of *flbA*. Only *sfaE83* suppressed dominant activating mutations in *fadA*, indicating that *sfaE* may have a unique role in *fadA-flbA* interactions. Finally, none of these suppressor mutations bypassed *fluG* loss-of-function mutations in development-specific activation.

THE asexual life cycle of the filamentous ascomycete *Aspergillus nidulans* can be divided into two distinct phases, growth and reproduction. The growth phase involves formation of an undifferentiated network of interconnected cells, or hyphae, that form the mycelium. Under appropriate growth conditions, some of the hyphal cells can stop normal growth and begin asexual reproduction by forming complex multicellular conidiophores that produce multiple chains of uninuleate spores called conidia (for review, see Adams 1994; Adams *et al.* 1998). We showed previously that initiation of *A. nidulans* asexual reproductive development requires the ability to control proliferative growth in response to an extracellular signal that functions specifically in activating development (Lee and Adams 1994b; Yu *et al.* 1996). The production of this developmental signal that controls initiating of conidiation requires *fluG*, which apparently functions in part by activating the regulator of G-protein signaling (RGS) domain protein FlbA (Lee and Adams 1994b, 1996; Yu *et al.* 1996). Other development-specific regulatory genes required for sporulation in response to the FluG signal include *flbB*, *flbC*, *flbD*, *flbE*, and *brlA* (see Figure 1; Adams *et al.* 1988; Wieser *et al.* 1994; Wieser and Adams 1995; Lee and Adams 1996).

Corresponding author: T. H. Adams, Department of Biology, Texas A&M University, College Station, TX 77843-3258.
E-mail: tom@bio.tamu.edu

[1]*Present address:* Cereon Genomics, LLC, Bldg. 300, 1 Kendall Sq., Cambridge, MA 02139.

FlbA (fluffy low BrlA) has a major role in determining the balance between growth and sporulation through its ability to regulate FadA (fluffy autolytic dominant), the α subunit for a heterotrimeric G-protein (Yu *et al.* 1996). When FadA-dependent signaling is activated in response to some unknown factor it stimulates growth and blocks sporulation. FlbA has the ability to inactivate FadA, as with other RGS domain proteins (Berman *et al.* 1996; Watson *et al.* 1996) probably working as a GTPase activating protein (GAP), thus allowing development to proceed. Inactivation of *flb*A or constitutive activation of *fadA* (*fadA*^{G42R}, *fadA*^{R178L} *fadA*^{G183S}, *fadA*^{R178C}, and *fadA*^{Q204L}) causes uncontrolled growth and leads to proliferation of undifferentiated aerial hyphae ("fluffy") that autolyse as colonies mature (Yu *et al.* 1996; Wieser *et al.* 1997; this study). By contrast, overexpression of *flbA* or dominant interfering mutations in *fadA* (*fadA*^{G203R}) result in inhibited hyphal growth coupled with conidiophore development, even under growth conditions that normally interfere with sporulation (Lee and Adams 1994a; Yu *et al.* 1996). Interestingly, in addition to its requirement for development, FlbA-directed inactivation of FadA signaling is required for biosynthesis of the aflatoxin-like mycotoxin called sterigmatocystin (ST; Brown *et al.* 1996; Hicks *et al.* 1997). This has led us to propose that sporulation and production of the secondary metabolite ST share the need to inactivate the FadA-mediated proliferation signaling pathway. However, there is an important difference in the control of asexual development *vs.* ST biosynthesis. While *fluG*

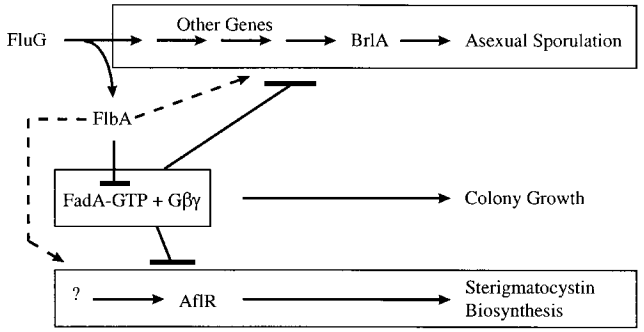

Figure 1.—*flbA* and *fadA* control *A. nidulans* growth, asexual sporulation, and ST biosynthesis. As described in the Introduction, we propose that two antagonistic signaling pathways control *A. nidulans* growth, asexual development, and ST production. When FadA (Gα) is active (GTP-bound), it signals to enhance proliferative growth and repress both asexual sporulation and ST production. This FadA-dependent growth signaling pathway is modulated by FlbA and FluG activities. FluG stimulates both development-specific events and activation of FlbA, which in turn inactivates FadA signaling. However, it is important to note that the main role of FluG in ST biosynthesis is apparently indirect, through activation of FlbA (see Introduction). The dotted arrows describe the observation that *flbA* overexpression in Δ*fadA* mutants has positive effects on asexual sporulation and ST production by an unknown mechanism (Yu *et al.* 1996; Hicks *et al.* 1997). Because no *flbA* suppressor mutations bypass *fluG* loss-of-function mutations for developmental phenotype except ST production, we propose that the major role for the products of *sfa* genes in activating asexual sporulation is through their effects on FadA-mediated growth signaling (see results and discussion).

deletion mutants fail to produce ST and do not sporulate, mutations that inactivate FadA suppress *fluG* deletion mutant defects in ST production but not sporulation. This implies that the main role of FluG in ST biosynthesis is activation of FlbA, which in turn inactivates FadA (Figure 1; Hicks *et al.* 1997).

While the most critical functions for FlbA involve inactivation of FadA, it is also clear that FlbA likely has other activities. Overexpression of *flbA* causes inappropriate sporulation and precocious ST production even in a Δ*fadA* mutant (Yu *et al.* 1996; Hicks *et al.*, 1997). This raises the possibility that FlbA could interfere with the activity of other Gα proteins, Gβγ signaling, or have a different role in activating sporulation and ST-specific genes (see Figure 1). These FlbA activities all appear to require an intact RGS domain (J.-H. Yu and T. H. Adams, unpublished results) but FlbA is also known to share at least one other conserved region that directly precedes the RGS domain and is ~80 amino acid residues in length (Ponting and Bork 1996). This domain is called DEP (**d**ishevelled, *egl-10*, **p**leckstrin) and is predicted to be a globular domain with an α + β topology (Ponting and Bork 1996). It is interesting that many other RGS domain proteins, including Sst2, RGS7, Egl-10, and Ya8c, have the DEP domain (Ponting and Bork 1996). Both RGS proteins and pleckstrin are known to function in negatively regulating critical signaling pathways, and it has been postulated that the DEP domains might regulate protein-protein interactions, but no such activity has been functionally demonstrated.

