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Analysis of *fluG* Mutations That Affect Light-Dependent Conidiation in *Aspergillus nidulans*

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ABSTRACT

Conidiation in *Aspergillus nidulans* is induced by exposure to red light but can also be induced by blue light in certain mutant strains. We have isolated a mutation in the *fluG* gene that abolishes responsiveness to red light but does not affect the response to blue light. It has been shown that the *veA1* (*velvet*) mutation allows conidiation to occur in the absence of light. We have identified three other *fluG* mutations that suppress the *veA1* phenotype; these double mutants do not conidiate in the dark. The mutations described here define two new phenotypic classes of *fluG* alleles that display abnormal responses to light. We have characterized these mutations with respect to their molecular identity and to their effect on *fluG* transcription. Although it has been shown that *fluG* is required for the synthesis of an extracellular factor that directs conidiation, we do not detect this factor under conditions that promote conidiation in the *veA1* suppressors. Furthermore, extracellular rescue is not observed in *fluG* deletion strains containing the wild-type *veA* allele. We propose that a genetic interaction between *fluG* and *veA* influences the production of the extracellular signal and regulates the initiation of conidiation.

CONIDIATION in the filamentous ascomycete *Aspergillus nidulans* involves the elaboration of haploid, uninucleate spores on a complex multicellular apparatus, called a conidiophore, that consists of a stalk, vesicle and sterigmata (metulae and phialides). Although the production of conidia results from the coordinate expression of several hundred genes, only three genes, *brlA*, *abaA*, and *wetA*, are required for the transition from vegetative growth to asexual sporulation (Martinelli and Clutterbuck 1971; Timberlake 1980; Boylan *et al.* 1987; Adams *et al.* 1988; Mirabito *et al.* 1989; Marshall and Timberlake 1991). These three genes form a sequentially expressed pathway, the activation of which is dependent upon at least six genes, designated *fluG*, *flbA*, *flbB*, *flbC*, *flbD*, and *flbE.* These latter genes were identified by mutations resulting in the proliferation of undifferentiated masses of vegetative hyphae that give colonies a "fluffy" appearance (Adams *et al.* 1992; Lee and Adams 1994a,b; Wieser *et al.* 1994; Wieser and Adams 1995). One of these genes, *fluG*, has been cloned and shown to encode an 864-amino acid polypeptide of unknown function that shares limited identity with type I prokaryotic glutamine synthetases (GSI) (Lee and Adams 1994a). It has been proposed that *fluG* is responsible for the synthesis of a diffusible, low molecular weight factor that controls the initiation of sporulation (Lee and Adams 1994a). Evidence for this was obtained by demonstrating that the *fluG* mutant phenotype could be suppressed by growing the mutant strain next to a wild-type strain, producing a strong band of conidiation between the two colonies. Furthermore, the phenotypic rescue of the *fluG* mutant strain occurred even if the two colonies were separated by dialysis membrane having a pore size of 6–8 kD.

The onset of conidiation is complex and controlled by both genetically determined factors and environmental conditions. It has been previously shown that conidiation is normally inhibited in submerged culture but readily takes place when mycelia are exposed to an air interface (Axelrod 1972; Axelrod *et al.* 1973). An important but often neglected environmental condition that also influences conidiation is light (Mooney and Yager 1990). Light dependence is determined by the allelic state of the *veA* (*velvet*) gene. Wild-type strains conidiate in the light but are aconidial in the dark, whereas strains bearing the *veA1* mutation conidiate in the absence of light.

Conidiation is elicited by exposure to red light in the range 690–710 nm (Mooney and Yager 1990). Champe *et al.* (1994) showed that light also affects sexual sporulation, causing a delay in the formation of cleistothecial foci, and that this delay is elicited by the same range of wavelengths that induce conidiation. In addition, these researchers identified a mutation, designated *bliA1* (blue light inducible; formally *edbA1*), that causes delayed cleistotheciation if colonies are exposed to blue

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light at 436 nm. We have shown that conidiation is also elicited by blue light in strains carrying the *bliA1* mutation (L. N. Yager, unpublished results).

To help elucidate how light influences development, we have isolated and characterized a mutation in the *fluG* gene that abolishes sensitivity to red light but maintains the ability to respond to blue light. In addition, three extragenic suppressors of the *veA1* mutation (Mooney *et al.* 1990) are shown to be alleles of *fluG.* The data presented indicate that *fluG* and *veA* are involved in the interpretation of the light signal and suggest a genetic interaction between these two genes.

MATERIALS AND METHODS

Aspergillus strains, genetic techniques and growth conditions: The strains used are listed in Table 1. Genotypes are designated as in Clutterbuck (1984). Standard genetic techniques were used throughout this study (Pontecorvo *et al.* 1953; Clutterbuck 1977; Käfer 1977).

The complete growth medium and general culture techniques that were employed are described in Yager *et al.* (1982) and Butnick *et al.* (1984). Incubations were performed either at 32° or 42°. Surface-grown colonies requiring illumination with white light were incubated in incubators (model I-35LL or model I-30BL; Percival Mfg. Co., Boone, IA) equipped with General Electric 20-W broad-spectrum fluorescent light bulbs positioned at a distance of 20 cm from the agar surface (average fluence rate was 35 $\mu E\ m^{-2}\ sec^{-1}$). Dark conditions were obtained by loosely wrapping single plates in aluminum foil. Broad wavelength red light (610 to 720 nm) was produced either by wrapping General Electric 20-W broad-spectrum fluorescent light bulbs housed in the Percival incubator with GAMCOLOR sharp cutoff gelatin filters or by placing a sheet of Red Shinkolite (Argo Plastic, Los Angeles, CA) between the bulbs and petri plates. In both cases, the average fluence rate was 20 $\mu E\ m^{-2}\ sec^{-1}$. All fluence rates were measured with a photometer (Li-Corp. Inc., Lincoln, NE), model LI-189.

