MOLECULAR AND CELLULAR BIOLOGY, Sept. 1992, p. 3827–3833
0270-7306/92/093827-07$02.00/0

Vol. 12, No. 9

Isolation of a Gene Required for Programmed Initiation of Development by *Aspergillus nidulans*

THOMAS H. ADAMS,[1]* WINSTON A. HIDE,[2]† LAWRENCE N. YAGER,[2] AND BEE NA LEE[1]

Department of Biology, Texas A&M University, College Station, Texas 77843,[1] and Department of Biology, Temple University, Philadelphia, Pennsylvania 19122[2]

Received 3 February 1992/Returned for modification 29 May 1992/Accepted 10 June 1992

In contrast to many other cases in microbial development, *Aspergillus nidulans* conidiophore production initiates primarily as a programmed part of the life cycle rather than as a response to nutrient deprivation. Mutations in the *acoD* locus result in "fluffy" colonies that appear to grow faster than the wild type and proliferate as undifferentiated masses of vegetative cells. We show that unlike wild-type strains, *acoD* deletion mutants are unable to make conidiophores under optimal growth conditions but can be induced to conidiate when growth is nutritionally limited. The requirement for *acoD* in conidiophore development occurs prior to activation of *brlA*, a primary regulator of development. The *acoD* transcript is present both in vegetative hyphae prior to developmental induction and in developing cultures. However, the effects of *acoD* mutations are detectable only after developmental induction. We propose that *acoD* activity is primarily controlled at the posttranscriptional level and that it is required to direct developmentally specific changes that bring about growth inhibition and activation of *brlA* expression to result in conidiophore development.

The asexual life cycle of the filamentous fungus *Aspergillus nidulans* can be divided into two distinct phases, growth and reproduction. Following germination of an asexually derived spore, termed a conidium, hyphae grow and branch outwards to form an undifferentiated network of interconnected cells, termed the mycelium. At a fixed time after germination, some of the hyphal cells making up the mycelium become capable of differentiating conidiophores and are described as developmentally competent (5, 11). These cells initiate development when exposed to an air-water interface and give rise to conidiophores that then produce thousands of mitotically derived spores. The mechanisms controlling the acquisition of developmental competence followed by a switch from undifferentiated growth to conidiophore development are largely unknown but apparently are genetically determined (5, 11), with competence arising as a programmed event in the life cycle rather than as a response to unfavorable environmental conditions (21).

Several mutant strains that exhibit morphological defects suggesting that they may be altered in their ability to undergo programmed switching from undifferentiated growth to conidiophore development have been described (10, 23, 26). In fact, when mutant strains of *A. nidulans* defective in asexual reproduction but having normal vegetative growth were classified by Martinelli and Clutterbuck (16) according to the stage of development affected, 83% of the mutants were altered in their ability to initiate development. Thus, if mutation frequency accurately reflects the number of genes directly and indirectly involved in a process, then initiation is the most genetically complex step in development.

The most commonly described phenotype for such putative switching mutants is "fluffy." *A. nidulans* fluffy mutants characteristically proliferate as undifferentiated masses of vegetative cells to form large cottonlike colonies (13, 16, 23). One typical fluffy mutant results from mutation of the *acoD* gene and is defined by a temperature-sensitive mutation, *acoD684* (26). Using temperature shift analysis, Yager et al. (26) showed that *acoD* mutations block development shortly after cells become competent to differentiate conidiophores. We have begun a molecular analysis of *acoD* and of other genes defined by mutations that result in fluffy *A. nidulans* colonies in an effort to understand the cellular mechanisms controlling the switch from proliferative growth to development. In this paper, we describe the isolation and initial characterization of the wild-type *acoD* gene. We show that under normal circumstances *acoD* is required for expression of the primary regulator of the pathway underlying conidiophore development, *brlA* (1, 18). This requirement for *acoD* can be partially overcome by limiting growth conditions. *acoD* is the first gene characterized at the molecular level that has been shown to play a role in the complex events leading up to *brlA* expression.

MATERIALS AND METHODS

Fungal strains and growth conditions. *A. nidulans* strains used in this study are shown in Table 1. For the *acoD684* and *acoD102* temperature-sensitive mutants, 42°C was used as the restrictive temperature and 32°C was used as the permissive temperature. Minimal medium was prepared as described by Käfer (15). Complete medium is standard minimal medium containing 5 g of yeast extract per liter. Developmental cultures were induced as described elsewhere (3, 24).

Growth rates of *acoD* mutant and wild-type colonies were determined by inoculating single spores in the center of a piece of cellulose membrane placed on the surface of an agar plate containing either complete or minimal medium. Plates were incubated at 37°C, and after appropriate times, the diameter of each colony was determined, fungal material was scraped and removed from the membrane, and the wet weight was determined. This material was dried under a vacuum for 24 h before the dry weight was determined. Four separate samples were used for each time point, and less than 10% variation in weight was observed. Statistical values for comparing data sets were obtained with the general linear

* Corresponding author.
† Present address: Institute of Molecular Genetics, Baylor College of Medicine, Houston, TX 77030.

