flbD encodes a Myb-like DNA-binding protein that coordinates initiation of *Aspergillus nidulans* conidiophore development

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The timing of asexual fruiting body formation during *Aspergillus nidulans* colony development is precisely regulated so that conidiophores are typically produced 1–2 mm behind the growing edge of the colony. Mutations in any of four *A. nidulans* genes, *flbB*, *flbC*, *flbD*, or *flbE*, result in colonies that are delayed at least 24 hr in their ability to initiate conidiophore development resulting in fluffy colonies with conidiophores forming in the center, at least 12–15 mm behind the growing edge. The requirement for each of these four genes in determining the timing of developmental initiation precedes transcriptional activation of the primary developmental regulatory gene *brlA*, indicating a possible role for each gene in developmentally regulated activation of *brlA* expression. The wild-type *flbD* gene was isolated and shown to encode an ~1.6-kb mRNA that is present throughout the *A. nidulans* life cycle. The deduced FlbD protein sequence predicts a 314-amino-acid polypeptide with significant identity at its amino terminus to the DNA-binding domain of the Myb family of transcription factors indicating that FlbD probably functions as a sequence-specific transcriptional activator. Although conidiophore development does not normally occur in submerged culture, forced overexpression of *flbD* in submerged hyphae caused inappropriate activation of *brlA* expression and resulted in production of complex conidiophores that produced all of the distinct cell types observed in wild-type conidiophores including viable spores. This ability of *flbD* overexpression to activate conidiation requires *brlA*, *flbB*, and *flbA* (another early developmental regulator) but does not require *flbC* or *flbE*. We propose that FlbD functions during normal development by activating transcription of other genes required for development (such as *brlA*) and that FlbD activity is normally controlled post-transcriptionally by an unknown mechanism.

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Asexual reproduction in the filamentous fungus *Aspergillus nidulans* is a continual progression that begins with newly forming hyphae at the growing edge of the colony and progresses through differentiation of specialized cells 1–2 mm behind the leading edge of the mycelium and gives rise to multicellular spore-bearing structures, termed conidiophores (Navarro-Bordonaba and Adams 1994). The initiation of *A. nidulans* conidiophore development, in contrast to initiation of sporulation in many other microorganisms, is not normally triggered by unfavorable environmental conditions but instead occurs as a precisely timed, genetically programmed event in the life cycle in response to internal and external cues (Pastushok and Axelrod 1976; Champe et al. 1981; Adams et al. 1992). The molecular genetic events regulating onset of conidiation are not fully understood but ultimately result in the controlled activation of a transcriptional cascade leading to the expression of hundreds of essential (Clutterbuck 1969; Marti-

nelli and Clutterbuck 1971; Stringer et al. 1991) and nonessential genes (Aramayo et al. 1989). Several lines of evidence indicate that a primary activator of sporulation-specific gene expression is *BrlA*, a C2H2 zinc finger nucleic acid-binding protein (Adams et al. 1988, 1990; Chang and Timberlake 1992). *brlA* mRNA is present at extremely low levels in hyphae and begins to accumulate shortly after developmental induction (Boylan et al. 1987; Han et al. 1993; Prade and Timberlake 1993). *brlA* is required for activation of other sporulation-specific genes including at least two developmental regulatory genes, *abaA* and *wetA* while *brlA* null mutants produce abnormal developmental structures that initially resemble conidiophore stalks but grow indeterminately, failing to differentiate other specialized cell types (Clutterbuck 1969; Boylan et al. 1987). The pivotal role for *brlA* in controlling activation of the conidiation pathway is most strongly supported by the observation that forced activation of *brlA* in vegetative hyphae re-

sults in misscheduled activation of other genes, including *abaA* and *wetA*, and causes hyphal tips to differentiate into reduced conidiophores that produce viable conidia (Adams et al. 1988). These results focus the question of what activates conidiation on what induces *brlA* expression.

We have identified and begun to characterize six separate loci, designated *fluG*, *flbA*, *flbB*, *flbC*, *flbD*, and *flbE*, that are independently required for normal activation of *brlA* expression (Wieser et al. 1994). Mutations in any one of these genes result in colonies that fail to make the programmed switch from undifferentiated vegetative hyphal growth at the edge of the colony to conidiophore development within. These mutants have diverse phenotypes, but all produce large masses of undifferentiated aerial hyphae that give the colony a “fluffy” or cotton-like appearance. Fluffy mutants resulting from loss of *fluG* function can be distinguished from strains with mutations in any of the other five genes in at least two ways. First, although *fluG* mutants are unable to activate *brlA* expression normally, the requirement for *fluG* can be partially overcome by limiting growth nutritionally (Adams et al. 1992). Second, the *fluG* mutant phenotype is suppressed when *fluG* mutants are grown next to wild-type colonies, even if the two colonies are separated by a dialysis membrane with a 6000- to 8000-dalton pore size (Lee and Adams 1994a). These results led us to propose that *fluG* is responsible for the synthesis of a low-molecular-weight factor that functions as an extracellular signal in activating the major programmed sporulation pathway. In the absence of *fluG*, however, development can be activated by a mechanism that involves sensing growth rate or nutrient status directly, bypassing the need for *FluG*.

Mutations in the *flbA* gene result in colonies that have abnormal hyphal morphology as well as being altered in their ability to produce conidiophores (Lee and Adams 1994b). The hyphae found at the leading edge of *flbA* mutant colonies grow close together in tight clumps rather than in a well-separated branching pattern as is observed for wild-type and other fluffy mutant colonies. In addition, *flbA* mutant colonies produce tightly interwoven aerial hyphae that autolyse beginning in the center of the colony so that by 5 days postinoculation the entire colony has disintegrated. The predicted *flbA* product is related to the *Saccharomyces cerevisiae* *SST2* gene product that has been implicated in regulating the activity of the G-protein-mediated signal transduction pathway that controls yeast mating (Dietzel and Kurjan 1987). The similarity observed between *flbA* and *SST2* has led to the proposal that *FlbA* could function to regulate the activity of a signal transduction pathway for responding to the putative *fluG* signal for conidiation (Lee and Adams 1994a,b). Although this hypothesis has not been tested directly, we have shown that *flbA*, like *brlA*, does have a direct role in activating development because overexpression of *flbA* in submerged culture is sufficient to cause activation of *brlA* expression and the production of viable spores (Lee and Adams 1994b).