Strains of *Aspergillus nidulans* that are responsive to blue light display a sharp peak of sensitivity at 436 nm (Champe *et al.* 1994; L. N. Yager, unpublished results). Blue light was produced by passing white light from either a projector lamp bulb (DFD, 120 V, 1000 W; Philips Lighting Co., Somerset, NJ) or an Osram tungsten-halogen incandescent lamp (FA4A, 120 V, 500 W) through a Corion (Holliston, MA) 436 ± 10-nm interference filter. Heat from the lamp was dissipated by directing forced air from a Shaded-Pole blower (model no. 4C441A; Dayton) onto the lamp. Heat was further reduced by passing the light generated from the lamp through a glass vessel (10 × 6 × 15 mm) containing 1.5 mm $CuSO_4 \cdot 5\ H_2O$ and focusing the light through a lens (U.S. Precision Lens, Inc.) onto the interference filter. The interference filter was fitted in a mask of black poster board cut to cover the lid of the petri dish. A piece of the same black poster board was also placed on the bottom of the dish, and the edges were wrapped with aluminum foil during incubation to reduce light scattering. The intensity of focused light was controlled by connecting the light source to a variable autotransformer (Powerstat model 1126 or 3PN16B). The average fluence rate of blue light produced by this apparatus was 5 $\mu E\ m^{-2}\ sec^{-1}$.

Colonies do not normally conidiate in submerged culture but do so readily after they have acquired developmental competence and are exposed to air (Axelrod *et al.* 1973). The transfer of mycelia from liquid to solid culture, termed induction, is used to initiate and synchronize conidiation (Timberlake 1980). For conidial yield determinations and RNA analysis, colonies were induced at 36 hr after inoculation of spores into liquid medium, which is well after the acquisition of competence, exposed to the appropriate illumination condition and then harvested at the designated time. Conidial yield determinations were performed as described in Yager *et al.* (1982). Radial colonial growth rates, which obey a linear growth relationship, were determined as described in Yager *et al.* (1982). The time of conidiophore vesicle appearance was scored by microscopic examination using the method of Axelrod *et al.* (1973).

Isolation of a red light-insensitive mutant: Approximately 5×10^4 conidia of strain WIM 126 (*yA2 pabaA1*; *veA*$^+$ (*bliA1*)) were suspended in 10 ml of deionized water and irradiated with ultraviolet light at a dosage that produced 10% survival (700 $\mu W\ cm^{-2}$ for 40 sec). A total of 5×10^5 conidia were mutagenized, plated at a density of 100 conidia/plate, and incubated in broad spectrum red light for 3 days at 32°. Approximately 500 presumptive aconidial colonies were selected and screened for their responses to white and blue light. Two mutants that failed to conidiate in red light, but conidiated in white and blue light, were isolated. However, only one mutant was selected for study; the other displayed a leaky aconidial phenotype that interfered with analysis. The selected isolate was backcrossed to TU 95 (*nicB8*; *veA*$^+$ (*bliA1*)) before further analysis.

Nucleic acid manipulations: *A. nidulans* DNA-mediated transformation was performed as described by Yelton *et al.* (1984) and Miller *et al.* (1985). Standard methods for construction, maintenance, and isolation of recombinant plasmids were used (Sambrook *et al.* 1989). All wild-type *fluG* genomic DNA fragments were obtained from plasmid pFM1 (Adams *et al.* 1992), which contains the entire *fluG* gene within a 7-kb *Bam*HI-*Hin*dIII fragment. Fragments tested for mutant complementation following transformation were subcloned into pPK1 or pSM3. Plasmid pPK1 was constructed by replacing the f1 origin of replication located at the *Ssp*I site in pBluescript SK$^-$ (Stratagene, La Jolla, CA) with the *argB* gene contained in the 1.9-kb *Bam*HI fragment from pSALARGB (Berse *et al.* 1983). Plasmid pSM3 was constructed by replacing the f1 origin of replication located at the *Ssp*I site in pBluescript SK$^-$ with the *pyroA* gene contained in the 1.9-kb *Xba*I-*Bam*HI fragment from p14 (May *et al.* 1989).

To isolate the *fluG701* allele, a genomic library was first constructed from strain TU 114 (*yA2 pabaA1*; *fluG701*; *veA1*). Fragments between 6 and 9 kb were isolated from *Bam*HI-*Hin*dIII-restricted DNA and inserted into pGEM3Zf(−) (Promega, Madison, WI). The probe used to screen this library was prepared by first isolating the 2.5-kb *Xho*I fragment from pFM1 and then radiolabeling the fragment with ^{32}P by random priming. The recovered plasmid was designated pJZ1.

DNA sequencing on isolated clones was performed by the chain-termination method using the Sequenase V2.0 kit (Amersham, Clearbrook, IL) following the procedure provided by the manufacturer. Uncloned genomic DNA was sequenced using the Sequitherm Cycle Sequencing kit (Epicentre Technologies, Madison, WI) according to the manufacturer's directions. The primers used for both procedures are listed below and designated as follows (name of oligonucleotide, sequence, location of 5′ end, annealing temperature used for TAQ cycle sequencing): TA141-8, CGTCGGCTGATCAATTG, 32, 54°; TA141-3, AGGTCTCCCCATTCATC, 125, 50°; TA141-12, GCAGATCATATCTGAGG, 406, 48°; FLUG-04, ATGAC GAGCTTTGGT, 513, 44°; FLUG-07, TTTGGGAGTCGTTCT, 789, 42°; BN1, CAGCAGATTGATGTC, 1115, 42°; ACODFUS1, GCCAGATATCCAGCCTC, 1222, 54°; PEP-8, CTGCATTCTG CTGAT, 1424, 42°; PEP-4, TCAATGGTATAATCACC, 1507,

44°; FLUG-15, TGGTGCCCAGACGAT, 1609, 46°; FLUG-18, TTATACCAGACCTAT, 1851, 38°; PEP-7, CCAAACTCGTCT TTGAG, 2008, 48°; PEP-1, ACCCATCAACAGGAGAGG, 2064, 54°; PEP-6, TTGTCAGGTGGGAGGATG, 2260, 54°; FLUG-23, TTCCATCTCCCCTTCAA, 2395, 46°; PEP-5, TTAACGCGGT CGTAGC, 2506, 48°; FLUG-26, CTCACTATCAAGGATT, 2687, 42°; BN3, GATTAGACTCAATACCTC, 3000, 46°; and FLUG-30, AGGCCGTGGAAATATT, 3036, 44°. The location of each oligonucleotide refers to its position relative to the sequence of the *fluG* gene shown in Lee and Adams (1994a). FLUG oligonucleotides were derived in this laboratory. TA, BN, PEP, and ACODFUS1 oligonucleotides were donated by Thomas Adams. Any differences observed between mutant and wild-type sequences for those results obtained from uncloned DNA were verified by repeating the sequencing from three independently isolated batches of DNA. Additional confirmation was obtained by sequencing both strands of DNA around the location of each mutation.