TABLE 1. *A. nidulans* strains used in this study

Strain	Genotype	Source
TU33	*yA2 pabaA1 pyrG89 acoD102 pyroA4 veA1*	This study
TU35	*yA2 pabaA1 pyrG89 acoD684 pyroA4 veA1*	This study
TU43	*yA2 pabaA1 pyroA4 veA1*	This study[a]
PW1	*biA1 argB2 methG1 veA1*	P. Weglenski
FGSC237	*pabaA1 trpC801 veA1*	FGSC[b]
TTA125.1	*biA1 acoD::argB$^+$ methG1 veA1*	This study[c]
TTA127.2	*pabaA1 ΔacoD::trpC$^+$ veA1*	This study[d]
FGSC26	*biA1 veA1*	FGSC[a]

[a] Transformant of TU35 with pAD4.
[b] FGSC, Fungal Genetics Stock Center.
[c] Transformant of PW1 with pTA125.
[d] Transformant of FGSC237 with pTA127.

model and analysis-of-variance procedure of SAS (SAS Institute, Carey, N.C.). Samples of cultures grown on complete medium were taken 48, 60, 72, 84, and 96 h after inoculation. Samples of cultures grown on minimal medium were taken 72, 84, 96, 108, and 120 h after inoculation.

To measure conidial production during the temperature shift experiment with the wild-type and *acoD* mutant strains, confluent lawns were produced by plating 3 ml of 1.0% top agar containing 10^5 conidia per ml onto complete-medium plates. Sets of plates were incubated at 32°C (permissive temperature) and were then upshifted to 42°C (restrictive temperature) at various times. Plates were incubated for a total of 60 h, and then five punches were taken randomly from a plate with an 11-mm-diameter steel test tube cap and the fungal material was placed into 5 ml of 0.2% Tween 80. This material was homogenized for 1 min in a Wheaton ground-glass homogenizer. The homogenate was diluted 1:10 in 0.1% Tween 80, and conidial counts were determined with a hemacytometer.

Genetic techniques. Standard *A. nidulans* genetic (12, 22) and transformation (17, 27) procedures were used.

Microscopy. All light microscopy was done with an Olympus SZ-11 stereomicroscope and transmitted light.

Nucleic acid isolation and manipulation. Total RNA was isolated as described previously (1). Total RNA (10 μg per lane) was separated by electrophoresis on formaldehyde-agarose gels and transferred without pretreatment to nylon membrane (Hybond-N; Amersham Corp., Arlington Heights, Ill.). Hybridization to ^{32}P-labeled random primed probes was done according to procedures recommended by the membrane manufacturer. Plasmid pBS2.5 containing the 2.5-kbp *Bam*HI-*Sal*I fragment from the *brlA* gene region was used as a *brlA*-specific probe (8). A 2.5-kb *Xho*I fragment from within the 7-kb *Bam*HI-*Hin*dIII *acoD* gene region cloned in Bluescript KS$^-$ (pFM1; Stratagene, La Jolla, Calif.) was used as an *acoD*-specific probe (see Fig. 2).

The *acoD* disruption vectors pTA125 and pTA127 were generated from pFM1 by standard techniques. pTA125 was constructed by inserting a 2-kb *Eco*RI fragment carrying the *A. nidulans argB*$^+$ (7) gene from pSalargB into a unique *Eco*RI site in the center of the predicted *acoD* coding region in pFM1 (see Fig. 2). pTA127 was constructed by first deleting the 2.5-kb *Xho*I fragment from pFM1 and then inserting a 4-kb *Xho*I fragment that contains the *A. nidulans trpC*$^+$ (27) gene in its place.

RESULTS

***acoD* is required at the time conidiophore development initiates.** Figure 1 shows conidiophores formed by a wild-type *A. nidulans* strain and the mycelial mass formed by an *acoD684*^{ts} mutant grown at the restrictive temperature. We have shown that the fluffy colony morphology exhibited by the *acoD684*^{ts} mutant strain is the same phenotype that results from complete loss of *acoD* function (see Results below). To determine the time during which the *acoD* gene product is required for conidiation, confluent lawns of the *acoD684*^{ts} mutant were grown for various times at the permissive temperature and then shifted to the restrictive temperature. Figure 1C shows that beginning about 24 h after germination, *acoD* was no longer needed for development to proceed. The requirement for *acoD* occurred only after the strain had become competent to develop and was exposed to an air interface (data not shown). Under the conditions used, cells became competent to develop approximately 18 h after germination, indicating that the *acoD* gene product was required during a critical period of about 6 h. After this critical time had passed, conidiation proceeded as with the wild type.

Isolation and physical mapping of the *acoD* gene. Standard procedures were used to transform *A. nidulans* TU35 (*acoD684*^{ts}) (Table 1) with purified DNA from an *A. nidulans* genomic DNA library constructed in the plasmid vector pGM3 (25). This library contains 6- to 9-kbp inserts in a vector (pGM3) that carries the *Neurospora crassa pyr4* gene as a selectable marker. *pyr4* can complement mutations in the *A. nidulans pyrG* gene which result in pyrimidine auxotrophy (6). Approximately 500 pyrimidine prototrophs were screened visually for the ability to conidiate at the restrictive temperature, and one strain that displayed normal development, TU43, was isolated for further characterization.

Genomic DNA was isolated from TU43, subjected to Southern blot analysis, and shown to contain a single copy of integrated vector sequences (data not shown). The integrated sequences were recovered from TU43 by restriction of genomic DNA with an enzyme (*Bgl*II) that does not cut in pGM3, followed by ligation and transformation of *Escherichia coli* and selection for ampicillin-resistant transformants. Restriction enzyme analysis of plasmid DNA isolated from several independent ampicillin-resistant *E. coli* transformants showed that all the plasmids were identical. One of these plasmids, pAD4, was isolated for further study. Forty-three of fifty transformants of TU35 with pAD4 produced wild-type conidiophores at the restrictive temperature.

pAD4 was then used to screen an ordered cosmid library of *A. nidulans* genomic DNA constructed in the vector pLorist2 (3a, 14), and four cosmids, pL12F7, pL12H9, pL25H12, and pL28H7, were identified. Each of these cosmids was previously shown to contain DNA from *A. nidulans* chromosome III (9), which corresponds to the chromosome shown by mitotic mapping to carry the *acoD* gene (25a). Cotransformation of TU35 with pL12F7 and a plasmid containing the *N. crassa pyr4* gene (pRG3) (20) demonstrated that pL12F7 contains all the sequences necessary to complement the *acoD684*^{ts} mutation at high frequency. A 12-kbp region of pL12F7 corresponding to sequences in pAD4 was mapped and used for further experiments aimed at localizing the *acoD* gene (Fig. 2A).

