Identification of Developmental Regulatory Genes in *Aspergillus nidulans* by Overexpression

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Manuscript received September 21, 1994
Accepted for publication October 22, 1994

ABSTRACT

Overexpression of several *Aspergillus nidulans* developmental regulatory genes has been shown to cause growth inhibition and development at inappropriate times. We set out to identify previously unknown developmental regulators by constructing a nutritionally inducible *A. nidulans* expression library containing small, random genomic DNA fragments inserted next to the *alcA* promoter [*alcA(p)*] in an *A. nidulans* transformation vector. Among 20,000 transformants containing random *alcA(p)* genomic DNA fusion constructs, we identified 66 distinct mutant strains in which *alcA(p)* induction resulted in growth inhibition as well as causing other detectable phenotypic changes. These growth inhibited mutants were divided into 52 FIG (Forced expression Inhibition of Growth) and 14 FAB (Forced expression Activation of *brlA*) mutants based on whether or not *alcA(p)* induction resulted in accumulation of mRNA for the developmental regulatory gene *brlA*. In four FAB mutants, *alcA(p)* induction not only activated *brlA* expression but also caused hyphae to differentiate into reduced conidiophores that produced viable spores from the tips as is observed after *alcA(p)::brlA* induction. Sequence analyses of the DNA fragments under *alcA(p)* control in three of these four sporulating strains showed that in two cases developmental activation resulted from overexpression of previously uncharacterized genes, whereas in the third strain, the *alcA(p)* was fused to *brlA*. The potential uses for this strategy in identifying genes whose overexpression results in specific phenotypic changes like developmental induction are discussed.

OUR primary interest is in understanding the mechanisms regulating the switch from vegetative growth to initiation of the pathway controlling asexual reproductive development, or conidiation, in *Aspergillus nidulans*. It was observed previously that overexpression of two known pathway-specific regulators of conidiophore development, *brlA* and *abaA*, caused vegetative cells to stop growing and initiate development at inappropriate times (ADAMS *et al.* 1988; MIRABITO *et al.* 1989). This developmentally induced growth inhibition was shown to result from blocks at the transcriptional, translational and posttranslational levels for several genes required during vegetative growth (ADAMS and TIMBERLAKE 1990). From these results, we reasoned that if inappropriate *brlA* expression caused growth cessation, then activation of genes functioning before *brlA* in development also should inhibit growth if their expression resulted in *brlA* activation. With this in mind, we set out to identify and characterize genes that cause severe growth inhibition when overexpressed in *A. nidulans* vegetative hyphae. This was accomplished by transforming *A. nidulans* with an inducible *A. nidulans* genomic expression library and screening for mutant transformants having restricted growth on expression-inducing medium.

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This approach is likely to identify genes other than regulators of conidiophore development that inhibit vegetative growth when overexpressed. For example, overexpression of critical mitotic regulators such as *nimA* and *bimE* has been shown to cause cell cycle arrest in Aspergillus (OSMANI *et al.* 1988a,b). It also has been observed that overexpression of a diverse group of genes in *Saccharomyces cerevisiae* can result in lethality (ROSE and FINK 1987; HURT 1988; BURKE *et al.* 1989; LIU *et al.* 1992). Many of these genes encode cytoskeletal components and LIU *et al.* (1992) used this property of overexpression lethality as a screen in attempting to identify additional *S. cerevisiae* genes needed for proper cytoskeletal function. Besides identifying genes encoding actin (*ACT1*), β-tubulin (*TUB2*) and actin-binding protein I (*ABP1*), it was shown that overexpression of genes that code for a nuclear sequence recognition protein (*NSR1*), a cAMP-dependent protein kinase subunit (*TPK1*), a type 1 protein phosphatase (*GLC7*), a nonhistone protein B and several unknown open reading frames (ORFs) inhibited growth when overexpressed (LIU *et al.* 1992). We expect that if overexpression of a gene inhibits growth in Aspergillus because of developmental induction rather than interfering with some other cellular process, it will cause activation of developmentally specific genes such as *brlA*. This hypothesis is supported by the fact that several different genes that we have identified on the basis of

TABLE 1

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

Strain	Genotype	Source
TA046	*biA1; argB2; pryoA4; veA1, ΔbrlA*	ADAMS and TIMBERLAKE 1990
FGSC26	*biA1; veA1*	Fungal Genetics Stock Center
RMS010	*biA1; ΔargB::trpCΔB; methG1; veA1, trpC801*	STRINGER *et al.* 1991
TTA292	*biA1; argB::alcA(p)::brlA; methG1; veA1*	ADAMS *et al.* 1988
TPM1	*biA1; argB::alcA(p)::abaA; methG1; veA1*	MIRABITO *et al.* 1989
TTAARG	*biA1; methG1; veA1*	ADAMS *et al.* 1988
TTAP714	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study
TTAP715	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study
TTAP721	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study
TTAP71	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study
TTAP79	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study
TTAP730	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study
TTAP85	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study
TTAP22	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study
TTAP17	*biA1;ΔargB::trpCΔB; methG1; veA1, trpC801*	This study

Strains created in this study were obtained by transformation of RMS010 with the genomic *alcA(p)* expression library. All these strains are *argB*$^+$ and have an *alcA(p)* fusion integrated in the genome.

their requirement for conidiophore develoment and normal activation of *brlA* expression, can cause both growth arrest and induction of *brlA* when overexpressed (LEE and ADAMS 1994; J. WIESER, B. LEE, J. FONDON and T. ADAMS, unpublished results).