Mutations in *flbB*, *flbC*, *flbD*, or *flbE* result in a third,

distinct class of fluffy mutant colony described as delayed conidiation (Wieser et al. 1994). This phenotype differs from the fluffy phenotype observed for *fluG* and *flbA* mutants in that delayed conidiation mutant colonies always produce conidiophores in the center after extended incubation and the colony margin remains fluffy. In this paper we describe the isolation and initial characterization of one delayed conidiation gene, *flbD*. The *flbD* gene is predicted to encode a 314-amino-acid polypeptide with significant identity in its amino terminus to the DNA-binding domain found in a group of transcription factors that includes the human proto-oncogene *c-myc* (Majello et al. 1986; Lüscher and Eisenman 1990). Forced overexpression of *flbD* in submerged culture leads to the production of complete conidiophores that include stalks, vesicles, and sterigmata that produce viable spores. This contrasts with development observed following forced expression of *brlA* or *flbA* that results in formation of reduced conidiophores with spores forming directly from hyphal tips (Adams et al. 1988; Lee and Adams 1994b). Because *flbD* mRNA is present at relatively constant levels in vegetative hyphae and after development initiates in wild-type cells, we propose that the activity of the putative *FlbD* DNA-binding protein is regulated post-transcriptionally in response to early sporulation signals resulting in the transcriptional activation of other developmental regulators, such as *brlA*.

Results

flbD is required for normal timing of conidiophore production

Figure 1 shows the growth of wild-type (FGSC26) and *flbD* mutant (TJW30.1) colonies 2 and 3 days after inoculation on complete medium. Conidiophores were observed within the center of the wild-type colony by 24 hr after inoculation, and by two days after inoculation conidiophores were seen throughout the colony (Fig. 1A). In contrast, no conidiophores were observed in the fluffy *flbD* mutant colony 24 hr after inoculation and two days after inoculation only a small number of conidiophores were observed within the center of the colony (Fig. 1C). By 3 days postinoculation the area of central conidiation in the *flbD* mutant colony had expanded so that conidiophores were present throughout a larger area (Fig. 1D). The *flbD* mutant colony remained somewhat fluffy, however, and the margin of undifferentiated hyphae at the edge of the colony was 10–12 mm greater than the 1- to 2-mm margin of nonconidiating vegetative hyphae seen at the edge of the wild-type colony.

Isolation and mapping of the *flbD* gene

The wild-type *flbD* gene was isolated by complementation of an *flbD*[−] mutant as described in Yelton et al. (1984). RBN070 (*biA1;argB2;flbD14*) was transformed with an *A. nidulans* genomic DNA library made in a

Figure 1. *flbD* is required for normal timing of conidiophore development. A wild-type strain (FGSC26; A,B) and an *flbD* mutant (TJW30.1; C,D) were point inoculated on complete medium and allowed to grow for 48 hr (A,C) or 72 hr (B,D). The wild-type and mutant strains were photographed at the same magnification.

cosmid vector containing the wild-type *argB*⁺ gene to use as a selectable marker (pUI1; kindly provided by Dr. Bruce Miller, University of Idaho, Moscow). Approximately 2000 *argB*⁺ transformants were selected on minimal medium lacking arginine, and two developmentally wild-type transformants were identified by visual screening (TJW001 and TJW002). Twenty-seven cosmids with identical restriction patterns were recovered following in vitro packaging of genomic DNA from TJW002 (Yelton et al. 1985), and three of these cosmids (designated pJW8, pJW9, and pJW10) were selected for further analyses. Two of the recovered cosmids, pJW8 and pJW10, complemented the *flbD14* mutation in RBN070 at high frequency indicating that they probably contain the entire wild-type *flbD* locus. Although pJW9 had identical restriction enzyme digestion patterns to pJW8 and pJW10, it did not rescue the *flbD14* mutation, suggesting it contained the *flbD14* mutant allele. The *flbD* complementing region in pJW10 was localized to a 3.2-kb *Bam*HI–*Kpn*I fragment as described in Materials and methods (Fig. 2).

Disruption of the flbD gene results in a delayed conidiation fluffy phenotype

To determine whether the cloned sequences corresponded to the wild-type *flbD* gene, we disrupted the putative *flbD*-coding region by transformation of a developmentally wild-type strain (FGSC237) with a plasmid containing the entire *trpC* gene inserted into a unique *Xho*I site located within the *flbD* gene, 401 bp

downstream of the predicted FlbD translational start site (Fig. 2A,B; see results below). Seven of the 42 *trpC*⁺ transformants had a delayed conidiation phenotype like that observed for the original *flbD14* mutant strain (RBN021). Southern blot analysis with genomic DNA from these transformants showed that all seven delayed conidiation mutants tested contained the predicted insertion, whereas all 4 *trpC*⁺*flbD*⁺ transformants examined maintained the wild-type *flbD* gene. Despite repeated attempts, we have not been able to construct a heterozygous diploid strain between the *flbD14* mutation and the putative *flbD* disruption mutant to determine allelism. However, the two mutations did not complement in heterokaryons, and when one *flbD* insertion mutant (TJW30.1) was crossed with RBN070 (*flbD14*), all 500 meiotic progeny analyzed had a delayed conidiation phenotype. This result indicates that the cloned region is closely linked to the original *flbD14* mutation, strongly supporting the idea that the *flbD* gene was disrupted in TJW30.1.

FlbD is related to c-myb

The DNA sequence of the *flbD* gene region was determined and is presented in Figure 2B. The *flbD* gene is predicted to encode a 314-amino-acid polypeptide. We compared the predicted FlbD sequence with other sequences in various data bases using the BLAST search algorithm (Altschul et al. 1990) and found that the first 108 amino acids shared >40% identity with the DNA-binding domains found in several transcription factors typified by the human proto-oncogene *c-myb* (Fig. 2C) (Majello et al. 1986). This region contains two or three imperfect repeats with 51 or 52 amino acids that typically include three critical tryptophan residues spaced either 18 or 19 residues apart (Lüscher and Eisenman 1990; Saikumar et al. 1990). The related region of FlbD contains two repeats, but each contains only two tryptophans with a tyrosine found at the position expected for the third tryptophan in the first repeat and a isoleucine found at the position expected for the first tryptophan in the second repeat (Fig. 2C). Both of these substitutions have been observed in other Myb-like proteins that remain functional as transcriptional activators (Paz-Ares et al. 1987; Tice-Baldwin et al. 1989). Other residues in the Myb-like repeats that have been shown to be important in DNA binding were also conserved, including a highly conserved cysteine residue in the first repeat that has been shown to have a role in redox regulation of Myb DNA binding (Frampton et al. 1991; Guehmann et al. 1992; Myrset et al. 1993). Sequence analysis of the *flbD* coding region in pJW9, the recovered cosmid proposed to contain the *flbD14* mutant allele (see above), indicated that the mutation resulted from a single base-pair change converting glutamic acid 67 in the conserved DNA-binding domain to a lysine (Fig. 2C). No significant similarities were observed between the remainder of the *flbD* coding region and other sequences in the various data bases.