Several fragments from the 12-kbp *acoD* genomic region were subcloned into either pBluescript KS$^-$ or the *pyr4* transformation vector pRG3 and tested for their ability to complement the *acoD684*^{ts} mutation. A high level of *acoD*

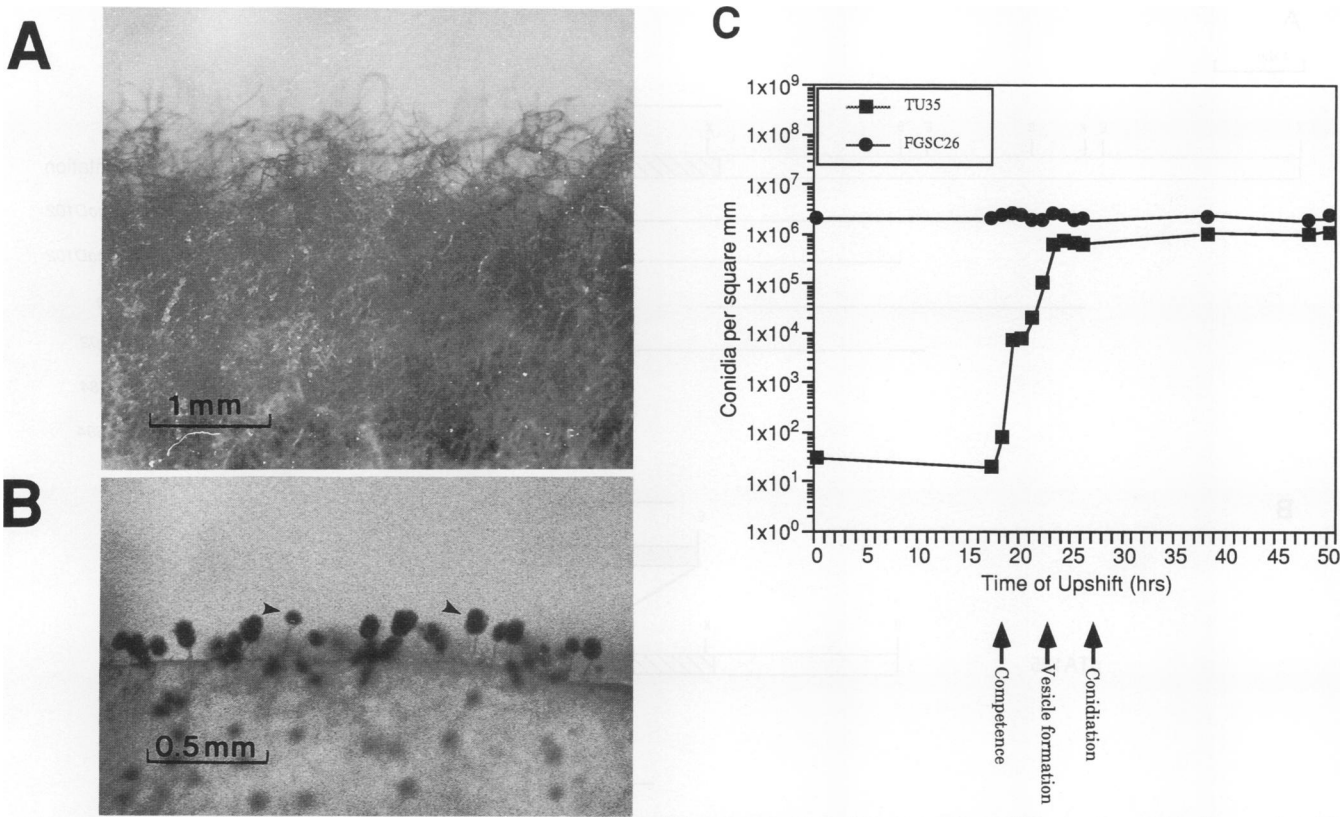

FIG. 1. *acoD* is required for normal conidiophore development. (A and B) Micrographs of aerial hyphae produced by an *acoD684*^{ts} mutant (A) and conidiophores produced by a wild-type strain (B) were prepared as described in Materials and Methods. Arrows indicate conidiophores produced by the wild-type strain. (C) *A. nidulans* FGSC26 (wild type) and TU35 (*acoD684*^{ts}) were grown as described in Materials and Methods at 32°C and upshifted to 42°C at the times indicated. After 60 h, samples were taken and the numbers of conidia were determined with a hemacytometer. Arrows indicate times when major developmental events take place in the wild-type strain.

complementation was observed by using a 7-kbp *Bam*HI-*Hin*dIII fragment cloned into pBluescript in cotransformation experiments with pRG3 to transform TU35 to uridine prototrophy and by observing the developmental phenotype (Fig. 2A). By probing Southern blots of genomic DNA from transformants having wild-type conidiophore morphology with the putative *acoD* gene region, we have shown that both homologous and heterologous integration of the 7-kbp *Bam*HI-*Hin*dIII fragment resulted in complementation of the *acoD684*^{ts} mutation (data not shown). When this fragment was divided by the central *Eco*RI site, fragments to the left failed to rescue the *acoD684*^{ts} fluffy phenotype, whereas fragments to the right did rescue but at a reduced frequency (Fig. 2A). By contrast, only fragments to the left of the central *Eco*RI site complemented the fluffy phenotype resulting from a second *acoD* mutant allele, *acoD102*^{ts}. This indicates that the gene extends to both sides of the *Eco*RI site. Southern blot analysis of wild-type transformants in both cases showed that integration must take place at the *acoD* locus to repair the mutation. From these results, we infer that the *acoD684*^{ts} mutation maps within the 800-bp *Eco*RI-*Xho*I fragment but that the gene extends to the left of the *Eco*RI site where the *acoD102*^{ts} mutation maps.