To better understand the complex role of FlbA in controlling growth and development we have isolated and begun to characterize suppressors of *flbA* loss-of-function mutations. We expect that understanding the roles of these suppressors will allow an unbiased approach toward identifying other elements in this multicomponent signaling pathway. We describe suppressor mutations identifying five distinct loci that can overcome *flbA* losses of function in both sporulation and ST biosynthesis. Characterization of these mutations and identification of one as a novel dominant inactivating allele of *fadA* are presented.

MATERIALS AND METHODS

Aspergillus strains, growth conditions, genetics, and transformation: The *A. nidulans* strains used in this study are listed in Table 1. Standard *A. nidulans* culture and genetic techniques were used (Pontecorvo *et al.* 1953; Käfer 1977). When appropriate (*e.g.*, deletion detection), genotypes of strains were confirmed by genomic Southern blot analysis. Standard *A. nidulans* transformation techniques (Yelton *et al.* 1984; Miller *et al.* 1985) were used with the slight modification of reducing the polyethylene glycol (PEG) treatment time from 20 min to 6 min. Whenever possible, genetic analysis was achieved via meiotic recombination. However, most of the primary isolates with *flbA* suppressor mutations were affected in their ability to form fertile cleistothecia with PW1 (or FGSC89) in meiotic crosses. In these cases, primary recombinants were produced via parasexual genetics. Dominance of each *sfa* mutation was tested by generating diploid strains (dJYA1–dJYE83; see Table 1). Each diploid strain was treated with the microtubule destabilizing agent benomyl (2 mg/ml DMSO, 6- to 9-μl/plate) to produce haploid sectors. At least 20 haploid progeny were isolated from each diploid strain and tested for auxotrophic markers and/or the deletion of *flbA*. To test whether suppressor mutations can bypass *A*, suppressor mutant strains with a *flbA* deletion (RJY8.9, RJY67.2, RJY82.4, and RJY83.6) were isolated from the haploid progeny of appropriate diploids. Suppressor mutant strains with Δ*fluG*;*flbA*98 (TJY8.G–TJY83.G) were generated by transforming each arginine auxotroph with a *fluG* deletion plasmid pJYGD4. Suppressor mutant strains with dominant activating alleles of *fadA* (TJY8.42R–TJY83.204L) were generated by transforming arginine auxotrophs with pJY8P2 (for G42R), pJYPK27 (for R178C), and pJYPK26 (for Q204L), respectively.

Mutagenesis and isolation of *flbA* suppressor mutations: Although *flbA* loss-of-function mutants autolyze and fail to sporulate when grown on normal minimal medium (70 mm $NaNO_3$, 1% glucose; Käfer 1997), this defect can be partially remediated by growth on medium containing a high concentration of salt (*e.g.*, 0.8 m NaCl or 0.6 m KCl). Conidiospores isolated from a *flbA* loss-of-function mutant (MJW98; see Table 1) grown on minimal medium with 0.8 m NaCl were mutagenized with NQO (4-nitroquinolin-1-oxide; 1 mg, 10 mg) as previously described (Wieser *et al.* 1994). Survival ratios after NQO treatment were from 0.1 to 10% depending on the length of NQO treatment. Survivors were visually screened for sporulation on complete medium (Käfer 1997) where the *flbA*98 mutant never sporulated. Among 100,000 survivors, 121

TABLE 1

***Aspergillus nidulans* strains used in this study**

Strain	Genotype[a]	Source
FGSC237	*pabaA1 yA2;trpC801*	FGSC[b]
FGSC26	*biA1*	FGSC
FGSC89	*biA1;argB2*	FGSC
PW1	*biA1;argB2;methG1*	P. Weglenski
MJW98	*pabaA1 yA2 flbA98*	Wieser *et al.* (1994)
SFA1	*pabaA1 yA2 flbA98;sfaA1*	This study
SFA8	*pabaA1 yA2 flbA98;fadA*^{R205H}	This study
SFA67	*pabaA1 yA2 flbA98;sfaC67*	This study
SFA82	*pabaA1 yA2 flbA98;sfaD82*	This study
SFA83	*pabaA1 yA2 flbA98;sfaE83*	This study
MSR123	*biA1* Δ*flbA::argB;argB2;pyroA4;sfaD123*	This study
MSR125	*biA1* Δ*flbA::argB;argB2;pyroA4;sfaD125*	This study
MSR126	*biA1* Δ*flbA::argB;argB2;pyroA4;sfaD126*	This study
MSR127	*biA1* Δ*flbA::argB;argB2;pyroA4;sfaD127*	This study
RBN119	*biA1;argB2* Δ*fluG::trpC;methG1*	Lee and Adams (1996)
RJH046	*biA1* Δ*flbA::argB;argB2;pyroA4*	J.K. Hicks and T.H. Adams, unpublished results
RJH057	*biA1* Δ*flbA::argB;argB2;trpC801*	J.K. Hicks and T.H. Adams, unpublished results
RJY98.22	*pabaA1 yA2 biA1 flbA98;argB2*	This study
RJY8.9	*biA1* Δ*flbA::argB;methG1;fadA*^{R205H}	This study
RJY67.2	*biA1* Δ*flbA::argB;methG1;sfaC67*	This study
RJY82.4	*biA1* Δ*flbA::argB;sfaD82*	This study
RJY83.6	*biA1* Δ*flbA::argB;sfaE83*	This study
RJY1.12	*pabaA1 yA2 flbA98;methG1;sfaA1*	This study
RJY8.22	*pabaA1 yA2 flbA98;argB2;fadA*^{R205H}	This study
RJY67.3	*pabaA1 yA2 flbA98;argB2;sfaC67*	This study
RJY82.6	*pabaA1 yA2 flbA98;argB2;sfaD82*	This study
RJY83.21	*pabaA1 yA2 flbA98;argB2;sfaE83*	This study
RJY8.6	*pabaA1 yA2 flbA98;methG1;fadA*^{R205H}	This study
RJY67.17	*pabaA1 yA2 flbA98;methG1;sfaC67*	This study
RJY82.14	*pabaA1 yA2 flbA98;methG1;sfaD82*	This study
RJY115.33	*yA2;pyroA4;fadA*^{G203R}	This study
RJY115.22	*biA1;argB2;methG1;fadA*^{G203R}	This study
RSR46.2	*pabaA1 yA2* Δ*flbA::argB*	This study
RSR126.1	*biA1* Δ*flbA::argB;argB2;trpC801;sfaD126*	This study
TBN39.5	*biA1* Δ*flbA::argB;argB2;methG1*	Lee and Adams (1994a)
TJY1.42R	*pabaA1 yA2 flbA98;methG1;sfaA1;fadA*^{G42R}*::argB*	This study
TJY8.42R	*pabaA1 yA2 flbA98;argB2;fadA*^{R205H}*;fadA*^{G42R}*::argB*	This study
TJY8.A	*pabaA1 yA2 flbA98;argB2;fadA*^{R205H}*;fadA*$^{+}$*::argB*	This study
TJY67.42R	*pabaA1 yA2 flbA98;argB2;sfaC67;fadA*^{G42R}*::argB*	This study
TJY82.42R	*pabaA1 yA2 flbA98;argB2;sfaD82;fadA*^{G42R}*::argB*	This study
TJY83.42R	*pabaA1 yA2 flbA98;argB2;sfaE83;fadA*^{G42R}*::argB*	This study
TJY83.178C	*pabaA1 yA2 flbA98;argB2;sfaE83;fadA*^{R178C}*::argB*	This study
TJY83.204L	*pabaA1 yA2 flbA98;argB2;sfaE83;fadA*^{Q204L}*::argB*	This study
TJY8.G	*pabaA1 yA2 flbA98;*Δ*fluG::argB argB2;fadA*^{R205H}	This study
TJY67.G	*pabaA1 yA2 flbA98;*Δ*fluG::argB argB2;sfaC67*	This study
TJY82.G	*pabaA1 yA2 flbA98;*Δ*fluG::argB argB2;sfaD82*	This study
TJY83.G	*pabaA1 yA2 flbA98;*Δ*fluG::argB argB2;sfaE83*	This study
dJYA1[c]	$\frac{+\ pabaA1\ yA2\ flbA98;\ +\ ;\ +\ ;\ sfaA1}{biA1\ +\ +\ \Delta flbA::argB;argB2;methG1;\ +}$	This study
dJYB8[c]	the same as DJYA1 except *sfaA1* → *fadA*^{R205H}	This study
dJYC67[c]	the same as DJYA1 except *sfaA1* → *sfaC67*	This study
dJYD82[c]	the same as DJYA1 except *sfaA1* → *sfaD82*	This study
dJYE83[c]	the same as DJYA1 except *sfaA1* → *sfaE83*	This study
dSRD123[c]	$\frac{biA1\ \ \Delta flbA::argB;argB2;pyroA4;\ +\ ;\ sfaD123}{biA1\ \Delta flbA::argB;argB2;\ +\ ;\ trpC801;\ +}$	This study
dSRD125[c]	the same as dSRD123 except *sfaD123* → *sfaD125*	This study
dSRD126[c]	the same as dSRD123 except *sfaD123* → *sfaD126*	This study
dSRD127[c]	the same as dSRD123 except *sfaD123* → *sfaD127*	This study