Figure 3. *flbD* mRNA is present throughout the *A. nidulans* life cycle and is required for normal timing of *brlA* expression. (A) Total RNA was isolated from vegetative and developing cultures of an *flbD* insertion mutant (TJW30.1, lanes 1–5) and from a wild-type strain (FGSC26, lanes 6–10) and fractionated on a formaldehyde-agarose gel. The resultant gel blot was probed with an *flbD*-specific fragment (A) or with a *brlA*-specific fragment (B). RNA was isolated 22 hr after inoculation (lanes 1,6), 4 hr after developmental induction (lanes 2,7), 8 hr after developmental induction (lanes 3,8), 12 hr after developmental induction (lanes 4,9), and 24 hr after developmental induction (lanes 5,10). Relative sizes of mRNA are indicated at right. (C) Equal loading of total RNA was confirmed by ethidium bromide staining.

mined by measuring *brlA* promoter-directed *lacZ* expression. Less than 5% of wild-type *brlA*-directed β -galactosidase activity was detected in *flbD* mutant strains following developmental induction (Wieser et al. 1994). To confirm that *flbD* influences the timing of *brlA* transcript accumulation, we isolated total RNA from wild-type and *flbD* mutant strains at various times after induction and examined *brlA* expression patterns (Fig. 3B). *brlA* message first appeared in the wild-type strain between 4 and 8 hr after developmental induction and accumulated to higher levels as development proceeded. In contrast, no *brlA* mRNA was detected in the *flbD* mutant strain until 24 hr after developmental induction. This corresponded to the time when conidiophores were first observed in the *flbD* mutant.

Overexpression of *flbD* causes production of a complex conidiophore in submerged culture

Conidiophore development is normally suppressed when *A. nidulans* is grown in submerged culture (Axelrod 1972; Law and Timberlake 1980). However, we have shown that other genes that have been identified in *A. nidulans* based on their requirement during normal conidiophore production are able to cause developmental induction inappropriately when overexpressed in submerged hyphae (Adams et al. 1988; Lee and Adams 1994b). To determine if *flbD* could activate conidiation

in submerged culture, we constructed strains containing one (TJW29.2) to several (TJW29.4) copies of the *flbD* gene fused to the nutritionally regulated *alcA* promoter (*alcA[p]*) and integrated into the *A. nidulans* genome at the *trpC* locus, leaving the wild-type *flbD* gene intact. *alcA* transcription is repressed in the presence of glucose and induced in the presence of threonine (Lockington et al. 1985; Gwynne et al. 1987). As expected, *flbD* mRNA could be detected in both TJW29.2 and TJW29.4 grown in glucose medium, but the *flbD* message levels increased at least fourfold by 3 hr after transferring TJW29.2 and TJW29.4 to threonine medium and remained high at all time points examined (not shown). *flbD* message could also be detected in both TTA11 (wild-type) and TTA292 (*alcA(p)::brlA*) grown in glucose medium, but the level of *flbD* expression did not increase in either strain after the shift to threonine medium, and *flbD* mRNA levels may have even decreased slightly in TTA292 (not shown).

brlA mRNA normally accumulates to detectable levels only after induction of development and has been shown to be required under all conditions for the production of conidiophores (Clutterbuck 1969; Adams et al. 1988). We therefore determined the effect that overexpression of *flbD* had on *brlA* expression. Figure 4 shows that in TJW29.2, *brlA* message could be detected by 6 hr after a shift to *alcA*-inducing medium, whereas in TJW29.4, *brlA* message could be detected 3 hr after the shift to induction medium. *brlA* message was also detected in TTA292 by 3 hr after the shift to *alcA*-inducing medium but was never detected in the wild-type (TTA11) strain grown in submerged culture.

Overexpression of *flbD* in hyphae growing in submerged culture resulted in rapid cellular changes that culminated in the production of complex conidiophores that included stalks, vesicles, metulae, phialides, and co-

Figure 4. Forced overexpression of *flbD* results in accumulation of *brlA* mRNA. (A) Total RNA was isolated from a developmentally wild-type *A. nidulans* strain (TTA11) or from strains containing an *alcA(p)::brlA* fusion (TTA292), multiple copies of the *alcA(p)::flbD* fusion (TJW29.4), or a single copy of the *alcA(p)::flbD* fusion (TJW29.2). The strains were grown in liquid minimal medium containing glucose for 14 hr (labeled 0 hr) or shifted from this medium to minimal medium with threonine and allowed to grow for an additional 3 hr, 6 hr, 9 hr, or 12 hr. The resulting gel blot was probed with a *brlA*-specific fragment. (B) Equal loading of total RNA was confirmed with ethidium bromide staining.

nidia. Figure 5B shows that by 3 hr after *alcA*-induced expression of *flbD*, the hyphae had become highly vacuolated. By 9 hr after induced overexpression of *flbD*, simple conidiophores were formed consisting of swellings at the tips of hyphae from which spores were produced (Fig. 5D), and by 18 hr after shifting to *alcA*-inducing medium, morphologically complex spore-producing conidiophores were present throughout the culture (Fig. 5E). These conidiophores contained all of the cell types seen in normal wild-type conidiophores including stalks, vesicles, sterigmata, and spores (Fig. 5, cf. E and J). The phenotypes of TJW29.2 and TJW29.4 were the same at all time points (not shown). By 3 hr after a shift to threonine medium, TTA292 (*alcA(p)::brlA*) became vacuolated and produced reduced conidiophores in which a spore formed directly from the hyphal tip (Fig. 5G). The TTA292 culture did not change with time and looked much the same at 18 hr after the shift to inducing medium as it did 3 hr after the shift from glucose to threonine medium (Fig. 5H). In contrast to TJW29.2, TJW29.4, and TTA292, the wild-type strain, TTA11, did not pro-

duce any conidiophores and the hyphae did not undergo any morphological changes other than those that occur during normal growth (Fig. 5I).