Disruption of the *acoD* gene results in a fluffy mutant phenotype. To demonstrate that sequences contained in pAD4 corresponded to the wild-type *acoD* gene and to exclude the possibility that pAD4 contains a suppressor, strain TTA125.1 was generated by disruption of putative *acoD* coding sequences (see results below). This strain, which is fluffy, was crossed with strain TU35 (*acoD684*^{ts}) and with strain TU33 (*acoD102*^{ts}). Several hundred meiotic progeny were analyzed from each cross and found either to be temperature-sensitive fluffy mutants or to have the same fluffy phenotype as the disruption strain TTA125.1. No developmentally wild-type progeny were observed in either cross, indicating that sequences in pAD4 are tightly linked to the original *acoD* mutations. Diploid strains were also generated with TTA125.1 or TTA127.2 (a putative *acoD* deletion strain) (Fig. 2B) and TU35. In both cases, the diploid phenotype was temperature-sensitive fluffy, as with TU35 and TU33. Taken together, these results support the notion that sequences contained within pAD4 correspond to the *acoD* gene.

The region corresponding to the *acoD* gene was further localized by probing RNA blots with DNA fragments from the complementing region. Both the 800-bp *Eco*RI-*Xho*I fragment containing the minimal *acoD684* complementing sequences and the adjacent 1.8-kbp *Eco*RI-*Xho*I fragment identified a single transcript of about 3.0 kilonucleotides (see Results below). Disruption of the *acoD* gene by insertion of a fragment containing the *argB* gene into the *Eco*RI site (Fig. 2B) resulted in a loss of this RNA and the appearance of an RNA species with decreased mobility that hybridized to the 1.8-kbp *Eco*RI-*Xho*I fragment but not to the 800-bp fragment (data not shown). This indicates that this 3-kilonucleotide message corresponds to *acoD* mRNA and that the gene is