Total RNA was isolated as described in Mooney and Yager (1990), separated by electrophoresis in formaldehyde-agarose gels, and transferred without pretreatment to nylon membranes (Hybond-N; Amersham, Clearbrook, IL). RNA was bound to membranes and hybridized to ^{32}P-labeled random primed probes according to the procedures recommended by the manufacturer. The 2.5-kb *Xho*I fragment from pFM1 was used as a *fluG*-specific probe. The 1.9-kb *Bam*HI fragment from pSALARGB was used as an *argB*-specific probe. Both fragments were gel purified before labeling.

RESULTS

Isolation and genetic analysis of a mutant that fails to conidiate in red light: Mutagenesis of the *veA*$^+$; *bliA1* strain, WIM 126 (Table 1), yielded a red light-insensitive mutant that was identified by its ability to produce normal developmental structures in either blue light or full spectrum white light, but not in red light. To demonstrate that this mutation behaved as a single Mendelian allele, the mutant strain was meiotically crossed to TU

TABLE 1

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

Designation	Genotype	Source
FGSC A4	*veA*$^+$ (*bliA*$^+$)	Fungal Genetics Stock Center (FGSC)
FGSC A283	*adE20 suA1adE20 yA2*; *AcrA1*; *galA1*; *pyroA4*; *facA303*; *sB3*;*nicB8*; *riboB2 veA1*	FGSC
FGSC A457	*proA1 biA1*; *galE9 sc12 dilA1 phenA2*; *choA1*	FGSC
FGSC A503	*proA1 yA2*; *methH2 dilA1*	FGSC
M3188	*biA1*; *argB2*; methG1; *veA1*	G. May
THL135.1	*yA2 pabaA1*; Δ*fluG::argB argB2*; *pyroA4*; *veA*$^+$	This study
TTA127.4	*yA2 pabaA1*; Δ*fluG::trpC*; *trpC801 veA1*	Lee and Adams (1994a)
TU1	*pyroA4*; *veA1*	This lab
TU5	*fluG102*; *pyroA4*; *veA1*	This lab
TU11	*fluG684*; *pyroA4*; *veA1*	This lab
TU 39[a]	*fluG10*^{sveA1}; *pyroA4*; veA1	Mooney *et al.* (1990)
TU 41[b]	*fluG20*^{sveA1}; *pyroA4*; *veA1*	Mooney *et al.* (1990)
TU 42[c]	*fluG30*^{sveA1}; *pyroA4*; *veA1*	Mooney *et al.* (1990)
TU 49[c]	*yA2 pabaA1*; *fluG30*^{sveA1}; *veA1*	Mooney *et al.* (1990)
TU 53[a]	*yA2 pabaA1*; *fluG10*^{sveA1}; *veA1*	Mooney *et al.* (1990)
TU 55[b]	*yA2 pabaA1*; *fluG20*^{sveA1}; *veA1*	Mooney *et al.* (1990)
TU 91	*yA2 pabaA1*; *fluG701*; *veA*$^+$ (*bliA1*)	This study
TU 95	*nicB8*; *veA*$^+$ (*bliA1*)	This lab
TU 110	*pabaA1*; *fluG701*; *veA*$^+$ (*bliA1*)	This study
TU 108	*pabaA1*; *fluG701*; *veA1*	This study
TU 114	*yA2 pabaA1*; *fluG701*; *veA1*	This study
TU 120	*fluG701*; *wA3 pyroA4*; *veA1*	This study
TU 135	*ya2 pabaA1*; *argB2*; *pyroA4*; *veA*$^+$	This lab
TU 136	*yA2 pabaA1*; *fluG701 argB2*; *veA1*	This study
WIM 009	*fluG102*; *veA*$^+$	Yager *et al.* (1982)
WIM 020	*fluG684*; *veA*$^+$	Yager *et al.* (1982)
WIM 064	*yA2 pabaA1*; *veA1*	S. P. Champe
WIM 126	*yA2 pabaA1*; *veA*$^+$ (bliA1)	S. P. Champe

TU and THL strains were derived in this laboratory, FGSC strains were obtained from the Fungal Genetics Stock Center, the M strain was obtained from Greg May, the TTA strain was obtained from Thomas Adams, and the WIM strains were obtained from Sewell P. Champe. Strains bearing the *bliA1* allele conidiate in response to blue light. This allele is indicated in parentheses because it has not been assigned to a specific locus. Only strains tested for blue light responsiveness were designated *bliA1* or *bliA*$^+$.

[a] The *suC1veA1* mutation has been renamed *fluG10*^{sveA1}. TU 53 is available from the FGSC as strain A861.

[b] The *suC2veA1* mutation has been renamed *fluG20*^{sveA1}. TU 55 is available from the FGSC as strain A863.

[c] The *suC3veA1* mutation has been renamed *fluG30*^{sveA1}. TU 49 is available from the FGSC as strain A858.

95. Analysis of 120 progeny showed that the mutation segregated from its respective wild-type allele in a 1:1 ratio (wild-type:mutant; 59:61; $\chi^2 = 0.034$, d.f. = 1, $P > 0.9$) and assorted independently from unlinked mutations ($P > 0.99$ for *nicB8*, *yA2*, and *pabaA1*; χ^2 test).

The red light-insensitive mutant displays an aconidial phenotype at 42° regardless of illumination in addition to its inability to respond to red light at 32°. Unlike the red light-insensitive phenotype, the temperature-sensitive aconidial phenotype is observed in strains carrying either veA^+ or *veA1* alleles. Both the red light-insensitive and temperature-sensitive aconidial phenotypes were found to cosegregate in an analysis of the progeny from the above cross and other crosses not shown. Although the genetic analyses described below were simplified by assaying for the temperature-sensitive phenotype, selected progeny from each cross were also examined for the red light-insensitive phenotype. In all cases, both phenotypes cosegregated and resulted in identical conclusions.