Genetic requirements for *flbD*-induced conidiation

By constructing double mutants in all pair-wise combinations among the FLB mutants, we showed that *flbB*, *flbD*, and *flbE* probably function in the same step of a pathway leading to conidiation and that this pathway is separate from the pathway in which *flbC* functions (Wieser et al. 1994). In addition, we showed that *flbA* and *fluG* were epistatic to *flbD*. The observation that overexpression of *flbD* in liquid culture resulted in developmental activation provided the opportunity to extend this classical epistatic data to include information on the genetic requirements for *flbD*-overexpression-induced development. We therefore constructed developmental mutant strains that contained the *alcA(p)::flbD* fusion and assessed the effects of *flbD* overexpression in these strains. Forced overexpression of *flbD* in liquid-grown hyphae failed to cause conidiophore production in *brlA*[−] (RJW223), *flbA*[−] (RJW193), and *flbB*[−] (RJW201) strains that contained a single copy of the *alcA(p)::flbD* fusion construct (Fig. 6B,C,G; Table 1). For all of these strains except the *flbB*[−] strain (RJW201), there were extreme changes in hyphal morphology including increased vacuolation and severe growth inhibition by 3 hr after shifting to *alcA*-inducing medium. Overexpression of *flbD* in an *flbE*[−] mutant (RJW197) led to the production of conidiophores with the same timing as observed in TJW29.2; however, conidiophore structures tended to be somewhat simpler (Fig. 6E). Overexpression of *flbD* in the *flbC*[−] mutant (RJW207; Fig. 6D) always resulted in very vacuolated hyphae, but the conidiophores produced were not as numerous nor as complex as those produced in the wild-type strain containing the *alcA(p)::flbD* construct (TJW29.2; Figs. 5 and 6H). Likewise, forced overexpression of *flbD* in a *fluG*[−] mutant (RJW194; Fig. 6F) resulted in conidiophore production that was neither as extensive nor as rapid as that observed in the wild-type background. As summarized in Table 1, *flbD*-induced conidiophore development was always associated with accumulation of *brlA* mRNA.

Discussion

A. nidulans strains bearing mutations in any of four different genes, *flbB*, *flbC*, *flbD*, and *flbE*, have a temporally altered developmental program resulting in fluffy colonies that, like wild-type strains, produce conidiophores within the center of the colony (Wieser et al. 1994). However, conidiation is delayed at least 24 hr in these mutants as compared with developmentally wild-type strains (Fig. 1). Genetic analysis of these delayed conidiation mutants indicated that they probably define separate branches of the pathway leading to developmental activation, with *flbB*, *flbD*, and *flbE* defining one branch and *flbC* defining the second (Wieser et al. 1994). Whereas elimination of one branch of the pathway

Figure 5. Overexpression of *flbD* causes conidiophore development. Strains TJW29.2 [*alcA(p)::flbD* (A–E)], TTA292 [*alcA(p)::brlA* (F–H)], and TTA11 [wild-type (I)] were grown in liquid minimal medium with glucose (A,F) and then shifted to minimal medium containing threonine and allowed to grow for an additional 3 hr (B,G), 6 hr (C), 9 hr (D), or 18 hr (E,H,I). (J) A wild-type conidiophore produced by TTA11 grown on the surface of an agar plate. Specimens were photographed using DIC optics. Arrows indicate conidiophores (c) or spores (s).

Figure 6. Overexpression of *flbD* in developmental mutant strains. Strains TTA11 [control (A)], RJW193 [$\Delta flbA::alcA(p)::flbD$ (B)], RJW201 [$flbB^-$, $alcA(p)::flbD$ (C)], RJW207 [$flbC^-$, $alcA(p)::flbD$ (D)], RJW197 [$flbE^-$, $alcA(p)::flbD$ (E)], RJW194 [$\Delta fluG$, $alcA(p)::flbD$ (F)], RJW223 [$\Delta brlA$, $alcA(p)::flbD$ (G)], and TJW29.2 [$alcA(p)::flbD$ (H)] were grown in liquid minimal medium containing glucose for 14 hr and then shifted from this medium to liquid minimal medium with threonine and allowed to grow for 12 hr (A,B,D,E,G,H) or 24 hr (D,F). Specimens were photographed using DIC optics.

causes a delay in conidiation, elimination of both branches causes a longer delay. However, conidiophores are still produced by the double mutant indicating that development can also be activated through other mechanisms (Adams et al. 1992; Wieser et al. 1994). We have shown here that at least one of these delayed conidiation genes, *flbD*, is directly involved in activating conidiophore development, because forced overexpression of *flbD* in submerged culture, a condition that normally inhibits conidiation, resulted in the formation of complex conidiophores that produced viable spores. These conidiophores had all of the cell types observed in normal conidiophores including stalks, vesicles, metulae, phialides, and spores, leading us to propose that *flbD* normally functions early in development, resulting in activation of all of the genes necessary to make a complete conidiophore.

The similarity observed between the amino-terminal portion of the predicted FlbD protein and the DNA-binding domain for proteins in the Myb family of transcription factors suggests that the function of *flbD* in development involves directly regulating transcription of other genes (Lüscher and Eisenman 1990). Like Myb,

FlbD apparently regulates the transition from cell proliferation to differentiation of new cell types. Because loss of *flbD* function results in a delay in conidiophore development and extensive uncontrolled growth, whereas overexpression of *flbD* in hyphae causes conidiophore development at inappropriate times, it seems likely that FlbD has a positive role in activating development-specific gene expression. Given that the early developmental regulatory gene *brlA* is required for *flbD* to cause production of conidiophores in submerged culture, one possible downstream target of FlbD is *brlA*. The *brlA* locus is complex, consisting of two overlapping transcripts designated *brlA α* and *brlA β* , both of which are required for normal conidiophore development (Han et al. 1993; Prade and Timberlake 1993). We showed that the delay in conidiation observed in *flbD* mutants was accompanied by a similar delay in accumulation of *brlA* mRNA, whereas forced overexpression of *flbD* resulted in the inappropriate activation of *brlA α* and *brlA β* expression (Fig. 4; data not shown). However, we have not yet demonstrated direct control of *brlA α* or *brlA β* transcription by *flbD*.

Because overexpression of *flbD* in *brlA* mutants is able to cause cellular changes that resemble those occurring following induction of normal development in *brlA* null mutants, we consider it likely that if *brlA* is regulated directly by FlbD, it is likely not the only such gene. The observation that overexpression of *flbD* in a wild-type strain resulted in formation of morphologically normal conidiophores whereas overexpression of *brlA* resulted in development of much simpler conidiophore-like structures also supports this hypothesis. The higher complexity of the conidiation structures produced following overexpression of *flbD* presumably reflects differences in timing, extent, and spatial organization of gene expression, but how these differences affect development will require identification of the specific targets for FlbD function.