Here we describe the construction of an *A. nidulans* expression library with random 2- to 10-kb genomic DNA fragments under the control of the nutritionally regulated inducible *alcA* promoter (GWYNNE *et al.* 1987). We analyzed ~20,000 primary transformants containing *alcA(p)* fusion constructs and identified 66 unique strains that each displayed a growth inhibited phenotype when plated on *alcA*-inducing medium but grew like wild type on *alcA*-repressing media. These growth inhibited mutants were categorized into two distinct groups designated FIG (Forced expression Inhibition of Growth) and FAB (Forced expression Activation of *brlA*) on the basis of whether or not *brlA* mRNA accumulates after *alcA* promoter induction. We have recovered *alcA(p)* fusion constructs from five transformed strains including three FAB mutants for which *brlA* expression after *alcA(p)* induction resulted in inappropriate sporulation. In one of these mutants, the *alcA(p)* was fused directly to the *brlA* gene indicating that this approach can be used to identify developmental regulatory genes.

MATERIALS AND METHODS

***A. nidulans* strains and growth conditions:** All *A. nidulans* strains used in this study are listed in Table 1. FGSC26 was used for isolation of genomic DNA for constructing the expression library. RMS010 (STRINGER *et al.* 1991) was used as the expression plasmid recipient in transformation experiments using standard *A. nidulans* techniques (YELTON *et al.* 1984). Standard *A. nidulans* genetic procedures were followed (PONTECORVO *et al.* 1953). All strains were grown in appropriately supplemented minimal medium (KÄFER 1977).

Expression vector and library construction and identification of growth-inhibited transformants: The *alcA(p)* expression vector pON24 (Figure 1) was constructed in three steps. First, a 500-bp *Bam*HI-*Bgl*II fragment from pALCA1 (ADAMS *et al.* 1988) containing the transcriptional promoter for the *A. nidulans* alcohol dehydrogenase gene [*alcA(p)*] was inserted into the *Bam*HI site of pBLUESCRIPT II SK$^-$ (Stratagene) to create pON2. Second, pON2 was digested with *Sal*I and religated to remove part of the polylinker and sequences in the *alcA(p)* fragment that include a *Xba*I site. Finally, a 3.3-kb *Xba*I fragment containing the entire *A. nidulans argB* gene (UPSHALL et al. 1986) was inserted into the unique *Xba*I site remaining in the pBLUESCRIPT II SK$^-$ polylinker present in pON23 to give pON24.

Genomic DNA was prepared from *A. nidulans* strain FGSC26 using conventional techniques (TIMBERLAKE 1986) and was used to construct the expression library. Genomic DNA (1 μg) was digested partially with *Mbo*I to give a range of fragment sizes from 2 to 10 kb and partially filled in with dGTP and dATP using the Klenow fragment of DNA polymerase I to leave a 2-bp overhang. The prepared genomic DNA then was ligated with 100 ng of pON24 vector DNA that had been restricted with *Xho*I, dephosphorylated using calf intestinal alkaline phosphatase and partially filled with dCTP and dTTP using Klenow fragment. The ligation mixture was divided into 10 aliquots that were used separately to transform competent DH5α *Escherichia coli* cells by electroporation using standard protocols (AUSUBEL *et al.* 1987). Approximately 10,000 total transformants resulted from each aliquot of the ligation mixture and these were treated as separate pools. Each transformant pool was suspended in LB medium containing 15% glycerol and stored at −80°.

Conditional growth-inhibited *A. nidulans* transformants were identified by transferring spores from primary transformants to agar solidified minimal medium containing either 50 mM glucose [*alcA(p)* repressing] or 100 mM L-threonine [*alcA(p)* inducing] as the sole carbon source. Colonies were screened visually to identify those with reduced growth on L-threonine medium but normal growth on glucose me-