The red light-insensitive mutation was tested for dominance by constructing a heterozygous diploid. Table 2 shows that this diploid was conidial and indistinguishable from a homozygous wild-type control, indicating that the red light-insensitive mutation (designated *fluG701* for reasons described below) is recessive to its wild-type allele. Diploids heterozygous for the red light-insensitive mutation and carrying multiple genetic markers were next constructed and subjected to mitotic haploidization. Analysis of the resulting progeny indicated that the red light-insensitive mutation is linked to chromosome *III.* Furthermore, analysis of progeny from meiotic crosses to chromosome *III* markers positioned the red light-insensitive mutation within 0.4% recombination distance from the *fluG102* mutation (4 wild-type recombinants recovered from 2000 total progeny) and <0.1% recombination distance from the *fluG684* mutation (0 wild-type recombinants recovered from 2000 total progeny). These latter two mutations display a temperature-sensitive aconidial phenotype with the concomitant production of fluffy, aerial hyphae (Yager *et al.* 1982; Adams *et al.* 1992). To show whether the red light-insensitive mutation is an allele of *fluG*, heterozygous diploids were constructed between the mutation and either *fluG102* or *fluG684.* Table 2 shows that both diploids display the fluffy, temperature-sensitive aconidial phenotype characterized by the homozygous diploid control and parental mutant strains. These data indicate that the red light-insensitive mutation is an allele of *fluG* and is thus designated *fluG701.*

TABLE 2

Phenotypes associated with *fluG* alleles in diploid strains

Genotype	Phenotype
+/*fluG701*	Conidial
fluG701/fluG701	Fluffy aconidial
+/+	Conidial
fluG701/fluG102	Fluffy aconidial
fluG701/fluG684	Fluffy aconidial
$fluG10^{sveA1}$/*fluG102*	Fluffy aconidial
$fluG10^{sveA1}$/*fluG684*	Fluffy aconidial
$fluG10^{sveA1}/fluG10^{sveA1}$	Fluffy aconidial
+/$fluG10^{sveA1}$	Conidial

Colonies were incubated at 42° in continuous white light at an intensity of 35 $\mu E\ m^{-2} sec^{-1}$ and then scored at 48 hr. All diploids were constructed in a homozygous *veA1* background.

Characterization of development and vegetative growth: Figure 1A shows that there is no significant difference in conidial yield between *fluG701* and wild-type strains for colonies incubated either in full spectrum white light or in blue light. However, incubation in red light decreases the conidial yield of the mutant strain by a factor of approximately 10^5 conidia per colony from that observed in the wild-type strain, a level equivalent to dark levels. As expected, conidial yields are negligible in all dark-grown colonies.

To show whether the *fluG701* mutation also affects vegetative growth, radial growth-rate measurements were performed on wild-type and mutant strains under different illumination conditions. The vegetative growth rate is not significantly affected by different illumination conditions or by the allelic state of *fluG* (Figure 1B). Furthermore, *fluG701* does not affect developmental kinetics. The time of conidiophore vesicle appearance under those conditions that promote conidiation is not significantly different between mutant and wild-type strains (6 ± 0.5 hr).

Suppressors of the *veA1* mutation are alleles of *fluG*: Mooney *et al.* (1990) identified six extragenic suppressors of the *veA1* mutation that restore light-dependent conidiation. Three of these suppressors, *suC1veA1*, *suC2-veA1*, and *suC3veA1*, define a single complementation group that is linked to chromosome *III* and whose *veA1* suppressors also display a temperature-sensitive aconidial, fluffy phenotype. We have previously shown that this temperature-sensitive phenotype cosegregates with restoration of the light-dependent conidiation phenotype (Mooney *et al.* 1990) and have used this temperature-sensitive characteristic to simplify the genetic analysis of these mutations. Construction of heterozygous diploids between *suC1veA1* and either *fluG102* or *fluG684* yield colonies displaying a temperature-sensitive aconidial, fluffy phenotype that is indistinguishable from homozygous diploid and parental strain controls (Table 2). Since these data indicate that *suC1veA1* is an allele of *fluG*, this mutation has been renamed $fluG10^{sveA1}$. Similarly, *suC2veA1* will be referred to as $fluG20^{sveA1}$, and *suC3veA1* will be referred to as $fluG30^{sveA1}$.

Table 3 classifies the known *fluG* alleles into five groups according to their conidial response to different illumination and temperature conditions. A comparison of the effect of the allelic state of the *veA* gene on

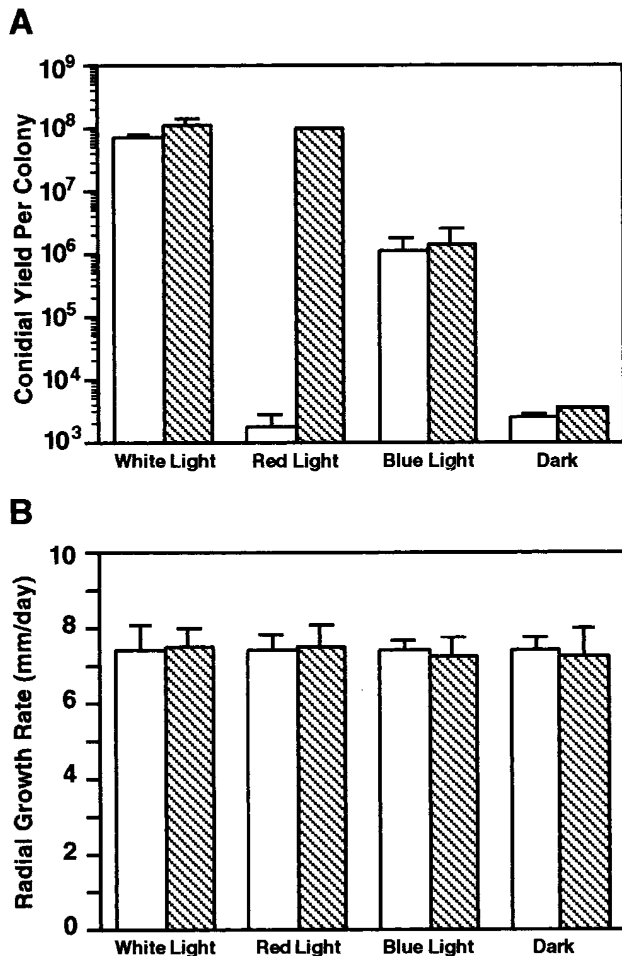

Figure 1.—Comparison of the effect of different wavelengths of light on conidiation and vegetative growth between *fluG701* and wild-type strains. Strains TU 91 (*fluG701*; open bars) and WIM 126 (*fluG*$^+$; hatched bars) were incubated on complete medium at 32° in either full-spectrum white light (35 μE m^{-2} sec^{-1}), red light (610 to 720 nm; 20 μE m^{-2} sec^{-1}), blue light (436 nm; 5 μE m^{-2} sec^{-1}), or the dark. (A) Conidial yield per colony was determined 24 hr after induction by the method of Yager *et al.* (1982). $N = 5$ for all determinations; means are $\pm$ standard deviation. (B) $N = 12$ for all radial growth-rate determinations; means are $\pm$ standard deviation. An ANOVA analysis indicated no significant effect of genotype on growth in different illumination conditions ($P > 0.05$) and no significant difference of growth rate between allelic states ($P > 0.05$).