If FlbD has a development-specific function in activating transcription of other genes like *brlA*, it is important to know how the activity of FlbD is controlled. As with other genes identified by fluffy mutations, *flbD* mRNA

Table 1. Effects of developmental mutations on the *flbD* overexpression phenotype

Strain ^a	<i>brlA</i> expression	Sporulation	Vacuolation
RJW193 (<i>flbA</i> ⁻)	-	-	+
RJW201 (<i>flbB</i> ⁻)	-	-	-
RJW207 (<i>flbC</i> ⁻)	+	+	+
RJW197 (<i>flbE</i> ⁻)	+	+	+
RJW194 (<i>fluG</i> ⁻)	+	+	+
RJW233 (<i>brlA</i> ⁻)	-	-	+
TJW29.2 (wild type)	+	+	+
TTA11 (control)	-	-	-

Strains were grown as described in Materials and methods and were observed at various times beginning 3 hr after induction and continuing through 24 hr postinduction.

^aAll strains contain a single copy of the *alcA(p)::flbD* fusion except the control strain TTA11.

accumulates to relatively constant levels throughout the *A. nidulans* life cycle (Adams et al. 1992; Lee and Adams 1994a,b). This might indicate that *flbD*, like *c-myc*, is probably regulated post-transcriptionally (Lüscher and Eisenman 1990). This brings up the question of why forced overexpression of *flbD* causes development in submerged culture. One explanation for this result could be that FlbD alone has a basal activity but it requires the products of other genes to reach the level required for developmental induction. If this were the case, overexpression of *flbD* mRNA could bypass the need for these other activities by increasing the concentration of FlbD. Similarly, the delay observed for conidiophore development in *flbD* loss-of-function mutants might be explained if the products of other genes can replace the need for FlbD once they have attained sufficient levels. Along these lines, it is interesting that the one other delayed conidiation mutant we have begun to characterize also results from loss of function in a gene (*flbC*) that encodes a putative transcription factor (J. Fondon and T.H. Adams, unpubl.). FlbC and FlbD apparently contribute independently to developmental activation because mutations in both *flbC* and *flbD* have an additive effect on development and *flbC* is not required for overexpression of *flbD* to cause development (Table 1). A second possible mechanism for regulating FlbD activity is through changes in the environment. Under normal conditions, *A. nidulans* does not go through conidiophore development unless hyphae are exposed to an air interface (Morton 1961; Axelrod et al. 1973; Timberlake 1980). This is particularly relevant because it has been demonstrated that changes in redox state can induce conformation changes in the Myb DNA-binding domain, altering its ability to stably associate with DNA (Guehmann et al. 1992; Myrset et al. 1993). Given that the change in redox is apparently sensed through a conserved cysteine residue in the first repeat for the DNA-binding

domain shown in Figure 2C, one possible explanation for the role of air in *A. nidulans* development is in controlling the activity of FlbD. In this model, increased expression of FlbD in submerged culture must in some way overcome the need for redox-induced changes in DNA binding.

To address the question of how FlbD activity is regulated, we have investigated the genetic requirements for FlbD-induced development, and a model summarizing the potential interactions between FlbD and other genes controlling *brlA* activation is presented in Figure 7. We proposed previously that FluG is responsible for the constitutive production of an extracellular signal for conidiation and that development only occurs once a threshold level of this FluG-dependent signal has been reached (Lee and Adams 1994a). This hypothesis is consistent with the fact that forced overexpression of *flbD* does not absolutely require *fluG* for the production of conidiophores in liquid (Fig. 6; Table 1). However, the conidiophores produced in a *fluG*⁻ mutant following *flbD* overexpression are not as complex or as numerous as those produced following *flbD* overexpression in a wild-type strain. This might be explained if the FluG activity contributes to activation of FlbD but is not absolutely required for it. For instance, FluG signal may be involved in modulating the activity of other genes (such as *flbA*) that work in conjunction with FlbD to activate development.

Analysis of double mutant strains lacking combinations of *flbD*, *flbB*, *flbC*, and *flbE* led to the hypothesis that *flbB*, *flbD*, and *flbE* function at the same step in a genetic pathway leading to the activation of *brlA* and that *flbC* functions in a separate pathway (Wieser et al. 1994). Because *flbE* is not required for overexpression of *flbD* to cause development in submerged culture but *flbB* is necessary, we can refine this model to include the gene order *flbE* → *flbD* → *flbB* for one branch of the

Figure 7. Genetic model for the role of *flbD* in regulating development. Our previous results led us to suggest that the *fluG* gene product is responsible for producing a signal that functions extracellularly in activating a pathway leading to conidiation and that the *flbA* product regulates development directly by controlling the response to this signal (Lee and Adams 1994a,b). The data presented in this paper indicate that *flbD* also has a direct role in activating *brlA* and development because overexpression of *flbD* is sufficient to cause sporulation in submerged culture. On the basis of epistasis analysis, *flbE* and *flbB* probably function at the same step as *flbD* in the genetic pathway leading to the activation of *brlA* and development, whereas *flbC* may function in a separate branch of the same pathway (Wieser et al. 1994). Because *flbB* is required for *flbD*-induced sporulation but *flbE* is not, we suggest that *flbE* acts before *flbD* whereas *flbB* functions downstream of *flbD*. *fluG* mutations are epistatic to *flbD* mutations (Wieser et al. 1994), and *fluG* is not needed for *flbD* overexpression to cause development as expected if *fluG* functions before *flbD* in regulating development. The placement of *flbA* in this pathway is somewhat uncertain. *flbA* mutations are epistatic to *flbD* mutations, but *flbA* is required for overexpression of *flbD* to activate development. We propose that *flbA* functions upstream of *flbD* in positively modulating FlbD activity, although it is also possible that *flbA* functions later in the pathway. Finally, because *flbA* mutations also affect vegetative growth, we also propose a second role for *flbA* in controlling aspects of colony development unrelated to conidiophore development.

pathway (Table 1; Fig. 7). The nature of these interactions will be made more clear by examining the roles of *flbB* and *flbE* in development more directly. As mentioned above, forced overexpression of *flbD* does not require *flbC* to cause conidiation in submerged culture although the number and complexity of the conidiophores produced is reduced in the *flbC* mutant. This result is consistent with *flbC* functioning in a separate pathway from *flbD* leading to the activation of *brlA*. Loss of the *flbC* pathway can be partially compensated for by the *flbE*, *flbD*, *flbB* pathway, resulting in delayed conidiation. Likewise, the *flbC* pathway can partially compensate for the loss of *flbD*, *flbB*, or *flbE* function again resulting in a delayed conidiation phenotype. Because some development still takes place in the absence of both pathways, there must also be other mechanisms for activation of the central conidiation pathway.