[a] All strains are *veA1*.
[b] Fungal Genetics Stock Center.
[c] Diploid strains (homozygous for *flbA*$^{-}$, heterozygous for *sfa*).

showed at least partial suppression of conidiation defects and 5 (SFA1, 8, 67, 82, and 83) of these mutants sporulated nearly as well as a wild type. These 5 mutants were further analyzed. Because characterization of these five suppressors identified five independent loci (no suppressor mutations were allelic to each other) an attempt was made to isolate additional suppressors to reach the saturation of mutations and/or to isolate possible alleles of the above-mentioned suppressor mutations. In a second set of mutagenesis, spores of a Δ*flbA* strain (RJH046; see Table 1) were mutagenized as mentioned above and among 50,000 survivors, 4 showed near complete suppression of conidiation defects (MSR123, 125, 126, 127). These 4 additional suppressors were also further characterized.

ST production analysis: ST production was examined by inoculating ~1 × 10^5 conidia in 3 ml of liquid complete medium (minimal medium with 2% glucose, 0.2% peptone, 0.1% yeast extract, and 0.1% casamino acids; Käfer 1977) in an 8-ml vial and incubated at 30° for 7 days (stationary culture) as previously described (Yu and Leonard 1995). ST was then extracted from 7-day-old cultures by adding 1.5 ml of $CHCl_3$ to the vials and then vortexed for 2 min. Vials were centrifuged at 500 × *g* for 5 min and the organic phase was collected, dried, resuspended in 50 μl of $CHCl_3$, and 4 μl of each concentrated sample was loaded for thin-layer chromatography (TLC) analysis described previously (Yu and Leonard 1995).

Nucleic acid manipulation: To determine the sequence of the *flbA*98 mutant allele the *flbA* coding region from MJW98 genomic DNA was amplified by the polymerase chain reaction (PCR) using the synthetic oligonucleotides CTGGTTTAGTC TGATTTTCGTC and TCGTCGTAATCTCACCGCA as primers. The resulting *flbA98* amplicon (~2.9 kb) was sequenced directly. The $fadA^{R178C}$ and $fadA^{Q204L}$ dominant-activating alleles were generated by site-directed mutagenesis with the synthetic oligonucloetides GTCCTACGCagctGTGTCAAGAAC or GAC GTTGGaGGcCtCCGTTCTGAG (lowercase letters represent mismatches), respectively (Kunkel 1985). These oligonucleotides introduce *Pvu*II or *Stu*I sites that were used for screening convenience. Each 3.15-kb *Pst*I fragment with the $fadA^{R178C}$ and $fadA^{Q204L}$ mutant alleles was then moved into pPK1 (Wieser and Adams 1995) to give pJYPK27 and pJYPK26, respectively. Resulting plasmids were used for transformation of suppressor mutant strains. The *fadA* gene from SFA8 was amplified by PCR with synthetic oligonucleotides ATGACTCTGCAGCGG GGCTATC and TCGCTGCTGCAGAGCGGCGAA. The resulting 3.15-kb amplicon was digested with *Pst*I and cloned into pPK1 (for *fadA* gene structure, see Yu *et al.* 1996). Four independent clones were isolated and used for transformation of RJY8.22. Two of these clones were sequenced and the mutation was also confirmed by directly sequencing the PCR product. A *fluG* disruption vector (pJYGD4) containing the wild-type *argB* gene as a selective marker was constructed by replacing the $trpC^+$ fragment in pTA127 (Lee and Adams 1994b) with an *Xho*I-digested *argB* fragment.

Microscopy: Photomicrographs presented in this study were taken using an Olympus BH2 compound microscope and differential interference contrast optics. All other microscopy was carried out using an Olympus SZ-11 stereo microscope and transmitted light.