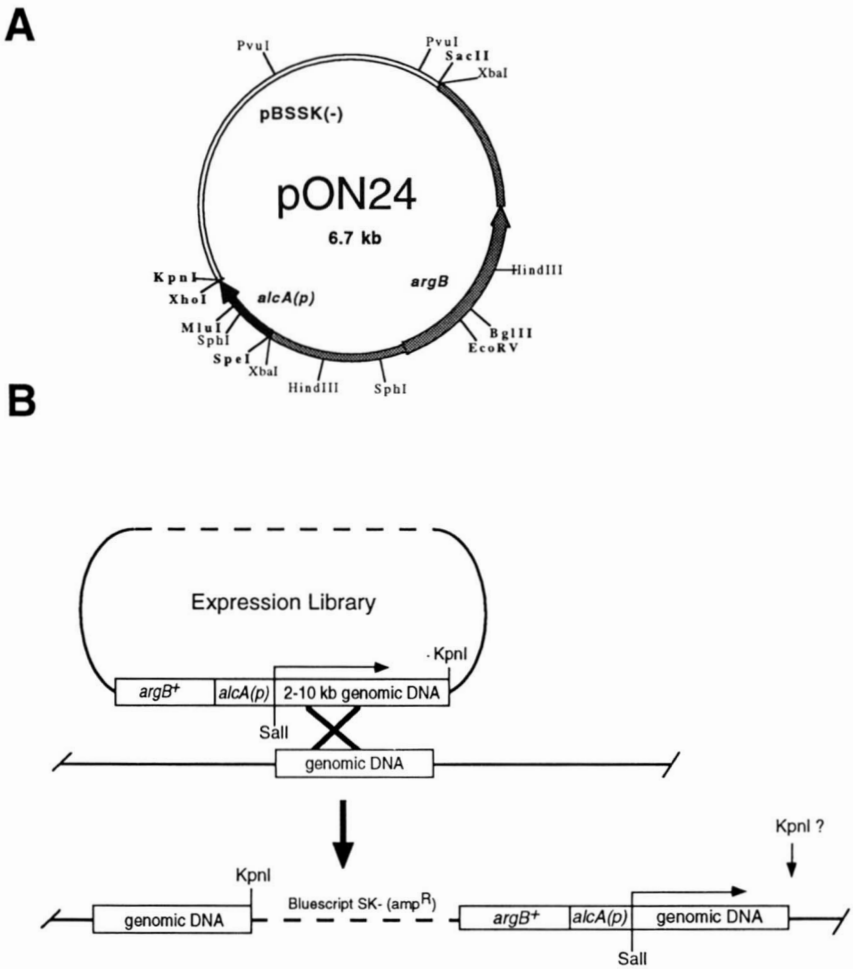

FIGURE 1.—(A) Restriction map of the genomic library expression vector pON24. Sites for enzymes that cleave the vector once or twice or were important in this work are shown; unique restriction sites are shown in bold letters. The promoter from the *A. nidulans* alcohol dehydrogenase gene [*alcA(p)*] is shown as a solid arrow pointing in the direction of transcription. The *A. nidulans argB* gene is shown as a gray bar with the arrow depicting the protein coding region and direction of transcription. (B) Predicted restriction pattern if the transforming plasmid integrated into the genome by homologous recombination at the site of the cloned insert.

dium and examined microscopically to observe formation of abnormal structures. For *alcA(p)* induction time course experiments, spores were inoculated at a density of 1×10^6 spores/ml in minimal medium containing 50 mM glucose and shaken at 300 rpm and 37° for 14 hr. 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.

Nucleic acid manipulations: Genomic DNA was isolated from each *A. nidulans* transformant, individually digested with *Sal*I, *Kpn*I and *Mlu*I, separated electrophoretically on a 1% agarose gel and transferred to Hybond N^+ membrane as recommended by the manufacturer (Amersham). The resulting blot was hybridized to randomly ^{32}P-labeled pBLUESCRIPT II SK$^-$ (Stratagene). If two transformants had the same hybridization pattern with all three restriction digests they were classified as the same.

Total RNA was isolated as previously described (ADAMS *et al.* 1988), separated by electrophoresis on formaldehyde-agarose gels and transferred without pretreatment to Hybond N^+ membrane. The 2.5-kbp *Bam*HI-*Sal*I fragment from the *brlA* gene was randomly ^{32}P-labeled for use as a *brlA*-specific probe (BOYLAN *et al.* 1987).

Recovery of integrated plasmids from Aspergillus: Genomic DNA (5 μg) that had been purified from transformants using standard protocols (TIMBERLAKE 1986) was digested to completion with *Kpn*I and then allowed to ligate with itself in a total volume of 100 μl incubating at 15° for 48 hr with 1 unit T4 DNA ligase. The ligation mixture was precipitated and 500 ng of the ligated genomic DNA was used to transform DH5α by electroporation. Typically, 10–100 ampicillin-resistant colonies containing plasmid were recovered.

Microscopy and photography: All light microscopy was done using an Olympus BH-2 microscope using differential interference contrast optics.

RESULTS

Isolation and characterization of conditional growth-inhibited mutants: We had observed previously that forced expression of the *A. nidulans* early developmental regulatory gene *brlA* in vegetative hyphae activated inappropriate sporulation and caused severe growth inhibition (ADAMS *et al.* 1988; ADAMS and TIMBERLAKE 1990). This led us to hypothesize that one way to identify genes controlling activation of *brlA* expression was to identify genes that caused growth inhibition (and perhaps *brlA* induction) when overexpressed in vegetative hyphae. Because we were not certain when mRNAs for developmental inducers most likely would be expressed and we assumed that mRNAs for many regulatory genes would be present at low

TABLE 2

Forced expression mutant phenotypes

Description	Total Strains	*brlA* Activation		Sporulation
		+	−	
Spore/Hyphal Swelling	12	4	8	0
Nongermination	2	0	2	0
Curly Hyphae	5	3	2	1
Inhibited Growth				
Severe	8	4	4	1
Moderate	39	3	36	2
Total	66	14	52	4

Descriptions are of growth phenotype observed on solid *alcA(p)*-inducing medium. The relative level of *brlA* mRNA accumulation: + high levels, or − no detectable *brlA* mRNA accumulation after 9 hr growth in induction media. Spores were observed forming from hyphal tips under *alcA(p)*-inducing conditions.

levels relative to other genes, we concluded that the best way to assure randomness in our expression library would be to fuse genomic DNA fragments, rather than cDNAs, to the inducible *alcA* promoter (see MATERIALS AND METHODS).

Strain RMS010 (Δ*argB*) was transformed separately using DNA obtained from each of the 10 distinct plasmid pools (each containing 10,000 plasmids) and selected for arginine prototrophy on media that caused repression of the *alcA* promoter (e.g., glucose; PATEMEN *et al.* 1983). Resulting transformants were replicated to media with *alcA(p)*-repressing or -inducing (e.g., L-threonine) carbon sources and screened for strains that grew like wild type on *alcA*-repressing media but had inhibited growth on *alcA(p)*-inducing media. In this way we have examined 20,000 total transformants (1000–3000 from each pool) and recovered 101 conditional growth inhibited mutants. We described this phenotype as FIG.

Because each of the FIG mutants was generated by transformation with a pool of plasmids, it was necessary to determine if these transformants had different plasmids inserted in their genomes. Genomic DNA was prepared from each transformant, digested with *Sal*I, *Mlu*I or *Kpn*I and Southern blots were probed with radioactively labeled plasmid DNA. By comparing hybridization patterns, we determined that 66 of the 101 initial FIG mutants contained unique clones (Table 2). This analysis also allowed us to determine if more than one plasmid had been inserted in the genome of a particular transformant. Because the portion of pON24 recognized by the probe has a single *Sal*I site, a single *Kpn*I site and no *Mlu*I sites, strains containing a single plasmid integrated into the genome should have a single hybridizing plasmid band of variable size for each genomic digest. As shown in Figure 2, while several mutants had a single vector band (33 of 66) in the genome, others had more than one and some had several.