Because FlbA is related to the *S. cerevisiae* SST2 gene product and SST2 functions in regulating the activity of the G-protein-mediated signal transduction pathway that controls yeast mating, we have suggested that FlbA could function by regulating the activity of a signal transduction pathway for responding to the putative *fluG* signal for conidiation (Lee and Adams 1994b). However, the relationship between *flbA* and the other genes that are normally required for activating *brlA* expression is somewhat complicated (Wieser et al. 1994). *flbA* mutations are epistatic to mutations in all of the other *flb* loci as predicted if *flbA* functions early in development, before *flbB*, *flbC*, *flbD*, or *flbE*. In contrast, *flbA* is required for conidiophore development induced by overexpression of *flbD* in submerged culture. This could be explained if *flbA* functions upstream of *flbD* and is required for bringing about events leading to FlbD activation. Alternatively, FlbD activity may be required for activation of *flbA*. It is interesting that overexpression of *flbA*, like overexpression of *flbD*, has been shown to activate *brlA* expression and development in submerged culture. The structures produced following forced overexpression of *flbA* in submerged culture are much simpler than the complex conidiophores made following overexpression of *flbD* (Fig. 5), consisting of simple phalide-like structures bearing chains of spores (Lee and Adams 1994b). One interpretation of this difference in conidiophore complexity is that *flbD* functions upstream of *flbA* in regulating initiation of conidiophore development. However, *flbD* overexpression can result in *brlA* mRNA accumulation by 3 hr after induction, whereas *flbA* overexpression does not cause *brlA* accumulation until between 6 and 9 hr postinduction. Clearly, the details of these interactions require a more complete understanding of the functions of FlbD, FlbA, and the products of other genes regulating initiation of *A. nidulans* conidiophore development.

Materials and methods

Fungal strains, growth, and genetics

All *A. nidulans* strains used in this study are listed in Table 2.

MBN021 is the original *flbD*⁻ mutant strain and was isolated from FGSC26 following NQO mutagenesis (Wieser et al. 1994). RBN021 was isolated as the meiotic progeny of FGSC237 and MBN014 and was crossed to PW1 to yield RBN070, an *argB*²⁻, *flbD*¹⁴⁻ mutant strain. TJW001 (*argB*⁺ *flbD*⁺) was isolated following transformation of RBN070 with an *A. nidulans* genomic DNA library constructed in the *argB*⁺-containing cosmid vector pUI1 (kindly provided by Dr. Bruce Miller, University of Idaho). TJW30.1 was constructed by transformation of FGSC237 with the *flbD* insertion plasmid pJW30. TJW29.2 and TJW29.4 were constructed by transformation of FGSC237 with the *alcA(p)::flbD* fusion plasmid pJW29. TJW29.2 has a single copy of pJW29 integrated at the *trpC* locus, whereas TJW29.4 has multiple copies of the plasmid integrated at *trpC*. RJW193, RJW194, RJW197, RJW201, RJW207, and RJW223 were constructed by crossing TJW29.2 to TBN39.5, RBN119, RJW120, RJW012, RJF003, or RSH94.4, respectively, to produce *flbA*⁻, *fluG*⁻, *flbE*⁻, *flbB*⁻, *flbC*⁻, and *brlA*⁻ mutant strains, each containing a single copy of the *alcA(p)::flbD* fusion construct integrated at the *trpC* locus as determined by Southern blot analysis.

Minimal medium with appropriate supplements was prepared as described (Käfer 1977). Complete medium is standard minimal medium with 5 grams of yeast extract per liter. Developmental cultures were grown on appropriately supplemented complete medium, and induction was performed as described previously (Han et al. 1993). For the *alcA(p)* induction experiments, spores were inoculated at a density of 1×10⁶ spores/ml in minimal medium containing 50 mM glucose and shaken at 300 rpm and 37°C for 14 hr. Aconidial mutant strains were inoculated using either a hyphal macerate or as 1×10⁶ ascospores/ml of culture. Following growth in liquid medium, hyphal cells were harvested by pouring through sterile Miracloth (Calbiochem), washed twice with minimal medium without glucose, transferred to minimal medium containing 100 mM L-threonine, and incubated as above. Samples were taken at the time points indicated for microscopic observation and RNA isolation.

Genetic techniques and microscopy

Standard genetic (Pontecorvo et al. 1953; Clutterbuck 1974) and transformation techniques (Yelton et al. 1984; Miller et al. 1985) were used. Photomicrographs of hyphal development were taken using an Olympus BH2 compound microscope and differential interference contrast optics.

flbD sequence analysis

Because *flbD* mRNA was present at constant levels in vegetative hyphae and following induction (Fig. 3), we were able to identify an *flbD* cDNA clone by screening an *A. nidulans* λUNI-ZAP cDNA library made from RNA isolated from a vegetatively growing wild-type culture (O'Donnel et al. 1991). The 3.2-kb fragment in pJW18 was used to screen 3×10⁵ plaques, and three positive clones were identified and rescued as phagemids. pJW25 was determined to have a 1.6-kb insert as observed for the *flbD* message, and the entire sequence was determined by the chain-termination method (Fig. 2B). The 3.2-kb fragment from pJW18 was also sequenced and found to be colinear with that of the cDNA. Finally, pJW9 was used to determine the sequence of the region corresponding to the FlbD14 mutant coding region.

Nucleic acid isolation and manipulation

Total RNA was isolated as described in Adams et al. (1988) and then was separated (10 μg per lane) by electrophoresis on a form-

Table 2. *A. nidulans* strains used in this study

Strain	Genotype	Source
TJW001	<i>biA1; veA1</i>	this study
TJW002	<i>biA1; veA1</i>	this study
TJW30.1	<i>pabaA1, yA2; flbD::trpC⁺, veA1</i>	this study
FGSC26	<i>biA1, veA1</i>	FGSC ^a
TJW29.2	<i>pabaA1, yA2; trpC::alcA(p)::flbD, veA1</i>	this study
TJW29.4	<i>pabaA1, yA2; trpC::alcA(p)::flbD, veA1</i>	this study
TTA11	<i>pabaA1, yA2; veA1</i>	Adams et al. (1988)
TTA292	<i>biA1; argB⁺::alcA(p)::briAα; methG1; veA1</i>	Adams et al. (1988)
RJW193	<i>pabaA1, ΔflbA::argB; trpC::alcA(p)::flbD, veA1</i>	this study
RJW194	<i>pabaA1; ΔfluG; trpC::alcA(p)::flbD, veA1</i>	this study
RJW197	<i>pabaA1, yA1, flbE58; methG1; veA1</i>	this study
RJW201	<i>pabaA1; flbB7; trpC::alcA(p)::flbD, veA1</i>	this study
RJW207	<i>pabaA1; flbC8; trpC::alcA(p)::flbD, veA1</i>	this study
RJW223	<i>pabaA1; ΔbriA, trpC::alcA(p)::flbD, veA1</i>	this study
TBN39.5	<i>biA1, ΔflbA::argB; methG1; veA1</i>	Lee and Adams (1994)
RJW012	<i>biA1; flbB7; veA1</i>	Wieser et al. (1994)
RJF003	<i>pabaA1, yA2; argB2; veA1; flbC8</i>	Wieser et al. (1994)
MBN014	<i>biA1; flbD14, veA1</i>	Wieser et al. (1994)
RBN021	<i>pabaA1, yA2; flbD14, veA1</i>	Wieser et al. (1994)
RBN070	<i>biA1; argB2; flbD14, veA1</i>	Wieser et al. (1994)
RBN119	<i>biA1, yA2; ΔfluG, argB2; methG1; veA1</i>	Lee et al. (1994)
RSH94.4	<i>methG1; ΔbriA, veA1</i>	S.T. Han (unpubl.)
RJW120	<i>flbE58; methG1; veA1</i>	Wieser et al. (1994)
PW1	<i>biA1; argB2; metG1; veA1</i>	P. Weglenski (unpubl.)
FGSC237	<i>pabaA1, yA2; trpC801, veA1</i>	FGSC ^a