RESULTS

Isolation of *flbA* extragenic suppressors that identify five distinct loci: We set out to identify extragenic suppressors of *flbA* using a mutant strain with the *flbA98* allele (strain MJW98). We used this strain because its sporulation defect was less severe than for an *flbA* deletion mutant, suggesting partial function. By using this partial-function *flbA98* allele we expected that we could identify both allele-specific and bypass suppressor mutations. Conidiospores from MJW98 were treated with NQO as described in materials and methods and 100,000 survivors were screened to identify suppressors. From this approach, 121 at least partially sporulating strains were isolated, and 5 of these mutants (SFA1, 8, 67, 82, and 83) that sporulated nearly as well as wild types (Figure 2) were selected for further studies. Suppressor loci are designated as either sfa^S (mutant alleles) or sfa^{WT} (wild-type alleles).

To determine if suppression resulted from mutations within *flbA* or from mutations in unlinked genes we attempted to cross each primary suppressed mutant strain with a developmentally wild-type strain (PW1) to look for segregation of the *flbA98* phenotype. However, only two of these mutants (SFA67 and SFA82) produced fertile cleistothecia when crossed with PW1. For each of these cases, ~25% of the progeny had the *flbA98* phenotype, indicating that *sfa67* and *sfa82* were extragenic *flbA* suppressors. In both cases, the other 75% of the progeny were developmentally wild type, indicating that neither *sfa67* nor *sfa82* likely caused phenotypic abnormalities in a $flbA^+$ strain.

After repeated unsuccessful attempts to cross SFA1, SFA8, and SFA83 with different wild-type strains (PW1 or FGSC89), we chose to examine linkage of mutations to *flbA* via parasexual (mitotic-cross) analysis. Diploids were generated from heterokaryons formed between each sfa^S;*flbA98* mutant (each SFA) strain and either PW1 or FGSC89. These diploid strains were then treated with the microtubule-destabilizing agent benomyl to generate haploid progeny. In every case *flbA98;*sfa^S/$flbA^+$;sfa^{WT} diploids produced haploid sectors with the *flbA98* phenotype as expected if the suppressor mutations were not linked to *flbA*. All other haploid progeny appeared wild type and several of these strains were purified and used in meiotic crosses to determine their genotype. Interestingly, several strains of each type that formed fertile crosses with wild-type strains yielded 25% fluffy progeny, indicating that the parent genotype was sfa^S;*flbA98*. Thus, the sexual defect in the primary mutant strains was apparently not linked to the *sfa* mutation and it is important to note that each suppressor segregated as a single trait. As above, none of the *sfa* mutations caused phenotypic abnormalities in $flbA^+$ strains and no further attempts to distinguish sfa^S; $flbA^+$ from sfa^{WT};$flbA^+$ were made. Finally, pairwise crosses were made between different sfa^S;*flbA98* mutant strains to determine how many different suppressor loci had been identified. In every case, ~25% of the progeny had the *flbA98* phenotype, indicating that every mutation defined a distinct locus, four of which were designated as *sfaA*, *sfaC*, *sfaD*, *sfaE*, and the fifth was a new allele of *fadA* ($fadA^{R205H}$; see below).

Dominance relationships of *sfa* mutations: Because

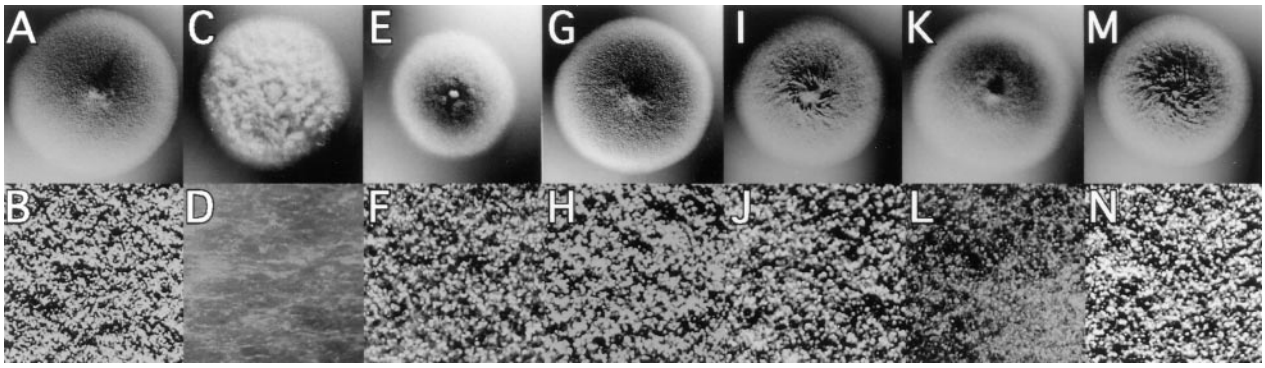

Figure 2.—Phenotypes of *flbA* suppressors. Photographs of wild-type and suppressor mutant colonies (top) and the close-up views of conidiation are shown (bottom). Panels are wild type (A and B), *flbA98* (C and D), *flbA98;sfaA1* (E and F), *flbA98;fadA*^{R205H} (G and H), *flbA98;sfaC67* (I and J), *flbA98;sfaD82* (K and L), and *flbA98;sfaE83* (M and N).

flbA loss-of-function mutations (*e.g.*, *flbA98* and Δ*flbA*) are recessive to the wild-type *flbA* gene, dominance of each suppressor mutation needed to be tested in homozygous *flbA*$^{-}$ diploid. Such diploids were generated from heterokaryons resulting from fusion between the *sfa*^{S};*flbA98* strains and a *sfa*^{WT};Δ*flbA* (TBN39.5) mutant strain. Resulting heterokaryons were predominantly conidiating and four of the diploid strains isolated (dJYB8, dJYC67, dJYD82, dJYE83; see Table 1) sporulated like wild types when grown at 37°. These diploid strains yielded fluffy haploid sectors when treated with benomyl, confirming that the suppressors had a dominant activity. The *sfaA1;flbA98*/*sfaA*$^{+}$;Δ*flbA* diploid strain (dJYA1; see Table 1) also sporulated but remained somewhat fluffy, so that this suppressor mutation was characterized as semidominant. Interestingly, we found that when these diploid strains were incubated at 25° the phenotype reverted to fluffy, indicating that the dominant suppressor mutant phenotype was cold sensitive (see Table 2). However, haploid *sfa*^{S};Δ*flbA* and *sfa*^{S};*flbA98* strains remained conidial when grown at 25°.