the phenotype of the different *fluG* alleles is also shown. The first class, defined by the single member *fluG701*, contains the only mutation that fails to conidiate in red light at 32°. Placing this mutation into a *veA1* background allows conidiation to occur both in the dark and in red light. The second class contains mutations of *fluG* that suppress the *veA1* phenotype. These mutations restore light-dependent conidiation in strains bearing the *veA1* mutation. Placing these mutations into a *veA*$^+$ background does not affect the conidial phenotype. The third class contains mutations that display a temperature-sensitive aconidial phenotype but do not show an abnormal response to light at the permissive temperature. Their conidial phenotype at 32° is identical to the wild type in both *veA*$^+$ and *veA1* backgrounds. The fourth class is defined by a *fluG* deletion mutation that is aconidial in all illumination and temperature conditions and whose conidial phenotype is unaffected by the allelic state of the *veA* gene. These four classes of *fluG* mutations all show an aconidial phenotype at 42°. The fifth class describes the phenotype of the wild-type *fluG* allele. Although *fluG*$^+$; *veA*$^+$ strains display light-dependent conidiation at 32°, conidiation occurs at 42° regardless of the presence or absence of light.

Although the allelic state of *veA* determines the conidial response to light, it also affects colony morphology. Table 3 shows that all *fluG* mutant strains that contain the *veA1* allele produce fluffy, aerial hyphae under conditions that suppress conidiation, a common characteristic of all previously described *fluG* mutations (Yager *et al.* 1982; Adams *et al.* 1992). However, fluffy, aerial hyphae are not observed under conditions that result in an aconidial phenotype either in *fluG701* or the *veA1* suppressor strains that contain the *veA*$^+$ allele.

Molecular identification of *fluG* mutations: Transformation events in *A. nidulans* can result in ectopic integration of exogenous DNA into the genome (Yelton *et al.* 1984). Nevertheless, a mutation can be "repaired" by a recombinational event that replaces the faulty region with wild-type sequences, if a homologous recombinational event can be selected at the endogenous target gene. Complementation of the mutant phenotype after transformation with different restriction enzyme fragments derived from the wild-type *fluG* clone, pFM1, was used to localize the various light-dependent mutations to specific regions within the *fluG* gene.

The *fluG* gene is contained within the 7-kb *Bam*HI-*Hin*dIII restriction fragment shown in Figure 2A. This fragment complements the *fluG701*, *fluG10*^{sveA1}, *fluG20*^{sveA1}, and *fluG30*^{sveA1} mutations. Both *fluG701* and *fluG10*^{sveA1} are contained within the 1.9-kb *Xho*I-*Eco*RI fragment as indicated by complementation with the 4.3-kb *Bam*HI-*Eco*RI and 2.8-kb internal *Xho*I fragments but not with the 2.7-kb *Eco*RI-*Hin*dIII fragment. Similarly, the *fluG30*^{sveA1} mutation is contained within the 0.9-kb *Eco*RI-*Xho*I fragment as demonstrated by complementation with the 2.8-kb internal *Xho*I and 2.7-kb *Eco*RI-*Hin*dIII fragments. The *fluG20*^{sveA1} mutation is located within the 2.8-kb internal *Xho*I fragment as indicated by complementation only by this subfragment from the entire 7-kb *Bam*HI-*Hin*dIII restriction fragment.

The molecular identity of each *fluG* light-dependent mutation was determined by DNA sequencing of the smallest overlapping region among restriction fragments that complement each mutant phenotype. In the case of *fluG701*, the *Bam*HI-*Hin*dIII fragment containing the mutant sequences was first cloned from strain TU 114 (Table 1) and the 2.8-kb internal *Xho*I fragment was later sequenced. As shown in Figure 2B, the *fluG701*

TABLE 3

Phenotypes associated with *fluG* alleles

Class	Allele	Genotype	Phenotypes 32° WL	RL	BL	D	42° WL	RL	D
Red light insensitive	*fluG701*	*fluG701*; *veA*$^+$	C	A	C	A	A	A	A
		fluG701; *veA1*	C	C	C	C	FA	FA	FA
veA1 suppressor	*fluG10*^{sveA1}	*fluG10*^{sveA1}; *veA*$^+$	C	C	ND	A	A	A	A
	fluG20^{sveA1}	*fluG10*^{sveA1}; *veA1*	C	C	ND	FA	FA	FA	FA
	fluG30^{sveA1}								
Temperature sensitive	*fluG102*	*fluG102*; *veA*$^+$	C	C	ND	A	FA	FA	FA
	fluG684	*fluG102*; *veA1*	C	C	ND	C	FA	FA	FA
Null	Δ*fluG*	Δ*fluG*; *veA*$^+$	FA	FA	ND	FA	FA	FA	FA
		Δ*fluG*; *veA1*	FA	FA	ND	FA	FA	FA	FA
Wild type	*fluG*$^+$	*fluG*$^+$; *veA*$^+$	C	C	C	A	C	C	C
		fluG$^+$; *veA1*	C	C	C	C	C	C	C

Colonies were incubated either in white light (WL; 35 μE m^{-2} sec^{-1}), red light (RL; 610 to 720 nm; 20 μE m^{-2} sec^{-1}), blue light (BL; 436 nm; 5 μE m^{-2} sec^{-1}), or the dark (D). All phenotypes were examined on complete medium. 42° in blue light was not done. The *fluG701* and wild-type *veA*$^+$ strains contain the *bliA1* allele. The symbols represent the phenotype of colonies possessing the indicated allele. C, conidial; A, aconidial; FA, aconidial with fluffy, profuse aerial hyphae; ND, not done.

mutation results from a G to A transition at nucleotide position 1739 that changes Lys467 to Glu. The identities of *fluG10*^{sveA1}, *fluG20*^{sveA1}, and *fluG30*^{sveA1} were determined by PCR amplification followed by DNA sequencing of genomic DNA from those selected regions shown to contain each mutation. Figure 2B indicates that *fluG10*^{sveA1} is caused by a T to G transversion mutation at nucleotide position 1032 that converts Leu248 to a stop codon. The *fluG20*^{sveA1} mutation is caused by a T to C transition that changes Leu570 to Ser. The *fluG30*^{sveA1} mutation is caused by a T to C transition that abolishes the normal termination codon such that translation continues to the next stop codon. This results in the addition of two amino acids, arginine and valine, to the carboxyl-terminal end of the FLUG protein.