^aFungal Genetics Stock center.

aldehyde-agarose gel and transferred to a nylon membrane (Hybond-N, Amersham Corp., Arlington Heights, IL). Hybridization to ³²P-labeled random primed probes was done according to the procedures recommended by the manufacturer. A 1.9-kb *HindIII*-*SalI* fragment from pTA39 containing the *briA* coding sequence was used as a *briA*-specific probe. A 1.3-kb *NcoI* fragment from the cDNA clone pJW25 that contains the *flbD* coding sequence was used as the *flbD*-specific probe. Hybridization to RNA blots was quantified using a Fujix BAS2000 phosphorimaging system.

The *flbD* gene region was localized in the following way: Eight *Bam*HI subclones were made from pJW10, the cosmid that originally complemented the *flbD* mutation, by digesting pJW10 with *Bam*HI and inserting each of the eight fragments into pPK1, a vector that contains the *argB* gene as a selectable marker for *Aspergillus* transformation (kindly provided by Pat Kennedy and Dr. Larry Yager, Temple University, Philadelphia). One of the *Bam*HI subclones, pJW17, contained a 5.3-kb insert that complemented the *flbD* mutation at a high frequency. The insert from pJW17 was divided into two fragments: a 3.2-kb *Bam*HI-*KpnI* fragment and a 2.1-kb *KpnI* fragment, and each of the fragments was inserted into pPK1 to give pJW18 and pJW20, respectively. pJW18 complemented the *flbD* gene at high frequency, whereas pJW20 did not complement the mutation at all, indicating that the entire *flbD* gene was probably contained in the 3.2-kb insert of pJW18.

The *flbD* disruption plasmid pJW30 was constructed by inserting a 4.1-kb *XhoI* *trpC* fragment from pTA11 into an *XhoI* site 410 bp downstream of the putative *FlbD* translation start site. The *alcA(p)::flbD* fusion plasmid was made by inserting the 1.5-kb *KpnI*-*EcoRI* fragment of pJW25 downstream of the *alcA* promoter in pON2 to create pJW26. The 2.0-kb *alcA(p)::flbD* fragment from pJW26 was then moved as an *SstI*-

KpnI fragment into the *SstI* and *KpnI* sites of pSH96 (S.T. Han and T.H. Adams, unpubl.). pSH96 is a Bluescript II KS(-) derivative in which the f1 replication origin was removed by digestion with *SspI* and replaced with the 5' 2556 bp of *trpC* (Yelton 1984) that had been blunt-ended by a fill-in reaction using Klenow fragment.

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References

- Adams, T.H., M.T. Boylan, and W.E. Timberlake. 1988. *briA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. *Cell* 54: 353-362.
- Adams, T.H., H. Deising, and W.E. Timberlake. 1990. *briA* requires both zinc fingers to induce development. *Mol. Cell. Biol.* 10: 1815-1827.
- Adams, T.H., W.A. Hide, L.N. Yager, and B.N. Lee. 1992. Isola-