Suppression of the *flbA* deletion mutation: To test whether these *flbA* suppressor mutations could bypass a complete lack of *flbA* function, we substituted the Δ*flbA* mutation for *flbA98* by recovering *sfa*^{S};Δ*flbA* haploid progeny from the *sfa*^{S};*flbA98*/*sfa*^{WT};Δ*flbA* diploid strain (dJYA1–dJYE83). For dJYB8, dJYC67, dJYD82, and dJYE83, conidiating Δ*flbA* haploid progeny were identified, indicating that *fadA*^{R205H}, *sfaC67*, *sfaD82*, and *sfaE83* are all able to bypass the complete loss of *flbA* function and are not allele specific. However, none of the conidial progeny from dJYA1 were Δ*flbA*, indicating that *sfaA1* cannot bypass the complete lack of *flbA* function and thus could be an allele-specific suppressor. Alternatively, the *sfaA1* mutation could be on chromosome *I*, the same chromosome as *flbA* (very little intrachromosomal recombination occurs in mitotic diploids). However, examination of the segregation pattern for other genes on chromosome *I* (*yA* and *biA*) indicated that this chromosome segregated freely among fluffy progeny.

Identification of a new *fadA* allele: Because we knew that some *fadA* mutations (*e.g.*, Δ*fadA* and *fadA*^{G203R}; Yu *et al.* 1996) suppress *flbA* loss-of-function mutations we tested the possibility that one of the suppressors was an allele of *fadA* by examining linkage to *fadA*^{G203R}. All but one suppressor (carried by SFA8) locus independently

TABLE 2

Characteristics of *flbA* suppressors

	Dominance[a]			Suppression			
Allele	37°	25°	ST[b] (30°)	*flbA98*	Δ*flbA*	Δ*fluG*	*fadA*$^{d+c}$
sfaA1	S	R	R	+	−	−	−
sfaC67	D	R	D	+	+	−	−
sfaD82	D	R	D	+	+	−	−
sfaE83	D	S	D	+	+	−	+
fadA^{R205H}	D	R	D	+	+	−	−
fadA^{G203R}	D	D	D	+	+	−	−/+
Δ*fadA*	R	R	R	+	+	−	−

[a] Domimance was determined using homozygous *flbA*$^{-}$ diploids. R, recessive; D,dominant; S, semidominant.
[b] Sterigmatocystin production at 30°.
[c] *sfaE83* was able to suppress all three dominant activating *fadA* mutations: G42R, R178C, and Q204L.

segregated from the *fadA* locus. The *fadA* genomic region from SFA8 was amplified by PCR and the sequence was determined to directly test if SFA8 carried an allele of *fadA*. We found that SFA8 carried a novel *fadA* mutant allele that resulted from a G-to-A transition causing conversion of Arg205 to His (*fadA*^{R205H}). This mutant allele was used to transform a *flbA*98 mutant strain and resulted in conidial transformants, indicating that *fadA*^{R205H} represents a novel dominant negative *fadA* allele (see discussion and Figure 5).

***sfaE83* can suppress dominant activating *fadA* mutations:** Because the primary function of the FlbA RGS domain protein antagonizes FadA-directed signaling (Yu *et al.* 1996), and because *sfa* mutations suppress *flbA* loss-of-function mutations, it was of interest to know if *sfa* mutations could also suppress *fadA*-activating alleles like *fadA*^{G42R}, which causes a dominant fluffy-autolytic phenotype. To address this question, suppressor mutant strains were transformed with the *fadA*^{G42R}-activating allele to produce *flbA98;fadA*^{G42R}/*fadA*$^{+}$;*sfa*^{S} mutant strains. More than 50% of *sfaA1*, *fadA*^{R205H}, *sfaC67*, and *sfaD82* transformants were fluffy autolytic as is observed when wild type is transformed with the *fadA*^{G42R} allele. However, no fluffy autolytic transformants were observed following transformation of the *flbA98;sfaE83* strain with the *fadA*^{G42R} allele. All *flbA98;sfaE83* transformants were able to conidiate and genomic DNA Southern blot analysis showed that about 50% of the total transformants had one to five copies of the *fadA*^{G42R} allele integrated into their genomes. To test whether *sfaE83* suppressed other dominant activating *fadA* alleles an *sfaE83;flbA98* strain was transformed with the *fadA*^{R178C} and *fadA*^{Q204L} alleles, respectively. Again, all transformants were conidiating and ~50% of them had from one to several copies of each *fadA* dominant activating mutant allele (see discussion).

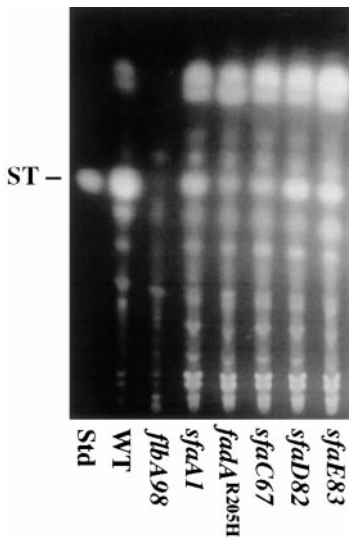

Figure 3.—Suppressors overcome *flbA* losses of function in ST biosynthesis. All *sfa*^{S} strains also suppress ST defects caused by *flbA* losses of function. The wild type (FGSC26; WT), *flbA98*, *flbA98;sfaA1*, *flbA98;fadA*^{R205H}, *flbA98;sfaC67*, *flbA98;sfaD82*, and *flbA98;sfaE83* strains (shown by relevant genotypes) were inoculated into 3 ml of liquid complete medium in 8-ml vials and incubated at 30° for 7 days (stationary culture). ST was extracted from each culture using chloroform, and samples were analyzed by thin-layer chromatography (Yu and Leonard 1995). ST standard is shown (Std).

***sfa* mutants regain the ability to produce a mycotoxin ST:** Because *flbA* suppressor mutations overcome a complete lack of *flbA* function for sporulation, it was of interest to test their ability to suppress defects in ST biosynthesis. As shown in Figure 3 and summarized in Table 2, we examined ST production from each suppressor mutant strain as previously described grown under conditions known to favor ST biosynthetic activities in wild type, and all suppressor mutant strains produced ST. Moreover, all suppressor mutants accumulated *stc* (sterigmatocystin gene cluster; Brown *et al.* 1996) transcripts with timing similar to that of the wild-type strain (data not shown).