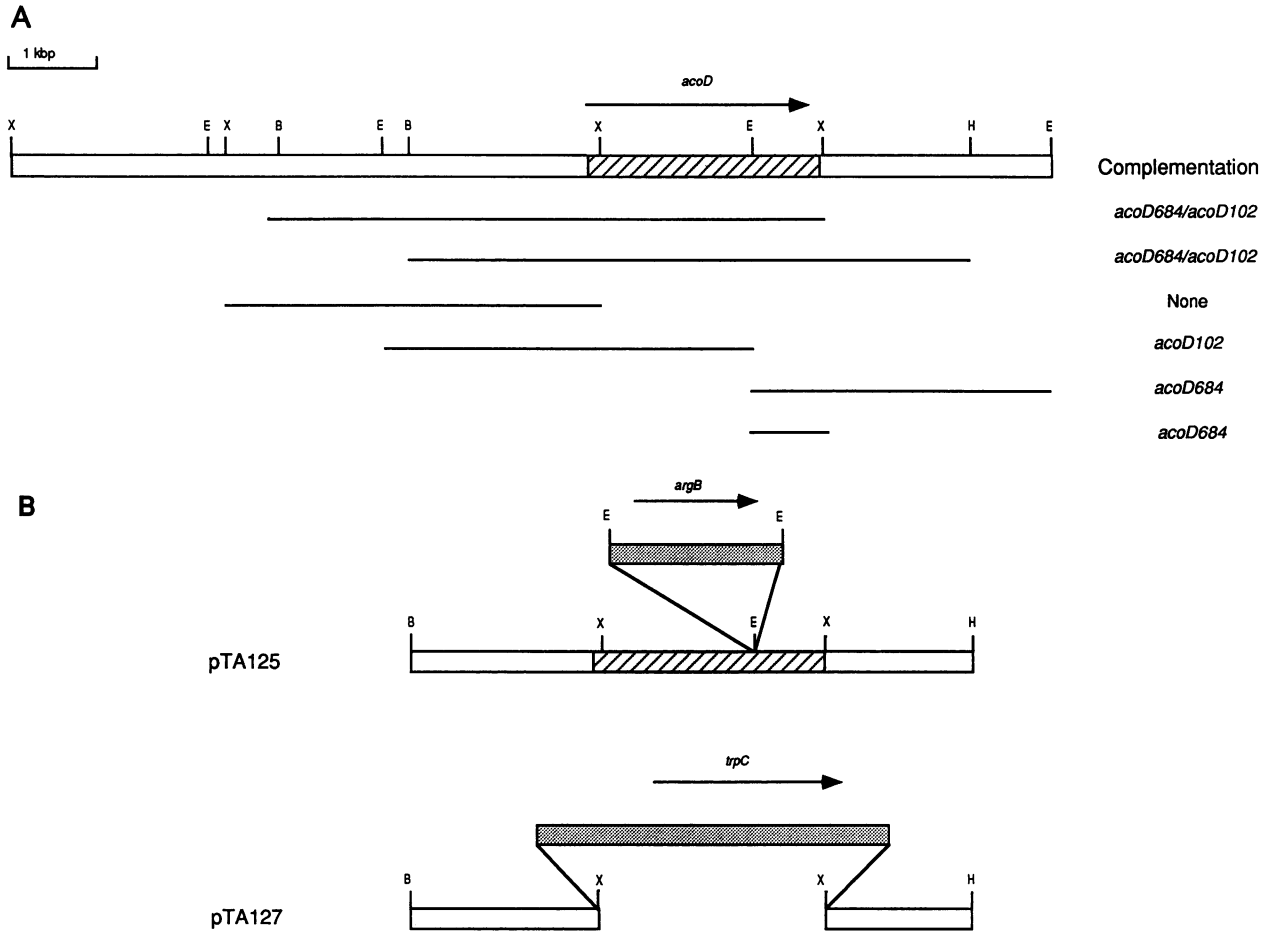

FIG. 2. Localization and mapping of the *acoD* gene. (A) Diagram of the *acoD* gene region. Top, restriction map of the DNA region that is able to complement an *acoD* mutation; bottom, DNA fragments used in transformation experiments to localize the *acoD* complementing region. Restriction sites: X, *Xho*I; E, *Eco*RI; B, *Bam*HI; H, *Hin*dIII. The direction of *acoD* transcription was determined with single-stranded specific DNA probes. (B) *acoD* disruption vectors. pTA125 and pTA127 were constructed as described in Materials and Methods and were used in transformation experiments to replace the wild-type *acoD* gene.

likely to be transcribed in the direction shown in Fig. 2. We have confirmed the direction of transcription by using single-stranded DNA probes (data not shown).

***acoD* mRNA is present throughout the life cycle.** The *acoD* gene product is apparently required after cultures have obtained developmental competence but prior to the elaboration of conidiophore structures (Fig. 1) (26). We investigated the possibility that, as with several other *A. nidulans* genes having developmentally specific functions, *acoD* transcription is regulated during development. Total RNA was isolated from submerged cultures both before and after the acquisition of developmental competence and from various times after development was induced. Figure 3 shows that *acoD* message was present within 12 h of inoculation and that transcript levels remained relatively unchanged after induction of development. However, no *acoD* mRNA was detected in mature spores.

Abnormal growth and development of *acoD* mutants. Both of the original *acoD* mutant strains were isolated as temperature-sensitive strains that conidiate like the wild type at the permissive temperature and are fluffy at the restrictive temperature (26). The construction of a strain containing a deletion of the *acoD* genomic region allowed us to investi-

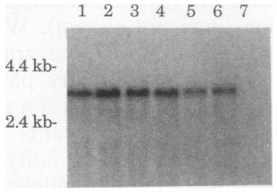

FIG. 3. *acoD* encodes a 3-kb message that is present in vegetative cells. Total RNA was isolated from a wild-type *A. nidulans* strain (FGSC26) at various times during development and fractionated on a formaldehyde-agarose gel. The resultant gel blot was probed with the 2.5-kbp *Xho*I fragment from the *acoD* gene region (Fig. 2). Lane 1, RNA isolated from an uninduced liquid culture 12 h after inoculation with spores and before developmental competence was reached; lane 2, RNA isolated from an uninduced liquid culture 22 h after inoculation and after acquisition of developmental competence; lanes 3 to 6, RNA isolated 3, 6, 12, and 24 h after induction of development; lane 7, RNA isolated from conidia. Equal loading of the RNA samples was determined by comparing intensities of ethidium bromide-stained rRNA bands.

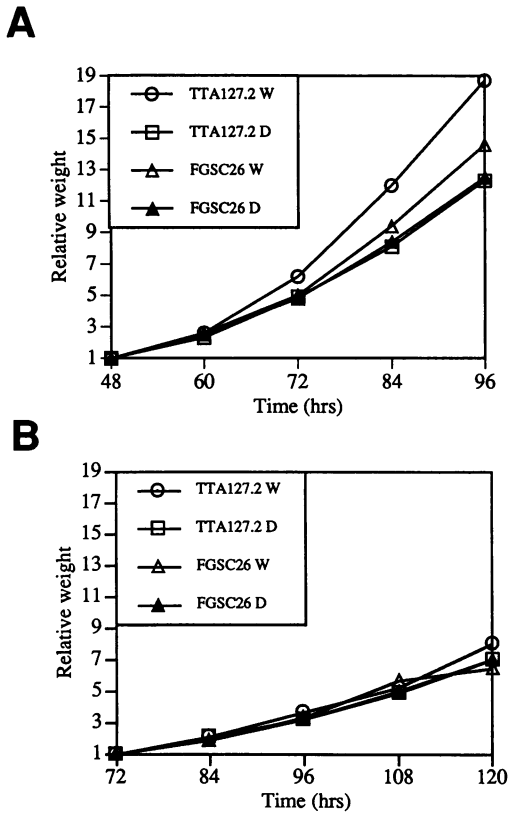

FIG. 4. *acoD* mutations result in altered growth. *A. nidulans* FGSC26 (wild type) and TTA127.2 (Δ*acoD*) were grown as colonies from single spores on cellulose membranes supported by solid complete (A) or minimal (B) medium as described in Materials and Methods. At the times indicated, the colonies were scraped off the filter and wet (W) and dry (D) weights were determined. Colony mass at each time relative to the colony mass at the first time point is reported and is the average for four samples from each time point. Less than 10% variation was seen within each data set (see Materials and Methods).

gate the effects of an *acoD* null mutation on growth and development. On complete medium, the *acoD* deletion mutant grew as a large mass of aerial mycelia while the wild-type strain produced mainly substrate mycelia that gave rise to conidiophores (Fig. 1). To determine whether the loss of development in *acoD* mutants corresponded to an increased growth rate, wild-type and *acoD* mutant strains were point inoculated as single spores onto cellulose membranes supported by solid complete medium and incubated for various times. Growth was determined by measuring colony diameter and by determining both the wet weight and the dry weight of the fungal material on the membrane at various times after inoculation (see Materials and Methods). The diameters of *acoD* mutant colonies increased at a slightly higher rate than did those of wild-type colonies, resulting in a 15% larger colony size by 6 days after germination (data not shown). As shown in Fig. 4A, the wet weight also increased at a significantly faster rate in the *acoD* deletion mutant than in the wild-type strain ($P > 0.001$) (see Materials and Methods). However, dry weight increased at the same rate in both strains, indicating that although the apparent growth of *acoD* mutant strains was increased above that of the wild type, there was no increase in accumulation of dry mass.