We further subdivided the 66 different FIG mutants into four groups on the basis of the morphological phenotypes observed on agar plates containing *alcA(p)*-inducing medium (Table 2). Twelve strains were described as "spore- or hyphal-swelling" mutants because spores from each of these mutants swelled to form large spheres when plated on induction media. When these spore swelling mutants formed germ tubes, the hyphae

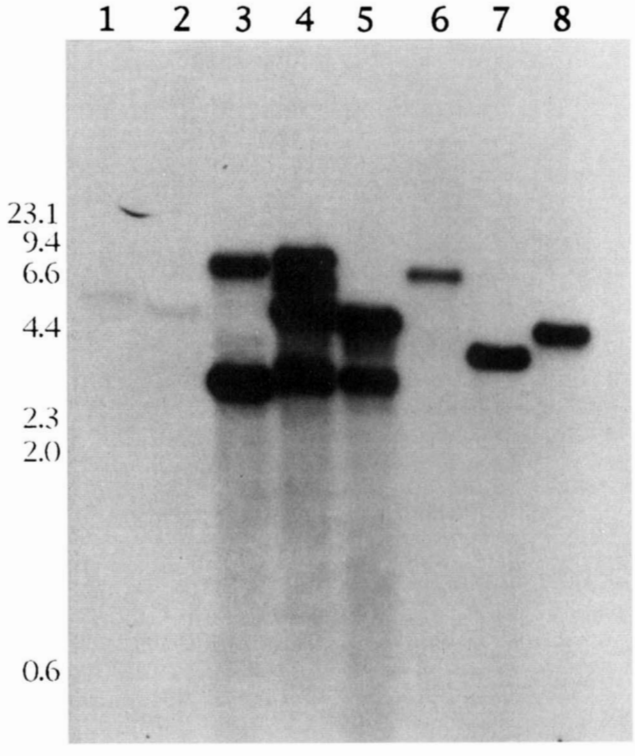

FIGURE 2.—Southern blot analysis of *A. nidulans* transformants. Genomic DNA from mutant strains TTAP15 (1), TTAP17 (2) TTAP71 (3), TTAP714 (4), TTAP715 (5), TTAP721 (6), TTAP730 (7) and TTAP85 (8) was digested with *Sal*I, separated electrophoretically on a 1% agarose gel, blotted and probed with radioactively labeled plasmid DNA. A single hybridization band represents a unique integration event.