***sfaC67* and *sfaD82* mutations cause inappropriate sporulation:** Because all suppressors were dominant we speculated that some of these gain-of-function suppressor mutations might behave like *fadA*^{G203R} dominant-interfering mutations and cause conidiation even in submerged culture where wild-type *A. nidulans* strains do not sporulate. In fact, *sfaC67* and *sfaD82* mutants elaborated complex conidiophores by 22 hr after inoculation in submerged culture (Figure 4). All of the other suppressor mutant strains grew like wild type in submerged culture and did not sporulate. As expected,

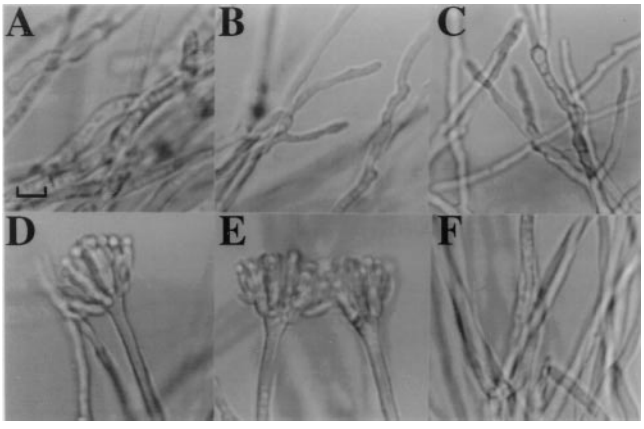

Figure 4.—*sfaC67* and *sfaD82* mutant strains produce conidiophores in submerged culture. Approximately 5×10^5 conidia/ml were inoculated into 100 ml of liquid minimal medium (with supplements and 0.1 g of yeast extract) in 250-ml flasks and incubated at 37° at 300 rpm. Micrographs of *flbA*98 (A), *flbA98;sfaA1* (B), *flbA98;fadA*^{R205H} (C), *flbA98;sfaC67* (D), *flbA98;sfaD82* (E), and *flbA98;sfaE83* (F) strains were taken at 22 hr after inoculation. Although wild-type *A. nidulans* strains occasionally formed conidiophores following prolonged incubation, only *sfaC67* and *sfaD82* strains produced conidiophores by 22 hr after inoculation and these structures were observed in every microscopic field examined.

mRNA corresponding to the developmental regulatory gene *brlA* accumulated in both *sfaC67* and *sfaD82* coincident with sporulation (data not shown).

***flbA* suppressors do not eliminate the need for *fluG* in sporulation:** The *A. nidulans fluG* gene is hypothesized to be required for production of a small diffusible extracellular factor that controls initiation of development, possibly by activating FlbA (Lee and Adams 1994b; Lee and Adams 1996). We showed previously that neither a dominant interfering *fadA* mutation (*fadA*^{G203R}) nor a deletion of *fadA* could overcome *fluG* loss-of-function mutations for asexual sporulation (Yu *et al.* 1996). This led us to propose that developmental activation requires *fluG* factor-mediated events that are distinct from inhibition of FadA-mediated growth signaling. Because *flbA* suppressors were identified on the basis of recovery of asexual sporulation, one possibility is that suppression results from hyperactivation of FadA-independent FluG signaling events. We have tested this possibility by examining the ability of *flbA* suppressor mutations to bypass the loss of *fluG* functions for sporulation. This was accomplished by transformation of each *flbA98;argB2;sfa*^{S} strain (except *sfaA1* due to the absence of appropriate strains) with pJYGD4 (containing a *fluG* deletion replaced by *argB*$^{+}$) and screening for the Δ*fluG* phenotype. Approximately 20% of transformants from each set of transformation experiment had the Δ*fluG* developmental phenotype, indicating that none of the suppressor mutations could bypass *fluG* loss-of-function mutations for this sporulation function. However, all Δ*fluG* phenotypic transformants were able to produce ST as expected, if the main role of FluG in ST biosynthesis was indirect, through activating FlbA (see Introduction and Figure 1; Hicks *et al.* 1997).

Additional suppressors identify alleles of *sfaD82*: Because all of the first *flbA* suppressor mutants identified different loci and most were bypass suppressors, we decided to screen for additional suppressor mutants beginning with a Δ*flbA* strain. Among 50,000 survivors, 2 showed partial suppression of conidiation defects and 4 sporulated nearly as well as wild type (MSR123, 125, 126, 127). Because a Δ*flbA* strain was used to isolate these primary suppressor mutants, these are expected to be extragenic bypass suppressors of *flbA* function. Meiotic crosses between these primary suppressor mutants and a developmentally wild-type strain (FGSC237) did not generate any distinguishable progeny, indicating that as with other *flbA* mutant suppressors, these mutations were silent in *flbA*$^{+}$ strains. No fluffy progeny arose from sexual crosses between these new suppressor mutant strains, indicating that the suppressor mutations were closely linked to one another. To test whether these suppressor mutations were alleles of previously identified *flbA98* suppressor mutations, mutant strains of the new series (MSR123 and MSR127) were crossed with *sfaA1*, *sfaC67*, *sfaD82*, *sfaE83*, and *fadA*^{R205H} mutant strains (RJY1.12, 67.3, 82.6, 83.21, and 8.22, respectively). Fluffy progeny were recovered from all crosses except MSR123 × RJY82.6 and MSR127 × RJY82.6 crosses, indicating that these additional suppressor mutations are likely to be alleles of *sfaD82* (or represent closely linked loci). We have tentatively called these mutations *sfaD123*, *sfaD125*, *sfaD126*, and *sfaD127*, respectively. Interestingly, MSR125 (*sfaD125*, not shown) sporulated in submerged culture like SFA82 (*sfaD82*; Figure 4), but the other mutants grew like wild types, indicating that all *sfaD* alleles are not identical for this trait. All of these mutations were dominant at 37° but recessive at 25°, similar to *sfaD82*, and all mutants regained the ability to produce ST (not shown).

DISCUSSION

We previously proposed that there are two antagonistic signaling pathways that coordinate *A. nidulans* growth, conidiation, and ST biosynthesis (see Introduction and Figure 1; Yu *et al.* 1996; Hicks *et al.* 1997). As a step toward understanding the multiple roles of FlbA in controlling growth, sporulation, and ST biosynthesis we have isolated a collection of mutants that sporulate and produce ST even in the absence of FlbA. These mutant strains carry dominant or semidominant mutations in any of five distinct loci designated *sfaA*, *sfaC*, *sfaD*, *sfaE*, and a novel *fadA* allele, *fadA*^{R205H}. Because *sfaA1*, *sfaC67*, and *sfaD82* mutations suppressed *flbA* loss-of-function mutations but could not suppress *fadA* dominant activating mutant alleles, we propose that the normal products of these genes most likely function prior to FadA activation or are required in some other way for FadA activity.