Transcription of *fluG* in wild-type and mutant strains: Lee and Adams (1994a) showed that *fluG* transcription is constitutive and not developmentally regulated in *veA1* strains. We obtain the same results in a *veA*$^+$ strain (data not shown). Relatively constant levels of *fluG* transcripts are observed throughout development regardless of whether cultures are incubated in white light, red light, or the dark. In addition, Lee and Adams (1994a) demonstrated that disruption of the *fluG* gene results in increased accumulation of altered *fluG* transcripts, indicating that *fluG* expression is negatively autoregu-

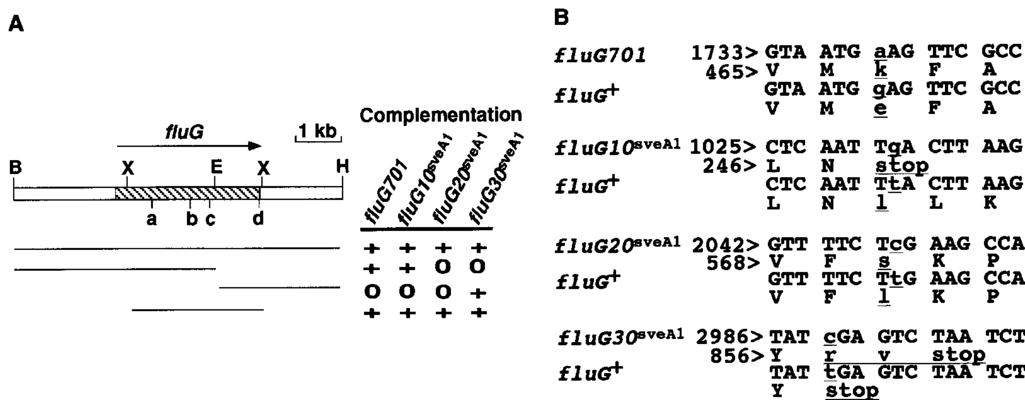

Figure 2.—Molecular identity of *fluG* mutations. (A) The 7-kb genomic region containing the *fluG* gene as defined by Adams *et al.* (1992) is shown. The hatched region and arrow indicate the transcription unit and direction of transcription. Uppercase letters indicate the location of the restriction enzymes sites for *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), and *Xho*I (X). The indicated restriction fragments were cloned from plasmid pFM1 into pPK1 or pSM3 and transformed by the method of Yelton *et al.* (1984) into strains TU 39, TU 41, TU 42, and TU 136. The location of each mutation can be deduced by examining the smallest overlapping region among restriction fragments that complement each mutant phenotype. Lowercase letters indicate the location of each mutation: a, *fluG10*^{sveA1}; b, *fluG701*; c, *fluG20*^{sveA1}; and d, *fluG30*^{sveA1}. (B) The predicted mutant regions were sequenced and compared to the wild type. Differences in nucleotide and predicted amino acid sequences are indicated. The numbered position of nucleotides and amino acids refers to the *fluG* sequence shown in Lee and Adams (1994a).

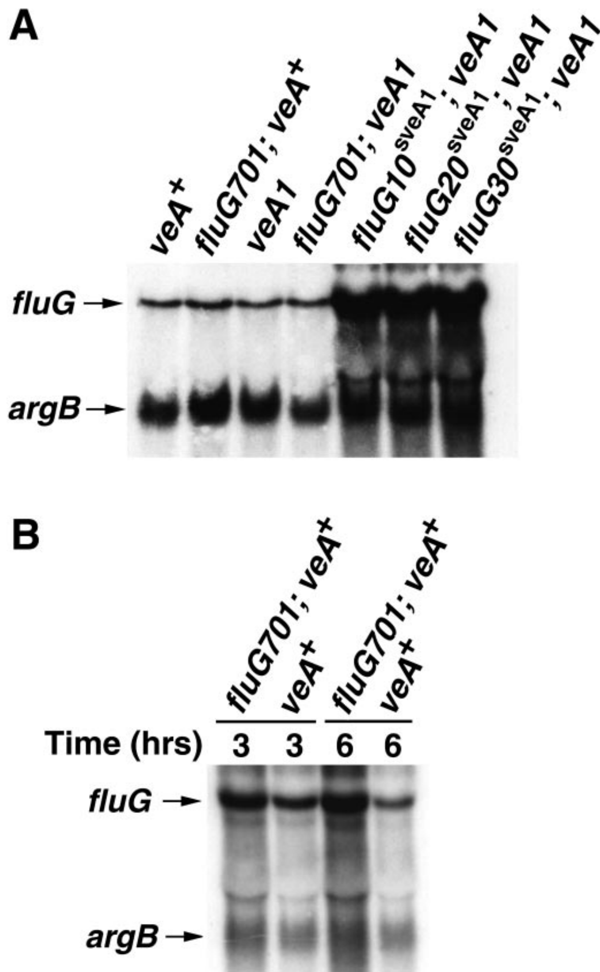

Figure 3.—Northern analysis of *fluG* mutants. Strains WIM 126 (*veA*$^+$), WIM 064 (*veA1*), TU 110 (*fluG701*; *veA*$^+$), TU 114 (*fluG701*; *veA1*), TU 53 (*fluG10*^{sveA1}; *veA1*), TU 55 (*flu G20*^{sveA1}, *veA1*) and TU 49 (*fluG30*^{sveA1}; *veA1*) were incubated either in submerged culture at 32° for 24 hr (A) or induced and exposed to red light (610 to 720 nm; 20 μE m^{-2} sec^{-1}) at 32° for the indicated time (B). Total RNA was extracted and 50 μg were loaded per gel lane. Blots were hybridized with *fluG*- and *argB*-specific probes. The *argB* gene, which serves as a control, has been shown to be expressed at a constant level throughout development (Yelton *et al.* 1983).