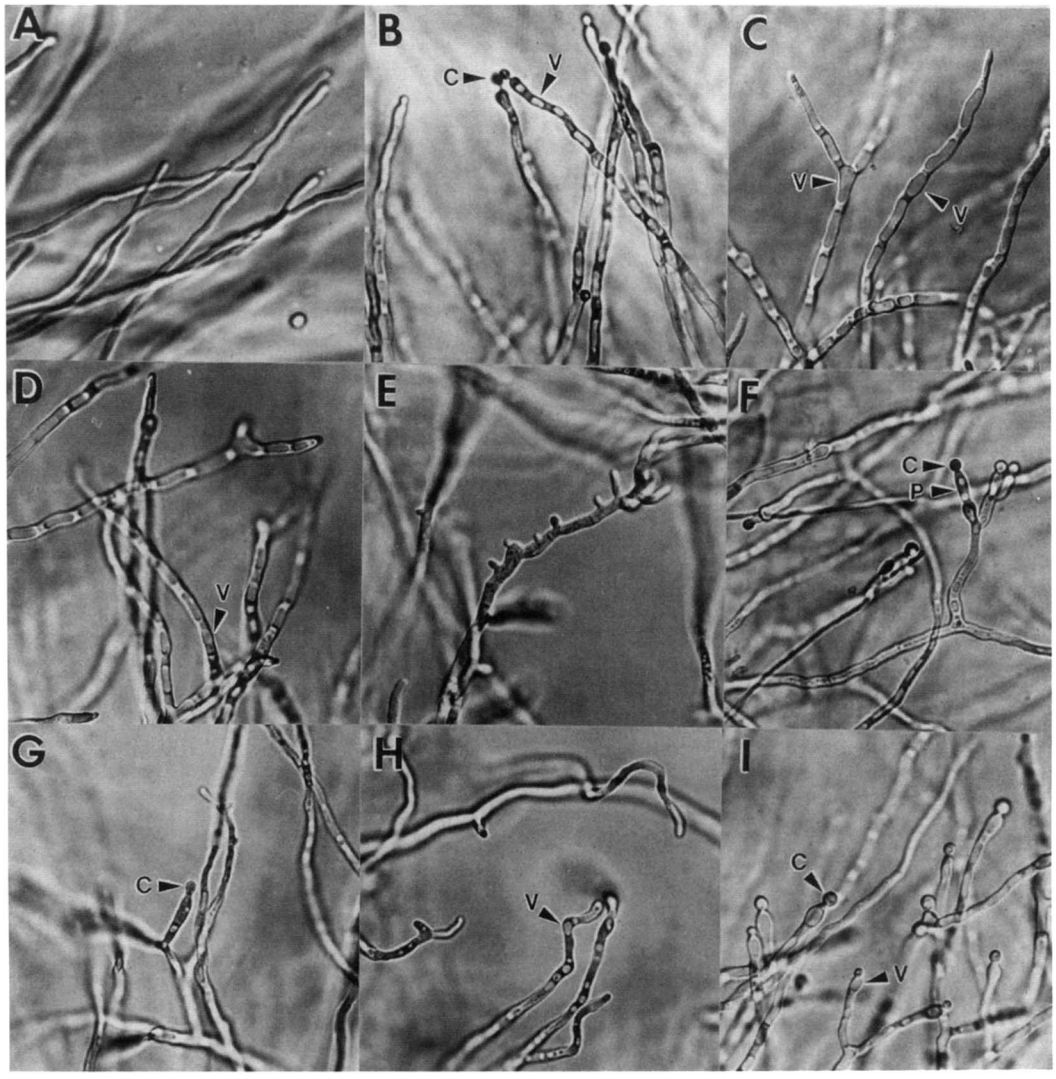

FIGURE 3.—Mutant phenotypes. Strains TTAARG (wild type, A); TTA292 [*alcA(p)::brlA*, B], TPM1 [*alcA(p)::abaA*, C], TTAP17 (D), TTAP22 (E), TTAP714 (F), TTAP715 (G), TTAP85 (H) and TTAP721 (I) were grown in liquid minimal medium containing 50 mM glucose as sole carbon source for 14 hr at 37° and then transferred to minimal medium containing 100 mM L-threonine as sole carbon source to induce *alcA(p)* expression. Photographs were taken 12 hr after transfer. Cell types and structures labeled are: conidia, C; vacuoles, V; and phialides, P.

produced were swollen and made bulbous structures. Two mutants were categorized as "nongerminating" because their spores never germinated on induction medium. However, the defects in these mutants were not germination specific because when mutant spores were allowed to germinate on *alcA*-repressing media and then transferred to *alcA*-inducing media, growth stopped immediately. Five mutants were described as "curly hyphal-growth" mutants because the hyphae produced after germination on inducing media grew in a curly wandering pattern. Finally, 47 mutants did not form novel structures and were classified as generally growth inhibited. The degree of growth inhibition varied from moderate to severe.

As shown in Figure 3, abnormal growth phenotypes also were observed for FIG mutants grown in submerged culture when spores were first allowed to germinate under noninducing conditions and then growing hyphae were transferred to *alcA(p)*-inducing media. TTAP714 (Figure 3, F), TTAP715 (Figure 3, G) and TTAP721 (Figure 3, I) all produced spores from hyphal tips much as was observed after *alcA(p)*-induced expression of *brlA* in vegetative hyphae (ADAMS *et al.* 1988; Figure 3, B). TTAP85 (Figure 3, H) also produced some spores from hyphal tips (not shown), but the predominant phenotype involved formation of curled, vacuolated hyphae. Finally, TTAP17 (Figure 3, D) and TTAP22 (Figure 3, E) did not produce spores but went through distinct morphological changes. Hyphae of strain TTAP17 were strongly growth inhibited and became highly vacuolated as was also observed after *alcA(p)*-induced expression of the developmental regu-

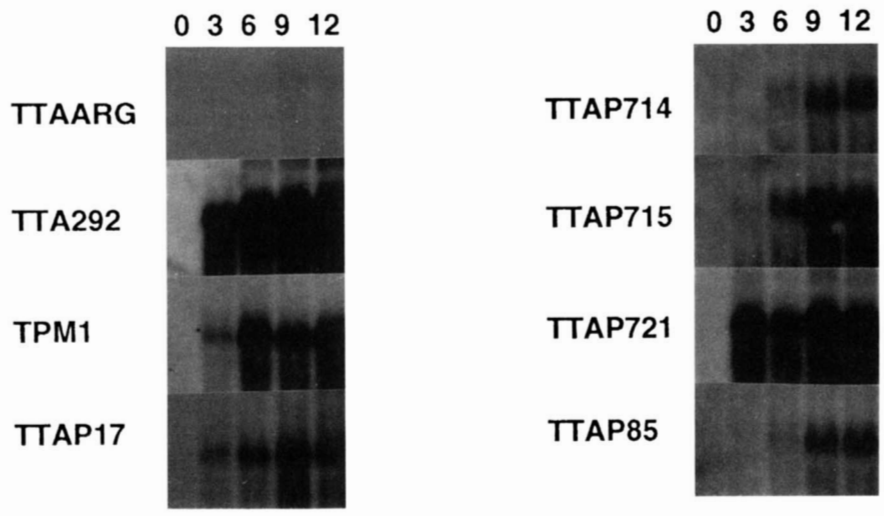

FIGURE 4.—*brlA* mRNA accumulation in overexpression mutant strains. Strains TTA292 [*alcA(p)::brlA*], TPM1 [*alcA(p)::abaA*], TTAARG (wild-type), TTAP17 (swollen spore mutant), TTAP714 (growth-inhibited mutant), TTAP715 (growth-inhibited mutant), TTAP721 (growth-inhibited mutant) and TTAP85 (curly hypha mutant) were grown in liquid *alcA*-repressing media for 14 hr and then transferred to liquid *alcA*-inducing media. Total RNA (10 μg/lane) was isolated from samples taken 0, 3, 6, 9 and 12 hr after transfer to *alcA*-inducing media and fractionated on formaldehyde-agarose gels. The resultant blots were probed with a radioactively labeled *brlA* gene fragment.

latory gene *abaA* (MIRABITO *et al.* 1989; Figure 3, C). Hyphae of strain TTAP22 (Figure 3, E) were highly branched and somewhat swollen.

Characterization of *brlA* expression in growth-inhibited mutants: Conidiophore development and *brlA* expression are normally suppressed when wild-type *A. nidulans* is grown in submerged culture. We measured *brlA* mRNA accumulation in each of the 66 strains during growth in submerged culture after *alcA(p)* induction to determine if induced expression of sequences fused to the *alcA(p)* could activate inappropriate development-specific gene expression. Each of the strains was grown for 14 hr under *alcA(p)*-repressing conditions, then transferred to *alcA(p)*-inducing medium and grown for 9 hr before samples were taken for RNA isolation. As described in Table 2, we found that *brlA* mRNA accumulated to high levels in 14 strains, including all 4 of the strains that sporulated inappropriately. These mutants were classified as having a FAB phenotype. *brlA* mRNA was not detected at significant levels in any of the other 52 strains classified as FIG mutants.