One of the suppressor mutants (SFA8) turned out to represent a novel allele of *fadA* (*fadA*^{R205H}). This mutation has not been described in other Gα proteins but causes a dominant negative phenotype similar to the G203R mutation described earlier (Yu *et al.* 1996). Arg205, like Gly203, is predicted to be a part of alpha helix 2 in the switch II region of Gα proteins and could therefore be required for the conformational change that triggers disengagement of Gα and Gβγ following receptor-mediated GDP-GTP exchange on Gα (Noel *et al.* 1993; Sondek *et al.* 1996). Although *fadA*^{R205H} behaves as a dominant loss-of-function mutation, this mutation differs from the *fadA*^{G203R} mutation in that it failed to stimulate submerged sporulation (see Yu *et al.* 1996).

While we do not yet know what *sfaA*, *sfaC*, and *sfaD* encode, it is interesting to speculate that these could encode other elements of the heterotrimeric G-protein such as Gβ or Gγ. In many cases the Gβγ heterodimer can function in signaling downstream effectors like those stimulated by Gα-GTP (Clapham and Neer 1993; Neer 1995). If the Gβγ complex associated with FadA is also required to stimulate growth and block sporulation, then loss-of-function or dominant negative mutations in either the Gβ or γ subunits could suppress *flbA*$^{-}$

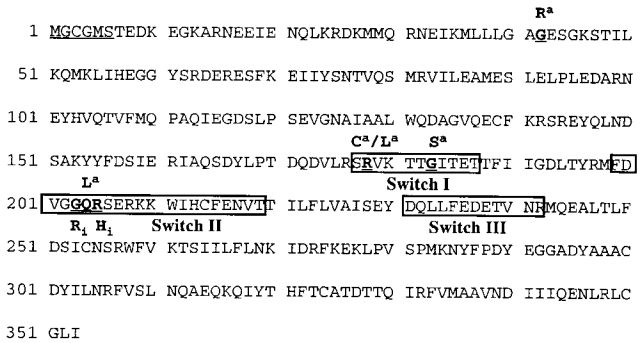

Figure 5.—*fadA* mutant alleles. FadA primary protein structure and known dominant activating (^{a}) and interfering ($_i$) mutations are shown. A consensus myristoylation site (MGXXXS) is underlined near the N-terminal end. Dominant activating mutations include G42R (Yu *et al.* 1996), R178C, R178L, (Wieser *et al.* 1997), G183S (Wieser *et al.* 1997), and Q204L, and dominant interfering mutations are G203R (Yu *et al.* 1996) and R205H. Switch domains that are important for the proper conformational changes are shown.

phenotype. We previously observed that the *fadA*^{G203R} dominant negative mutation caused submerged asexual sporulation, but Δ*fadA* mutations did not. Given that the expected effect of *fadA*^{G203R} is to block the conformational change in the switch II region of Gα, preventing dissociation from Gβγ, one possible explanation for the different phenotypes is that inhibition or loss of Gβγ is required for hyperactive sporulation to occur. In keeping with this hypothesis, both *sfaC67* and *sfaD82* alleles caused hyperactive sporulation (Figure 4) and might therefore identify Gβ or γ subunits.

Another possible role for *sfaA*, *sfaC*, or *sfaD* products is in post-translational modification of G-protein subunits. Like many Gα proteins, FadA contains a consensus amino acid sequence for myristoylation at its N terminus (Buss *et al.* 1987; Yu *et al.* 1996; see Figure 5). *N*-Myristoylation is known to be essential for Gα membrane association, proper Gα-Gβγ interaction, and receptor coupling (Song and Dohlman 1996; Song *et al.* 1996) so that mutations blocking myristoylation of FadA would be predicted to be like loss of *fadA* function. Similarly, prenylation of Gγ subunits is typically required for membrane localization and for efficient downstream signaling by Gβγ (Simonds *et al.* 1991; Clarke 1992; Muntz *et al.* 1992). Thus, loss of prenyltransferase activity could have similar effects to loss of Gβ or Gγ function.

sfaA1 differs from mutations in other suppressor genes in that it is semidominant and is unable to suppress a *flbA* deletion mutant. Sequence analysis of the *flbA98* allele that *sfaA1* mutation suppresses showed that a G-to-A transition occurred at the 3′ border of the third intron (GT - - - AGG→ AAG). This mutation is predicted to cause incorrect splicing and result in a frameshift affecting the last 50 amino acids at C terminus, including the end (16 amino acids) of the RGS domain. An interesting possibility is that the *flbA98* mutation results in a partially functional FlbA protein that lacks RGS-GAP activity and that the *sfaA1* mutation can suppress loss of GAP activity but not loss of other unknown FlbA functions. If this turns out to be true, *sfaA* might identify a unique activity that will help to define FlbA's additional roles.

sfaE83 differs from the other suppressor mutations in its ability to suppress not only *flbA* loss-of-function, but also dominant activating *fadA* mutations (G42R, Q204L, R178C). These dominant activating *fadA* mutations cause a loss of (or a dramatic decrease in) the intrinsic GTPase activity of Gα (FadA), which is essential for inactivating heterotrimeric G-protein signaling. Thus, *sfaE* mutations could either block activation of FadA by preventing GDP-GTP exchange or prevent transmission of downstream FadA-mediated signaling events. In the first case, it is possible that mutations that interfere with agonist-receptor sensitization (Stefan and Blumer 1994) would prevent GDP-GTP exchange and suppress both *flbA* loss-of-function and *fadA* dominant activating mutations. For the second case, many downstream effector molecules that are regulated by G-protein subunits have been described, including ion channels, phospholipase A2, protein kinases, adenylyl cyclases, and phospholipase C (for review, see Clapham and Neer 1993; Neer 1995). If any of these activities are essential for FadA-mediated growth activation and inhibition of sporulation, loss-of-function mutations would be predicted to suppress both *flbA* loss-of-function and *fadA* dominant activating mutations.

No *flbA* suppressors bypass the complete lack of *fluG* function. We proposed previously that *fluG* is required for: (i) activation of FlbA, which then inactivates FadA, and (ii) activation of development-specific functions that require the products of other genes, including *flB*, *flbC*, *flbD*, *flbE*, and *brlA* (see Figure 1; Adams *et al.* 1988; Wieser *et al.* 1994; Wieser and Adams 1995; Lee and Adams 1996). Our earlier finding that *fadA* deletion and *fadA*^{G203R} dominant interfering mutant alleles did not bypass *fluG* loss-of-function mutations in asexual sporulation led us to propose that both processes must occur if development is to proceed (Yu *et al.* 1996). Because none of the *flbA* suppressor mutations can suppress loss-of-*fluG* function, we propose that like FlbA, the major role for the products of *sfa* genes in activating asexual sporulation is indirect, through their effects on FadA-mediated growth signaling.