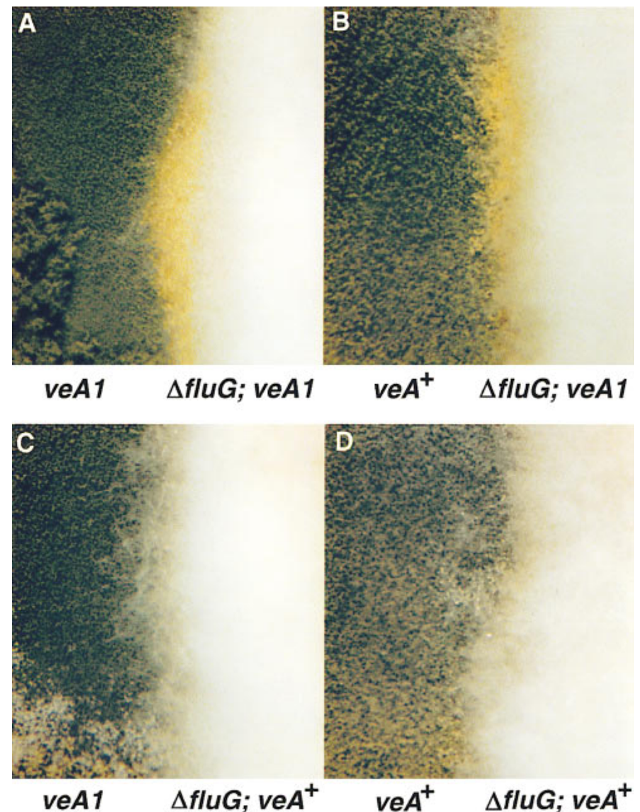

Figure 4.—The effect of the allelic state of the *veA* gene on extracellular rescue of the *fluG* mutant phenotype. The yellow-spored *fluG* deletion *veA1* strain, TTA 127.4, was grown adjacent to either the green-spored *veA1* strain, TU 1 (A), or the green-spored *veA*$^+$ strain, FGSC 4 (B). The yellow-spored *fluG* deletion *veA*$^+$ strain, THL 135.1, was grown adjacent to either the green-spored *veA1* strain, TU 1 (C), or the green-spored *veA*$^+$ strain, FGSC 4 (D). All incubations were performed on complete medium at 32° in full spectrum white light (35 μE m^{-2} sec^{-1}) and examined between 48 and 72 hr after inoculation. The *fluG* deletion strains were inoculated on the right side of each part and appear as white masses due to the aconidial fluffy mycelia growing out of the focal plane.

lated. We observe increased accumulation of *fluG* transcripts in vegetative (submerged) cultures in *fluG* mutations that suppress the *veA1* phenotype, but we do not detect increased accumulation of transcripts in the *fluG701* mutant (Figure 3A). Because the *fluG701* phenotype is only detectable in surface-grown cultures exposed to red light, we examined transcript accumulation in this mutant under this restrictive condition. As shown in Figure 3B, increased accumulation of mutant transcripts is still not detectable. Furthermore, we note that *fluG* transcript accumulation is not affected by the allelic state of the *veA* gene. Although changes in light-dependent activities are observed among different *fluG* and *veA* alleles, negative autoregulation of *fluG* transcription does not appear to be a light-related event.

Production of and response to the extracellular factor is affected by different strains and growth conditions: Conidiation can be rescued in a *fluG* deletion mutant by growing the mutant adjacent to a wild-type strain (Lee and Adams 1994a). In this experiment, both the strain that served as the producer of the extracellular factor and the recipient *fluG* deletion strain were genotypically *veA1.* We first sought to determine whether the allelic state of *veA* affects production of the extracellular factor by growing *veA*$^+$ and *veA1* strains next to a Δ*fluG*; *veA1* strain. As shown in Figure 4, A and B, both *veA*$^+$ and *veA1* strains are able to rescue conidiation in the yellow-spored deletion strain if incubation occurs in white light. However, if incubation occurs in the dark, a condition that suppresses conidiation in *veA*$^+$ strains, production of the factor is only observed in the *veA1* strain and not in the *veA*$^+$ strain (Table 4).

Lee and Adams (1994a) demonstrated that produc-

TABLE 4

Production of extracellular factor in wild-type and mutant strains

Genotype of producing strain	Illumiation condition	Rescue of conidiation in the Δ*fluG*; *veA1* recipient strain
veA^{+}	White light	+
veA^{+}	Red light	+
veA^{+}	Dark	0
veA1	White light	+
veA1	Dark	+
fluG701; *veA*$^{+}$	White light	+
fluG701; *veA*$^{+}$	Red light	0
fluG701; *veA*$^{+}$	Dark	0
fluG10^{sveA1}; *veA1*	White light	0
fluG10^{sveA1}; *veA1*	Dark	0
fluG20^{sveA1}; *veA1*	White light	0
fluG20^{sevA1}; *veA1*	Dark	0
fluG30^{sveA1}; *veA1*	White light	0
fluG30^{sveA1}; *veA1*	Dark	0

The Δ*fluG*; veA1 strain, TTA 127.4, was grown adjacent to either a *veA*$^{+}$ strain (FGSC 4), a *veA1* strain (TU 1), the *fluG701* mutant (TU 110), the *fluG10*^{sveA1} mutant (TU39), the *fluG20*^{sveA1} mutant (TU 41), or the *fluG30*^{sveA1} mutant (TU 42). All incubations were performed on complete medium at 32° in either full spectrum white light (35 μE m^{-2} sec^{-1}), red light (610 to 720 nm; 20 μE m^{-2} sec^{-1}) or the dark. Cultures were examined between 48 and 72 hr for the ability of thte producing strain to rescue conidiation in the Δ*fluG*; *veA1* strain.

tion of the extracellular factor is dependent on a functional *fluG* gene. Not surprisingly, the *fluG701* mutant produces the factor only in white light, which is the permissive condition that allows conidiation in this mutant (Table 4). However, the *veA1* suppressors all fail to produce the extracellular factor in conditions that promote conidiation (surface-grown cultures exposed to white light; Table 4).

We next inquired whether the allelic state of *veA* affects the ability of a *fluG* deletion strain to respond to the extracellular factor. As shown in Figure 4, C and D, rescue is not observed in the yellow-spored Δ*fluG*; *veA*$^{+}$ strain regardless of whether it is grown adjacent to a *veA*$^{+}$ or *veA1* strain and despite the fact that all cultures are exposed to full spectrum white light.

DISCUSSION

Light is an important environmental stimulus that can elicit conidiation in wild-type strains of *A. nidulans.* We describe the isolation and characterization of two new classes of *fluG* mutant alleles that display abnormal responses to light. One class is defined by a mutation that causes failure to conidiate in red light in a *veA*$^{+}$ strain that is normally red light responsive. The other class includes mutations that restore red light-dependent conidiation to the *veA1* mutant that normally conidiates without dependence on red light. The *fluG* gene encodes a polypeptide having unknown function that shares limited identity to GSI-type prokaryotic glutamine synthetases (Lee and Adams 1994a). The identification of *fluG* mutant alleles that affect responses to light suggests the possibility that *fluG* encodes the red light photoreceptor. At the very least, the identification of these two mutant classes indicates that *fluG* activity influences and/or is affected by light-dependent functions.