Figure 4 shows the patterns and levels of *brlA* mRNA accumulation for several representative FAB mutant strains and for strains containing *alcA(p)::brlA* (TTA292; ADAMS *et al.* 1988) or *alcA(p)::abaA* (TPM1; MIRABITO *et al.* 1989) fusions. *brlA* mRNA was not detectable in any of the strains grown for 14 hr in liquid medium containing glucose as a carbon source (Figure 4, 0 hr). However, *brlA* message was easily detectable in strains TTA292 [*alcA(p)::brlA*], TPM1 [*alcA(p)::abaA*], TTAP17, TTAP715 and TTAP721 by 3 hr after transfer to *alcA(p)*-inducing media. *brlA* mRNA also was detected in strains TTAP714 and TTAP85 but not until 6 and 9 hr after *alcA(p)* induction respectively. No *brlA* mRNA was detected in TTAARG, a wild-type control strain, even 12 hr after transfer to L-threonine medium.

Linkage analysis: Because the phenotypes of each of the mutants described were observed only if the strains were grown on *alcA(p)*-induction medium, we predicted that each mutant arose through integration of the transforming plasmid DNA into the genome rather than by spontaneous mutation. The observed phenotype should therefore be linked to the *argB*$^+$ gene in pON24. To test this hypothesis, and to determine if growth inhibition observed for each of the FAB mutants was dependent on the presence of a wild-type *brlA* gene, we crossed each of the 14 FAB mutants with an *argB*$^-$ Δ*brlA* strain (TA046) and examined meiotic progeny. In each case, all FAB mutant progeny were *argB*$^+$ and, for strains having a single plasmid integrated in the genome, all *argB*$^+$ progeny had the FAB phenotype. However, in strains with multiple plasmid integration events, some *argB*$^+$ progeny behaved like wild-type on *alcA*-inducing medium. For all 14 FAB mutant strains, the growth inhibition phenotype caused by the *alcA(p)* gene fusion was *brlA* independent because the effect was observed on *alcA*-inducing medium in both *brlA*$^+$ and *brlA*$^-$ strains. The *alcA(p)* gene fusion in one of the four sporulating FAB mutants (TTAP721) also caused *brlA*-independent sporulation.

Recovery and characterization of *fig* genes: *A. nidulans* transformation typically requires integration of the transforming DNA into the chromosome, necessitating a strategy for recovering the transforming plasmid to allow characterization of sequences under *alcA(p)* control (TIMBERLAKE and MARSHALL 1989). Because the

entire *argB* gene has been deleted from RMS010, integration of transforming DNA into the genome of RMS010 could occur at a heterologous site or through homologous recombination at the site of the cloned genomic fragment (Figure 1B) or at the *alcA(p)*. In the second two cases, this would result in a duplication of the target region separated by plasmid sequences as shown in Figure 1B. Because we knew that the transformation vector (pON24) had a single *Kpn*I site, we predicted that the plasmid vector and some unknown amount of the genomic DNA placed under *alcA(p)* control could be recovered intact by restricting genomic DNA from a transformant with *Kpn*I followed by ligation and bacterial transformation, selecting for the presence of the ampicillin resistance marker present in pON24. We tested this by isolating genomic DNA from two FIG mutant strains, TTAP22 and TTAP79 and recovered related plasmids (pTAP22 and pTAP79 respectively) from each strain that corresponded to pON24 with unique genomic sequences next to the *alcA(p)*. Each isolated plasmid was used to transform RMS010 to arginine prototrophy and in both cases, >90% of the transformants displayed the same *alcA*-inducible FIG phenotype as the original *A. nidulans* mutant from which the plasmid was recovered. This result confirmed that the FIG phenotype resulted from overexpression of genomic sequences recovered in the plasmid.

To begin to examine the mechanism of growth inhibition, we have determined the DNA sequences for the entire insert in pTAP22 (Figure 5) and for ~1 kb of DNA inserted next to the *alcA(p)* in pTAP79. A long ORF was identified in the pTAP22 insert that began with an AUG just downstream of the *alcA* transcription start site and extended for 980 codons before reaching a termination codon (Figure 5). When the predicted amino acid sequence for this ORF was used to search various databases using the BLAST algorithm (ALTSCHUL *et al.* 1990), we found it had several regions of identity to the C-terminal half of the predicted product of a *S. cerevisiae* gene (*BNI1*) isolated in a screen for mutants that were synthetically lethal in a *cdc12* mutant strain (Figure 6; J. PRINGLE and H. FARES, personal communication). No significant identities were observed between sequences cloned next to the *alcA(p)* in pTAP79 and any known genes.

Because the ORF in pTAP22 was located directly downstream of the *alcA(p)*, we predicted that the FIG phenotype resulted from overexpression of this region and the gene was tentatively designated *figA*. To determine if *alcA(p)*-induction in TTAP22 resulted in *figA* overexpression, total RNA was isolated from a wild-type strain and from TTAP22 grown under *alcA*-repressing or -inducing conditions and RNA blots were probed with labeled fragments corresponding to the predicted *figA* coding sequences. This probe hybridized to a large (>7 kb) RNA present in both wild type and TTAP22 under all growth conditions and also hybridized to an abundant 3.5-kb RNA present only in TTAP22 after *alcA*-induction (data not shown). These results support the hypothesis that the observed ORF corresponds to *figA* and raise the possibility that growth inhibition results from overexpression of a partial gene product.