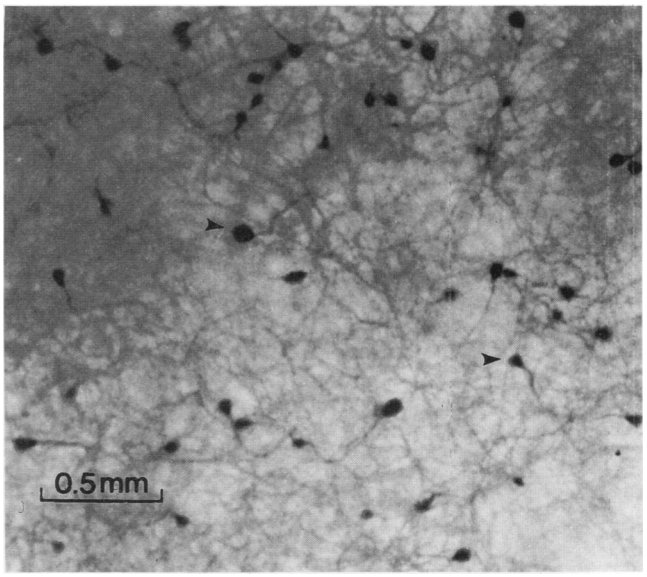

FIG. 5. Nutritional limitation results in conidiation by *acoD* mutant strains. TTA127.2 was grown for 3 days at 37°C on minimal medium and photographed as described in Materials and Methods. Arrows, conidiophores.

The growth rate of *A. nidulans* can be affected by a number of parameters including temperature and nutrient supply. Growth rates of wild-type and *acoD* deletion strains were reduced at least twofold on minimal medium compared with rates on complete medium (Fig. 4B). For wild-type strains, this change in growth rate had no demonstrable effect on the timing or extent of conidiophore development. By contrast, we found that the developmental deficiency of the *acoD* deletion strain was partially rescued by growing the strain on minimal medium to limit the nutrient supply (Fig. 5). The development observed in *acoD* mutants grown under these conditions was delayed at least 24 h compared with that of the wild type, and the number of conidiophores produced was greatly reduced.

***acoD* mutations affect accumulation of *brlA* message.** Differentiation of the multiple cell types that make up the *A. nidulans* conidiophore involves the sequential activation of several hundred genes. One of these genes, *brlA*, has been shown to be the primary activator of a linear dependent regulatory pathway that controls its own temporal and spatial expression and that of many other developmentally regulated genes (1, 18). In order to determine whether *acoD* was required for *brlA* expression, we isolated total RNA from wild-type and *acoD* deletion mutant *A. nidulans* strains grown in submerged culture or allowed to develop for various times and examined *brlA* expression patterns. Figure 6A shows that, when grown on complete medium, *brlA* mRNA first became detectable between 2 and 4 h after induction of development in wild-type strains. This corresponds to the time when conidiophore vesicles, the first cell type that can be distinguished during conidiophore development, were observed. By contrast, no *brlA* mRNA and no conidiophore vesicles were ever detected in the *acoD* deletion strain grown under these conditions.

As described above, *acoD* deletion mutants can be induced to form conidiophores when grown on minimal medium. Figure 6B shows that *brlA* mRNA was first detected in the *acoD* deletion strain between 6 and 9 h after induction of

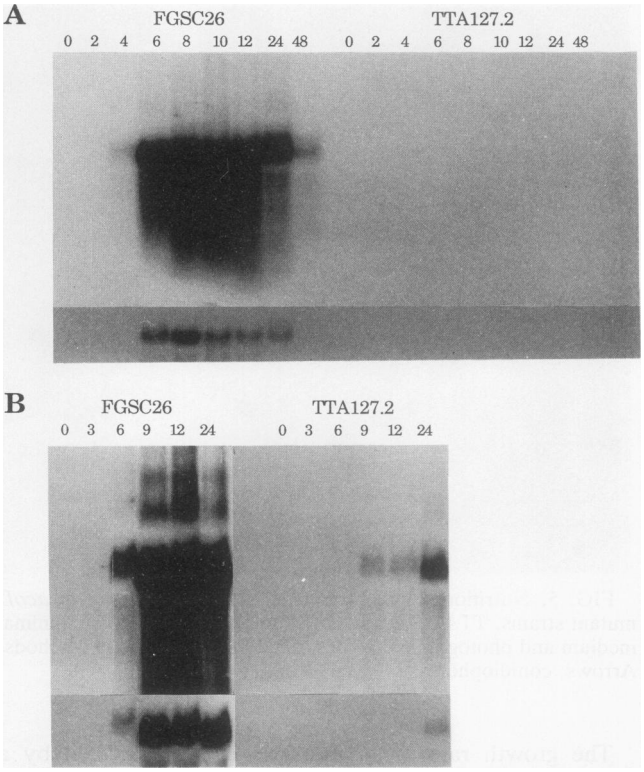

FIG. 6. *acoD* mutations result in altered control of *brlA*. Total RNA was isolated from FGSC26 (wild type) and TTA127.2 (Δ*acoD*) grown on complete medium (A) or supplemented minimal medium (B) at various times after induction of development and fractionated on a formaldehyde-agarose gel. The resultant gel blot was probed with a *brlA*-specific DNA fragment. Lane designations represent times (in hours) after developmental induction. Equal loading of the RNA samples was determined by comparing intensities of ethidium bromide-stained rRNA bands.