Lee and Adams (1994a) showed that the *fluG* gene is involved in the production of an extracellular factor that is necessary for the activation of the major programmed pathway of asexual development. Since the FLUG polypeptide is constitutively expressed in both vegetative and developing cultures, they proposed that the extracellular factor accumulates independent of environmental conditions and that conidiation is induced only after a threshold level of the sporulation-inducing factor is achieved. This hypothesis was suggested by experiments performed in strains containing the *veA1* mutation. However, the inability of *fluG*$^{+}$; *veA*$^{+}$ strains to produce extracellular factor in the absence of light, despite constitutive expression of *fluG* transcription, suggests that environmental conditions may influence the production of the factor. Lee and Adams (1994a) have shown that the absence of conidiation is not sufficient to inhibit synthesis of the extracellular factor, because both aconidial *brlA* deletion and *flbA* deletion strains are able to rescue conidiation in a *fluG* deletion. Thus, the failure of *veA*$^{+}$ strains to produce the factor in the dark could indicate that light-dependent *veA* activities either precede or are required for those *fluG* functions associated with synthesis of the extracellular signal.

Although the FLUG polypeptide shares significant homology with GSI-type prokaryotic glutamine synthetases, Lee and Adams (1994a) indicated that it is unlikely that FLUG has glutamine synthetase activity. Rather, it is possible that FLUG has a related enzymatic activity that is responsible for the production of the

extracellular factor. We have shown, not unexpectedly, that production of the extracellular factor requires that a strain contain a wild-type *fluG* allele or an allele that produces a functional gene product under permissive conditions. Strains containing a disrupted gene do not produce the factor. GSI homologous sequences are located in the carboxyl terminal 436 amino acids. This region shares 28% identity and 50% similarity with GSI from *Bacillus cereus.* The *fluG701*, *fluG20*^{sveA1}, and *fluG30*^{sveA1} mutations are all located within the GSI homologous region, suggesting that these mutations may be disrupting the postulated enzymatic activity of FLUG. Although the *fluG10*^{sveA1} mutation is located upstream of the GSI homologous domain, its identity as a nonsense mutation is consistent with the disrupted enzymatic activity of *fluG.*

The perception of the extracellular signal does not require *fluG* function, as evidenced by phenotypic complementation of a Δ*fluG*; *veA1* strain when inoculated adjacent to either *veA*$^+$ or *veA1* strains. But why can't conidiation be rescued in Δ*fluG*; *veA*$^+$ strains? An attractive explanation is that signal perception may be repressed in a *veA*$^+$ strain and that derepression requires *fluG* activity. The possibility for such an interaction between these gene products is supported by the identification of mutations in *fluG* that suppress the *veA1* phenotype. However, the possibility that *veA*$^+$ strains are simply unable to respond to the sporulation-inducing factor regardless of which *fluG* allele is present cannot be excluded, though it would seem unlikely that *veA*$^+$ strains that produce this factor would be constitutively unable to respond to it.

It is clear that *fluG* participates in the synthesis of the extracellular factor, but it is also apparent that *fluG* influences light-dependent activities associated with conidiation. Examples of *fluG*'s effect on light-dependent conidiation include the loss of red-light perception in the *fluG701* mutant and the identification of specific *fluG* mutations that suppress the *veA1* mutation. Although Lee and Adams (1994a) suggest that the enzymatic domain of FLUG involved with production of the extracellular factor lies within the carboxyl-terminal 436 amino acid, it is unclear what portion of the molecule participates in light-dependent functions. Since the *fluG10*^{sveA1} mutation introduces a stop codon at amino acid position 248, it is possible that the amino-terminal end of FLUG functions in light-related activities. However, a strain bearing a *fluG* mutant allele that encodes a truncated form of FLUG containing only its first 250 amino acids is phenotypically different from *fluG10*^{sveA1} and does not suppress the *veA1* mutation (unpublished data). The reasons for this difference are not clear at present, but it is possible that the phenotype of *fluG-10*^{sveA1} arises not from high-level production of a truncated polypeptide but from low-level production of an altered full-length polypeptide, *e.g.*, through low-level read-through of the premature termination codon by nonsense codon suppression.

The identification of *fluG* mutations that suppress the *veA1* phenotype suggests a genetic interaction between these two genes. A simple model to explain the relationship between the *fluG* and *veA* is that *veA* functions as an integrator of the light signal and modulates the expression of *fluG*, thereby regulating the extracellular signal. Mooney and Yager (1990) speculated that *veA* functions as a negative regulator of conidiation-specific gene expression. Thus, as suggested above, the expression of *fluG* could be derepressed in *veA1* strains. Alternatively, an interaction between FLUG and VEA might be necessary to effect *fluG* function. Other known signal integrators control the transcriptional activity of downstream regulatory elements (Bradford *et al.* 1996; Kamei *et al.* 1996). However, neither light nor the allelic state of *veA* appears to affect the level of *fluG* mRNA, suggesting that, if VEA has effects on *fluG* expression, then these effects are posttranscriptional.

It is unlikely that *veA* and *fluG* form a strict linear pathway. The ability of the extracellular factor to rescue conidiation in a Δ*fluG*; *veA1* strain, but not in a Δ*fluG*; *veA*$^+$ strain, suggests that *veA* activity could be also affecting the expression of downstream conidiation-specific genes. For example, *veA* could act as a general repressor of certain developmental functions. Downstream gene activation would require derepression by the *veA* signal integrator followed by initiation of functions that are regulated by the *fluG* signal. In a *veA1* strain, this repression is absent, and downstream gene activation requires only that these functions be initiated by the *fluG* signal. This dual activation mechanism would insure that conidiation proceeds only in response to correct environmental and internal preprogrammed signals.

Although certain developmental events occur in response to genetically preprogrammed signals, the initiation of conidiation is also responsive to specific external stimuli. Light appears to influence the activities of both the FLUG and VEA gene products. Conversely, the activities of these genes may also be affecting other light-related events. An understanding of how *veA* and *fluG* influence each other's function and how these two genes coordinate the activities of other conidiation-specific genes remain important questions to be solved.

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