Recovery and characterization of *fab* genes: We recovered the integrated plasmids from genomic DNA for three of the four strains (TTAP715, TTAP721 and TTAP85) for which *alcA(p)* induction resulted in inappropriate sporulation from hyphal tips during growth in submerged culture (Figure 3). Each isolated plasmid was used to transform RMS010 to arginine prototrophy and in all three cases, transformants were isolated that displayed the same FAB phenotype as the original *A. nidulans* mutants from which the plasmids were recovered. The sequences of the DNAs directly adjacent to the *alcA(p)* in each plasmid were determined to see if the fragments were derived from known *A. nidulans* developmental regulatory genes. We found that plasmids recovered from two strains (TTAP715 and TTAP85) contained unique, previously uncharacterized DNA fragments. However, in the third strain, TTAP721, the *brlA* coding region was inserted just downstream of the *alcA(p)* suggesting that growth inhibition and sporulation in strain TTAP721 resulted from forced induction of *brlA* as described previously (ADAMS *et al.* 1988). This result demonstrates that this approach can identify developmental regulators.

DISCUSSION

Induced expression of several well-characterized *A. nidulans* developmental regulators, including *abaA* and *brlA*, in vegetative cells activates development and blocks growth (ADAMS *et al.* 1988; MIRABITO *et al.* 1989; ADAMS and TIMBERLAKE 1990; LEE and ADAMS 1994). We have taken advantage of this observation in devising a scheme for identifying additional genes that regulate *A. nidulans* asexual sporulation. We constructed a genomic DNA expression library for *A. nidulans* that contains ~100,000 plasmid clones with 2–10 kbp fragments inserted next to the alcohol inducible *alcA(p)* in a transformation vector that contains the *argB*$^+$ gene for use as a selectable marker. This library was used to transform an Δ*argB A. nidulans* strain, and transformants selected on *alcA(p)*-repressing medium lacking arginine were screened on *alcA(p)*-inducing medium to identify conditionally growth inhibited strains. Through this approach, we identified 66 different mutant strains that were severely growth inhibited after induction of *alcA* expression. These 66 mutants included 14 strains called FAB mutants in which *alcA(p)*-induced growth inhibition was coupled to greatly increased accumulation of the development-specific *brlA* transcript under conditions that normally block *brlA* expression (Figure 4). Although

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                M  L  V  D  A  P  E  N  D  L  Q  L  R  C  H  I  R  A  Q  F  I  S  C  G  I  K  R  L  L  S  K  M  E  G  F  Q
1   GGATCCTCATTAACATGTTGGTGGATGCACCCGAGAATGACCTGCAGTTACGATGTCATATCCGAGCGCAGTTCATATCTTGTGGCATCAAACGACTTTTGTCGAAGATGGAAGGCTTTC  120

    Y  E  V  I  D  K  Q  I  E  H  F  R  E  N  E  A  I  D  Y  E  D  L  L  Q  R  E  S  S  S  T  K  D  S  I  E  G  E  V  K  D
121 AGTATGAAGTTATTGATAAACAGATTGAGCATTTTCGAGAGAATGAGGCCATTGATTACGAAGATTTACTGCAACGCGAGAGTAGCAGTACGAAGGATAGTATTGAGGGGAGGTTAAGG  240

    M  T  D  P  L  Q  I  T  D  A  I  A  S  R  L  N  G  T  R  A  H  D  Y  F  L  S  A  L  Q  H  L  L  L  I  R  E  N  S  G  E
241 ACATGACCGACCCTCTTCAGATCACTGACGCCATCGCGAGTCGCCTCAATGGAACGAGAGCCCACGATTACTTCCTTTCAGCTCTGCAGCATCTGCTTCTCATCCGGGAGAATTCTGGGG  360

    D  G  L  R  M  Y  Q  L  V  D  A  M  L  S  Y  V  A  M  D  R  R  L  P  D  L  D  L  R  Q  G  L  T  F  T  V  Q  S  L  L  D
361 AGGATGGTCTCAGAATGTACCAGCTCGTGGACGCTATGCTAAGCTATGTGGCTATGGATAGGAGGCTACCGGATCTTGACCTGCGGCAGGGATTGACATTCACTGTACAGAGCTTGTTAG  480

    R  L  H  T  D  A  E  A  R  R  A  Y  D  E  S  L  E  A  R  Q  I  A  E  A  A  L  A  E  R  D  E  M  K  A  Q  V  E  L  G  A
481 ATCGACTACATACGGATGCAGAAGCGAGACGAGCGTACGACGAATCTCTGGAAGCGCGCCAAATCGCAGAAGCCGCACTTGCTGAACGAGATGAGATGAAGGCGCAGGTTGAGTTAGGCG  600

    D  G  L  V  R  K  L  Q  K  Q  I  E  E  Q  T  G  I  I  E  L  Q  S  R  Q  N  E  M  L  K  A  E  L  A  D  V  Q  R  L  R  A
601 CGGATGGCCTGGTGAGAAAGTTACAGAAGCAGATTGAGGAGCAGACCGGTATAATTGAGCTACAGAGCAGGCAGAATGAGATGCTCAAGGCCGAGCTTGCCGATGTTCAGAGGCTTCGTG  720

    Q  E  L  Q  R  N  E  L  E  T  R  E  L  Y  L  M  L  R  D  A  Q  D  I  A  A  S  N  A  K  K  S  N  M  G  E  A  E  T  D  P
721 CTCAAGAGTTGCAGCGGAATGAGCTGGAGACGCGAGAACTCTACCTTATGCTTCGCGACGCTCAAGATATTGCGGCATCAAATGCGAAAAAGTCGAACATGGGAGAGGCCGAGACGGATC  840