development on minimal medium. *brlA* mRNA was first detected in wild-type strains grown under the same conditions between 3 and 6 h after developmental induction. The absolute levels of *brlA* mRNA are significantly higher in the wild type than in the *acoD* mutant. Under these conditions, conidiophore vesicles were first observed in the wild-type strain at about 4 h after induction of development but were not detected in the *acoD* deletion strain until 24 h postinduction.

DISCUSSION

The molecular genetic mechanisms controlling the initiation of *A. nidulans* conidiophore development are largely unknown but have been shown to involve at least two separate events. First, vegetative cells must become competent to undergo differentiation (5, 11). Developmental competence arises after cells have grown for a fixed time that, as with development, is apparently genetically determined rather than environmentally dependent. Second, conidiophore development does not occur when cells are grown in liquid medium but takes place only when hyphae are exposed to an air interface (4, 19, 24). Induction of development ultimately leads to activation of transcription of the *brlA* gene, which in turn results in the sequential activation of numerous genes controlling various aspects of development (1, 8, 18). Adams et al. (1) showed that forced expression of *brlA* at any time during the life cycle overrides the need for prior conidiation-specific events and directs development of vegetative cells into reduced conidiophores even in a liquid medium. These data demonstrate that activation of *brlA* expression is a major control point in initiation of the conidiation pathway.

We have described the isolation of the *A. nidulans acoD* gene and have demonstrated that *acoD* is required for normal control of development. In contrast to many other cases of microbial development, *A. nidulans* conidiophore development initiates primarily as a programmed part of the life cycle rather than as a response to an unfavorable environment (21). Strains lacking the *acoD* gene no longer make conidiophores as a normal part of their life cycle but now respond to growth-limiting conditions by initiating development.

Our results show that under optimal growth conditions on supplemented medium, *acoD* is required for activation of *brlA* expression but that this requirement is partially overcome when growth is limited nutritionally. We were unable to detect any *brlA* mRNA in cultures even 48 h after development was induced in an *acoD* deletion mutant grown on complete medium (Fig. 6). When the same strain was induced to develop on minimal medium, we observed detectable levels of *brlA* transcript within 9 h and conidiophores appeared several hours later. Although *acoD* mutant strains are able to accumulate *brlA* mRNA and produce conidiophores under these conditions, the time of appearance of *brlA* transcript is delayed and the final level of accumulation is reduced. From these results, we suggest that *brlA* can be activated by more than one mechanism. In wild-type *A. nidulans*, the major developmental pathway requires *acoD* and results in a programmed induction of *brlA* expression that occurs independently of growth conditions. In the absence of *acoD*, development is controlled by a mechanism that senses growth rate or nutritional status directly.

There is no measurable effect of *acoD* mutations on vegetative growth in liquid medium, indicating that the *acoD* gene product is apparently required only after cells have reached developmental competence. However, *acoD* transcript accumulation is not developmentally regulated. *acoD* mRNA is present beginning shortly after germination and persists throughout the life cycle, although it is absent in mature spores (Fig. 3). Developmental induction results in a slight increase in *acoD* transcript levels, but the results presented here suggest that a major mechanism controlling *acoD* activity is likely to be posttranscriptional. This makes *acoD* the first example of a gene required for *A. nidulans* conidiation that is not controlled primarily at the level of transcription. The disappearance of *acoD* mRNA in spores argues that some level of transcriptional control of *acoD* also takes place.

acoD mutant strains appear to grow faster than wild-type *Aspergillus* strains, resulting in colonies made up of large masses of vegetative aerial hyphae, which give the colony a cottonlike or fluffy appearance (Fig. 1 and 4). Despite the increased size and total wet weight of *acoD* mutant colonies as compared with those of the wild type, the total dry mass of *acoD* mutants increases at the same rate as in wild-type colonies. Thus, the increased size of *acoD* mutant colonies likely reflects a difference in mode of growth rather than an increased ability to utilize nutritional substrates. *acoD* mutant strains primarily produce vegetative mycelia, while wild-type strains produce large numbers of spores. The

differences observed in colony size and wet weight likely reflect differences in the properties of these cell types.

It was previously shown that artificial induction of development by forcing *brlA* expression leads to a complete cessation of vegetative growth and induction of the conidiation pathway (1, 2). Thus, it is possible that the increased vegetative growth observed in *acoD* mutant strains resulted from the loss of *brlA*-induced growth inhibition. In fact, *acoD* mutant strains respond to forced expression of *brlA* just as the wild type does, by stopping growth and forming functional conidiophores (data not shown). An alternative explanation for the increased colony growth rate observed in *acoD* mutant strains is that the *acoD* product itself is directly involved in the switch from vegetative growth to development. Developmentally programmed changes in growth induced by *acoD* may result in activation of *brlA* expression, thus causing initiation of the central conidiation pathway. If this is the case, *acoD*-controlled *brlA* activation may represent a refinement of the more primitive control observed when growth is limited by environmental conditions.

ACKNOWLEDGMENTS

This work was supported by NIH grant GM45252-01A1 to Thomas H. Adams and by NSF grant DMB 8701-897 to Lawrence N. Yager.