- tion of a gene required for programmed initiation of development by *Aspergillus nidulans*. *Mol. Cell. Biol.* **12**: 3827–3833.
- Altschul, S.F., W. Gish, W. Miller, W.W. Myers, and D.J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**: 403–410.
- Aramayo, R., T.H. Adams, and W.E. Timberlake. 1989. A large cluster of highly expressed genes is dispensable for growth and development in *Aspergillus nidulans*. *Genetics* **122**: 65–71.
- Axelrod, D.E. 1972. Kinetics of differentiation of conidiophores and conidia by colonies of *Aspergillus nidulans*. *J. Gen. Microbiol.* **73**: 181–184.
- Axelrod, D.E., M. Gealt, and M. Pastushok. 1973. Gene control of developmental competence in *Aspergillus nidulans*. *Dev. Biol.* **34**: 9–15.
- Boylan, M.T., P.M. Mirabito, C.E. Willett, C.R. Zimmerman, and W.E. Timberlake. 1987. Isolation and physical characterization of three essential conidiation genes from *Aspergillus nidulans*. *Mol. Cell. Biol.* **7**: 3113–3118.
- Champe, S.P., M.B. Kurtz, L.N. Yager, N.J. Butnick, and D.E. Axelrod. 1981. In *The fungal spore: Morphogenetic controls* (ed. G. Turian and H.R. Hohl), pp. 63–91. Academic Press, New York.
- Chang, Y.C. and W.E. Timberlake. 1992. Identification of *Aspergillus brlA* response elements (BREs) by genetic selection in yeast. *Genetics* **133**: 29–38.
- Clutterbuck, A.J. 1969. A mutational analysis of conidial development in *Aspergillus nidulans*. *Genetics* **63**: 317–327.
- . 1974. In *Handbook of genetics* (ed. R.C. King), pp. 447–510. Plenum Publishing, New York.
- Dietzel, C. and J. Kurjan. 1987. Pheromonal regulation and sequence of the *Saccharomyces cerevisiae* SST2 gene: A model for desensitization to pheromone. *Mol. Cell. Biol.* **7**: 4169–4177.
- Frampton, J., T.J. Gibson, S.A. Ness, G. Doderlein, and T. Graf. 1991. Proposed structure for the DNA-binding domain of the Myb oncoprotein based on model building and mutational analysis. *Protein Eng.* **4**: 891–901.
- Guehmann, S., G. Vorbrueggen, F. Kalkbrenner, and K. Moelling. 1992. Reduction of a conserved Cys is essential for Myb DNA-binding. *Nucleic Acids Res.* **9**: 2279–2286.
- Gwynne, D.I., F.P. Buxton, S. Sibley, R.W. Davies, R.A. Lockington, C. Scazzocchio, and H. Sealy-Lewis. 1987. Comparison of the *cis*-acting control regions of two coordinately controlled genes involved in ethanol utilization in *Aspergillus nidulans*. *Gene* **51**: 205–216.
- Han, S., J. Navarro, R.A. Greve, and T.H. Adams. 1993. Translational repression of *brlA* expression prevents premature development in *Aspergillus*. *EMBO J.* **12**: 2449–2457.
- Käfer, E. 1977. Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. *Adv. Genet.* **19**: 33–131.
- Katzen, A.L., T.B. Kornberg, and J.M. Bishop. 1985. Isolation of the proto-oncogene *c-myc* from *D. melanogaster*. *Cell* **41**: 449–456.
- Law, D.J. and W.E. Timberlake. 1980. Developmental regulation of laccase levels in *Aspergillus nidulans*. *J. Bacteriol.* **144**: 509–517.
- Lee, B.N. and T.H. Adams. 1994a. The *Aspergillus nidulans* *fluG* gene is required for production of an extracellular developmental signal. *Genes & Dev.* **8**: 641–651.
- . 1994b. 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.
- Lockington, R.A., H.M. Sealy-Lewis, C. Scazzocchio, and R.W. Davies. 1985. Cloning and characterization of the ethanol utilization regulon in *Aspergillus nidulans*. *Gene* **33**: 137–149.
- Lüscher, B. and R.N. Eisenman. 1990. New light on Myc and Myb. Part II. Myb. *Genes & Dev.* **4**: 2235–2241.
- Majello, B., L.C. Kenyon, and R. Dalla-Favera. 1986. Human *c-myc* proto-oncogene: Nucleotide sequence of cDNA and organization of the genomic locus. *Proc. Natl. Acad. Sci.* **83**: 9636–9640.
- Martinelli, S.D. and A.J. Clutterbuck. 1971. A quantitative survey of conidiation mutants in *Aspergillus nidulans*. *J. Gen. Microbiol.* **69**: 261–268.
- Miller, B., K. Miller, and W. Timberlake. 1985. Direct and indirect gene replacements in *Aspergillus nidulans*. *Mol. Cell. Biol.* **5**: 1714–1721.
- Morton, A.G. 1961. The induction of sporulation in mould fungi. *Proc. R. Soc. Lond. B* **153**: 548–569.
- Myrset, A.H., A. Bostad, N. Jamin, P. Lirsac, F. Toma, and O.S. Gabrielsen. 1993. DNA and redox state induced conformational changes in the DNA-binding domain of Myb oncoprotein. *EMBO J.* **12**: 4625–4633.
- Navarro-Bordonaba, J. and T.H. Adams. 1994. In *Growth, differentiation, and sexuality* (ed. J.G.H. Wessels and F. Meinhardt), pp. 333–349. Springer-Verlag, Berlin, Germany.
- O'Donnel, K.L., A.H. Osmani, S.A. Osmani, and N.R. Morris. 1991. *bimA* encodes a member of the tetratricopeptide repeat family of proteins and is required for the completion of mitosis in *Aspergillus nidulans*. *J. Cell Sci.* **99**: 711–719.
- Ohi, R., D. McCollum, B. Hirani, G.D. Haese, X. Zhang, J.D. Burke, K. Turner, and K.L. Gould. 1994. The *Schizosaccharomyces pombe cdc5+* gene encodes an essential protein with homology to c-Myb. *EMBO J.* **13**: 471–483.
- Oppenheimer, D.G., P.L. Herman, S. Sivakumaran, J. Esch, and M.D. Marks. 1991. A myb gene required for leaf trichome differentiation in *Arabidopsis* is expressed in stipules. *Cell* **67**: 483–493.
- Pastushok, M. and D.E. Axelrod. 1976. Effect of glucose, ammonium and media maintenance on the time of conidiophore initiation by surface colonies of *Aspergillus nidulans*. *J. Gen. Microbiol.* **94**: 221–224.
- Paz-Ares, J., D. Ghosal, U. Wienand, P.A. Peterson, and H. Saedler. 1987. The regulatory *c1* locus of *Zea mays* encodes a protein with homology to myb proto-oncogene products and with structural similarity to transcriptional activators. *EMBO J.* **6**: 3553–3558.
- Peters, C.W.B., A.E. Sippel, M. Vingron, and K.H. Klempnauer. 1987. *Drosophila* and vertebrate myb proteins share two conserved regions, one on which functions as a DNA-binding domain. *EMBO J.* **6**: 3085–3090.
- 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.
- Prade, R.A. and W.E. Timberlake. 1993. The *Aspergillus nidulans brlA* regulatory locus encodes two functionally redundant polypeptides that are individually required for conidiophore development. *EMBO J.* **12**: 2439–2447.
- Saikumar, P., R. Murali, and E.P. Reddy. 1990. Role of tryptophan repeats and flanking amino acids in Myb-DNA interactions. *Proc. Natl. Acad. Sci.* **87**: 8452–8456.
- Slamon, D.J., T.C. Boone, D.C. Murdock, D.E. Keith, M.F. Press, R.A. Larson, and L.M. Souza. 1986. Studies of the human *c-myc* gene and its product in human acute leukemias. *Science* **233**: 347–351.
- Stringer, M.A., R.A. Dean, T.C. Sewall, and W.E. Timberlake. 1991. Rodletless, a new *Aspergillus* developmental mutant

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- induced by directed gene inactivation. *Genes & Dev.* **5**: 1161–1171.
- Tice-Baldwin, K., G.R. Fink, and K.T. Arnt. 1989. *BAS1* has an Myb motif and activates *HIS4* transcription only in combination with *BAS2*. *Science* **246**: 931–935.
- Timberlake, W.E. 1980. Developmental gene regulation in *Aspergillus nidulans*. *Dev. Biol.* **78**: 497–510.
- 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.
- Yelton, M.M., J.E. Hamer, and W.E. Timberlake. 1984. Transformation of *Aspergillus nidulans* by using a *trpC* plasmid. *Proc. Natl. Acad. Sci.* **81**: 1470–1474.
- Yelton, M.M., W.E. Timberlake, and C.A.M.J.J. van den Hondel. 1985. A cosmid for selecting genes by complementation in *Aspergillus nidulans*: Selection of the developmentally regulated *yA* locus. *Proc. Natl. Acad. Sci.* **82**: 834–838.

flbD encodes a Myb-like DNA-binding protein that coordinates initiation of *Aspergillus nidulans* conidiophore development.

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