Finally, strategies for isolating the genes identified by these suppressors need to be discussed. The fact that all the suppressor mutations are dominant or semidominant at 37° but recessive at 25° (Table 2) provides two potential strategies for isolating the corresponding genes. In the first approach, the dominant nature of these mutations can be taken advantage of in constructing cosmid libraries from the suppressor mutant strains (*sfa*^{S};*flbA*$^-$) to transform *flbA98* or *flbA* deletion strains followed by screening for transformants that are

developmentally wild type at 37° but fluffy at 25°. Alternatively, it may be possible to take advantage of the temperature-sensitive nature of the suppressors by transforming the suppressor strains (*sfa*^{S}; *flbA98* or *sfa*^{S};Δ*flbA*) with a wild-type genomic DNA library and screening for transformants that are conidial at 37° but are fluffy at 25°. In any case, identification of each suppressor will lead us to better understand coordinate control of growth, development, and ST biosynthesis in *A. nidulans.*

We thank our colleagues in the lab for their many helpful suggestions. This work was supported by National Institutes of Health grant GM-45252 to T.H.A. and by Hellmuth Hertz Foundation and the Swedish Institute postdoctoral fellowship to S.R.

LITERATURE CITED

Adams, T. H., 1994 Asexual sporulation in higher fungi, pp. 367–382 in *The Growing Fungus*, edited by N. A. R. Gow and G. M. Gadd. Chapman & Hall, London.

Adams, T. H., M. T. Boylan and W. E. Timberlake, 1988 *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans.* Cell **54**: 353–362.

Adams, T. H., J. K. Wieser and J.-H. Yu, 1998 Asexual sporulation in *Aspergillus nidulans.* Microbiol. Rev. **62:** 35–54.

Berman, D. M., T. M. Wilkie and A. G. Gilman, 1996 GAIP and RGS4 are GTPase activating proteins (GAPs) for the G_i subfamily of G protein α subunits. Cell **86:** 445–452.

Brown, D. W., J.-H. Yu, H. S. Kelkar, M. Fernandes, T. C. Nesbitt *et al.*, 1996 Twenty-five coregulated transcripts define a sterigmatocystin gene cluster in *Aspergillus nidulans.* Proc. Natl. Acad. Sci. USA **93:**1418–1422.

Buss, J. E., S. E. Mumby, P. J. Casey, A. G. Gilman and B. M. Sefton, 1987 Myristoylated alpha subunits of guanine nucleotide-binding regulatory proteins. Proc. Natl. Acad. Sci. USA **84:** 7493–7497.

Clapham, D. E., and E. J. Neer, 1993 New roles for G-protein βγ dimers in transmembrane signalling. Nature **365:** 403–406.

Clarke, S., 1992 Protein isoprenylation and methylation at carboxyl-terminal cysteine residues. Annu. Rev. Biochem. **61:** 355–386.

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.

Kunkel, T. A., 1985 Rapid and efficient site-specific mutagenesis without phenotypic selection. Proc. Natl. Acad. Sci. USA **82:** 488–492.

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 and is related to prokaryotic glutamine synthetase I. 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.

Miller, B., K. Miller and W. E. Timberlake, 1985 Direct and indirect gene replacements in *Aspergillus nidulans.* Mol. Cell. Biol. **5:** 1714–1721.

Muntz, K. H., P. C. Sternweis, A. G. Gilman and S. M. Mumby, 1992 Influence of γ subunit prenylation on association of guanine nucleotide-binding regulatory proteins with membranes. Mol. Cell. Biol. **3:** 49–61.

Neer, E. J., 1995 Heterotrimeric G proteins: organizers of transmembrane signals. Cell **80:** 249–257.

Noel, J. P., H. E. Hamm and P. B. Sigler, 1993 The 2.2 Å crystal structure of transducin-α complexed with GTPγS. Nature **366:** 654–663.

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.

Ponting, C. P., and P. Bork, 1996 Pleckstrin's repeat performance: a novel domain in G-protein signaling? Trends Biochem. Sci. **21:** 245–246.

Simonds, W. F., J. E. Butrynski, N. Gautam, C. G. Unson and A. M. Spiegel, 1991 G protein βγ dimers: membrane targeting required subunit coexpression and intact γ C-A-A-X domain. J. Biol. Chem. **266:** 5363–5366.

Sondek, J., A. Bohm, D. G. Lambright, H. E. Hamm and P. B. Sigler, 1996 Crystal structure of a G_A protein βγ dimer at 2.1 Å resolution. Nature **379:** 369–374.

Song, J., and H. G. Dohlman, 1996 Partial constitutive activation of pheromone responses by a palmitoylation-site mutant of a G protein α subunit in yeast. Biochemistry **35:** 14806–14817.

Song, J., J. Hirschman, K. Gunn and H. G. Dohlman, 1996 Regulation of membrane and subunit interactions by N-myristoylation of a G protein α subunit in yeast. J. Biol. Chem. **271:** 20273–20283.

Stefan, C. J., and K. J. Blumer, 1994 The third cytoplasmic loop of a yeast G-protein-coupled receptor controls pathway activation, ligand discrimination, and receptor internalization. Mol. Cell. Biol. **14:** 3339–3349.

Watson, N., M. E. Linder, K. M. Druey, J. H. Kehrl and K. J. Blumer, 1996 RGS family members: GTPase-activating proteins for heterotrimeric G-protein α subunits. Nature **383:** 172–175.

Wieser, J. and T. H. Adams, 1995 *flbD* encodes a Myb-like DNA binding protein that regulates initiation of *Aspergillus nidulans* conidiophore development. Genes Dev. **9:** 491–502.

Wieser, J., B. N. Lee, J. W. Fondon and T. H. Adams, 1994 Genetic requirements for initiating asexual development in *Aspergillus nidulans.* Curr. Genet. **27:** 62–69.

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.

Yelton, M. M., J. E. Hamer and W. E. Timberlake, 1984 Transformation of *Aspergillus nidulans* by using a *trpC* plasmid. Proc. Natl. Acad. Sci. USA **81:** 1470–1474.

Yu, J.-H., and T. J. Leonard, 1995 Sterigmatocystin biosynthesis in *Aspergillus nidulans* requires a novel type I polyketide synthase. J. Bacteriol. **177:** 4792–4800.

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.

Communicating editor: R. H. Davis