We thank Jeff Mooney and Rodolfo Aramayo and our colleagues in the laboratory for many helpful discussions. We also thank Mike Plamann for critically evaluating the manuscript, Greg May for providing a plasmid genomic library, and Victoria Valadez and Foster McNair for providing technical assistance.

REFERENCES

1. **Adams, T. H., M. T. Boylan, and W. E. Timberlake.** 1988. *brlA* is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell **54**:353–362.
2. **Adams, T. H., and W. E. Timberlake.** 1990. Developmental repression of growth and gene expression in *Aspergillus*. Proc. Natl. Acad. Sci. USA **87**:5405–5409.
3. **Adams, T. H., and W. E. Timberlake.** 1990. Upstream elements repress premature expression of an *Aspergillus* developmental regulatory gene. Mol. Cell. Biol. **10**:4912–4919.

3a. **Adams, T. H., and W. E. Timberlake.** Unpublished data.

4. **Axelrod, D. E.** 1972. Kinetics of differentiation of conidiophores and conidia by colonies of *Aspergillus nidulans*. J. Gen. Microbiol. **73**:181–184.
5. **Axelrod, D. E., M. Gealt, and M. Pastushok.** 1973. Gene control of developmental competence in *Aspergillus nidulans*. Dev. Biol. **34**:9–15.
6. **Ballance, D. J., F. P. Buxton, and G. Turner.** 1983. Transformation of *Aspergillus nidulans* by the orotidine-5′-phosphate decarboxylase gene of *Neurospora crassa*. Biochem. Biophys. Res. Commun. **112**:284–289.
7. **Berse, B., A. Dmochowska, M. Skrzypek, P. Weglenski, M. A. Bates, and R. L. Weiss.** 1983. Cloning and characterization of the ornithine carbamoyltransferase gene from *Aspergillus nidulans*. Gene **25**:109–117.
8. **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.
9. **Brody, H., J. Griffith, A. J. Cuticchia, J. Arnold, and W. E. Timberlake.** 1991. Chromosome-specific recombinant DNA libraries from the fungus *Aspergillus nidulans*. Nucleic Acids Res. **19**:3105–3109.
10. **Butnick, N. Z., L. N. Yager, M. B. Kurtz, and S. P. Champe.** 1984. Genetic analysis of mutants of *Aspergillus nidulans* blocked at an early stage of sporulation. J. Bacteriol. **160**:541–545.
11. **Champe, S. P., M. B. Kurtz, L. N. Yager, N. J. Butnick, and D. E. Axelrod.** 1981. Spore formation in *Aspergillus nidulans*: competence and other developmental processes, p. 255–276. *In* G. Turian and H. R. Hohl (ed.), The fungal spore: morphogenetic controls. Academic Press, Inc., New York.
12. **Clutterbuck, A. J.** 1974. *Aspergillus nidulans*, p. 305–317. *In* R. C. King (ed.), Handbook of genetics. Academic Press, Inc. (London), Ltd., London.
13. **Dorn, G. L.** 1970. Genetic and morphological properties of undifferentiated and invasive variants of *Aspergillus nidulans*. Genetics **66**:267–279.
14. **Gibson, T. J., A. R. Coulson, J. E. Sulston, and P. F. R. Little.** 1987. Lorist2, a cosmid with transcriptional terminators insulating vector genes from interference by promoters within the insert: effect on DNA yield and cloned insert frequency. Gene **53**:275–281.
15. **Käfer, E.** 1977. Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. **19**:156–165.
16. **Martinelli, S. D., and A. J. Clutterbuck.** 1971. A quantitative survey of conidiation mutants in *Aspergillus nidulans*. J. Gen. Microbiol. **69**:261–268.
17. **Miller, B. L., K. Y. Miller, and W. E. Timberlake.** 1985. Direct and indirect gene replacements in *Aspergillus nidulans*. Mol. Cell. Biol. **5**:1714–1721.
18. **Mirabito, P. M., T. H. Adams, and W. E. Timberlake.** 1989. Interactions of three sequentially expressed genes control temporal and spatial specificity in *Aspergillus* development. Cell **57**:859–868.
19. **Morton, A. G.** 1961. The induction of sporulation in mould fungi. Proc. R. Soc. Lond. B **153**:548–569.
20. **Osmani, S. A., G. S. May, and N. R. Morris.** 1987. Regulation of the mRNA levels of *nimA*, a gene required for the G2-M transition in *Aspergillus nidulans*. J. Cell Biol. **104**:1495–1504.
21. **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.
22. **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.
23. **Tamame, M., F. Antequera, J. R. Villanueva, and T. Santos.** 1983. High-frequency conversion to a "fluffy" developmental phenotype in *Aspergillus* spp. by 5-azacytidine treatment: evidence for involvement of a single nuclear gene. Mol. Cell. Biol. **3**:2287–2297.
24. **Timberlake, W. E.** 1980. Developmental gene regulation in *Aspergillus nidulans*. Dev. Biol. **78**:497–510.
25. **Waring, R. B., G. S. May, and N. R. Morris.** 1989. Characterization of an inducible expression system in *Aspergillus nidulans* using *alcA* and tubulin-coding genes. Gene **79**:119–130.

25a. **Yager, L. N., and W. A. Hide.** Unpublished data.

26. **Yager, L. N., M. B. Kurtz, and S. P. Champe.** 1982. Temperature-shift analysis of conidial development in *Aspergillus nidulans*. Dev. Biol. **93**:92–103.
27. **Yelton, M. M., J. E. Hamer, and W. E. Timberlake.** 1984. Transformation of *Aspergillus nidulans* by using a *trpC* plasmid. Proc. Natl. Acad. Sci. USA **81**:1470–1474.