    A  H  M  R  G  I  L  D  R  E  K  L  L  T  R  L  E  K  Q  L  E  R  T  K  T  Q  F  K  L  E  G  K  V  W  G  Q  H  D  P  S
841 CCGCTCATATGCGAGGGATCCTAGATAGAGAGAAGCTGTTGACACGGCTAGAGAAGCAGCTTGAAAGGACAAAGACGCAATTCAAGTTGGAAGGTAAAGTTTGGGGCCAGCATGACCCAT  960

    D  R  L  R  E  L  R  E  Q  M  D  G  D  A  G  P  R  E  A  F  E  E  Q  A  R  L  N  L  S  L  N  P  V  G  S  V  Y  R  K  K
961 CGGACCGACTGCGTGAGCTCCGTGAGCAAATGGACGGCGATGCTGGGCCCAGAGAAGCCTTCGAAGAGCAAGCCGCCTGAACCTCAGCCTGAACCCAGTAGGATCGGTTTACCGCAAGA 1080

    T  Y  I  Q  G  M  E  D  T  A  T  E  E  L  G  Q  T  D  D  E  V  V  Y  A  K  A  R  L  V  D  L  H  R  P  R  M  D  P  E  Q
1081 AAACCTACATCCAGGGCATGGAGGATACTGCCACCGAGGAGCTAGGCCAAACTGATGATGAAGTCGTCTACGCTAAGGCCCGACTTGTGGATCTTCATCGGCCGCGTATGGACCCGGAAC 1200

    A  T  G  L  L  G  E  I  A  A  K  V  P  K  I  D  A  D  D  A  K  D  E  G  K  P  T  E  S  E  Q  P  A  E  G  A  A  T  K  G
1201 AAGCAACTGGCCTTCTTGGTGAGATTGCTGCCAAGGTTCCAAAGATCGATGCCGATGATGCGAAGGATGAGGGCAAACCAACTGAATCTGAACAGCCAGCAGAAGGTGCAGCCACCAAAG 1320

    D  E  Q  G  V  D  D  T  V  A  V  D  K  A  T  A  A  P  P  P  P  P  P  P  P  P  P  A  H  P  G  L  S  G  A  A  P  P  P  P  P
1321 GCGATGAACAAGGAGTGGACGATACCGTCGCTGTGGACAAAGCCACTGCGGCTCCTCCGCCGCCGCCTCCCCCTCCTCCCGCACATCCAGGATTATCTGGAGCAGCTCCGCCACCCCCAC 1440

    P  P  P  P  P  P  P  G  A  G  A  A  P  P  P  P  P  P  P  P  P  P  P  P  P  P  G  G  L  G  G  P  P  P  L  P  S  P  S  S  T  G
1441 CGCCGCCGCCTCCACCACCCCCAGGCGCAGGAGCAGCACCACCACCTCCTCCGCCGCCACCACCACCCCCTCCGGGTGGACTTGGCGGCCCCCCTCCCCTCCCCTCCTCCACCG 1560

    W  F  W  W  T  P  A  A  S  P  S  T  W  W  F  W  R  S  T  S  A  A  A  A  S  Y  W  W  C  I  C  V  P  P  P  P  P  P  P  G
1561 GGTGGTTTTGGTGGACCCCCGCCGCCTCCCCCTCCACCTGGTGGTTTTGGCGGTCCACCTCCGCCGCCGCCGCCTCCTACTGGTGGTGCATTTGCGTGCCGCCTCCACCTCCTCCACCAG 1680

    T  V  I  G  G  W  R  A  N  Y  L  A  S  Q  G  A  P  S  H  A  I  P  V  M  S  S  I  R  P  K  K  K  L  K  A  L  H  W  D  R
1681 GCACGGTTATCGGTGGATGGAGCAAATTACTTGGCTTCCCAAGGTGCCCCGTCTCACGCTATACCTGTGATGTCCTCTATCAGACCTAAGAAAAAACTCAAGGCCCTTCATTGGGACA 1800

    V  D  T  P  Q  V  T  V  W  A  T  H  G  T  T  P  Q  E  K  E  E  K  Y  V  E  L  A  K  R  G  V  L  D  E  V  E  R  L  P  M
1801 AGGTCGACACCCCGCAAGTTACAGTATGGGCAACTCACGGCACAACTCCCCAGGAGAAGGAGGAGAAGTACGTTGAGCTGGCCAAGAGGGGAGTCTTGGATGAAGTGGAGCGGCTATTCA 1920

    A  K  E  T  R  I  F  G  G  G  V  A  A  K  Q  R  K  D  K  K  Q  I  I  S  N  D  L  S  K  N  F  Q  I  A  L  S  K  F  S  Q
1921 TGGCCAAGGAGACCAGAATCTTCGGAGGTGGTGTGGCAGCAAAGCAACGAAAGGACAAGAAACAAATCATCTCCAACGACTTATCCAAAAACTTTCAGATTGCCTTGTCCAAATTTTCTC 2040

    F  P  A  E  E  V  V  R  R  I  I  H  C  D  A  E  I  L  D  N  M  V  V  M  E  F  L  Q  R  D  E  M  C  T  V  P  E  N  V  S
2041 AGTTTCCGGCTGAAGAGGTTGTCCGAAGGATCATACATTGCGACGCCGAGATTCTGGACAACATGGTTGTCATGGAATTCTTGCAGCGAGATGAAATGTGCACAGTACCGGAAAACGTAT 2160

    K  L  M  A  P  Y  S  K  D  W  T  G  P  D  A  A  N  T  E  R  E  Q  D  P  S  E  L  T  R  E  D  Q  I  Y  L  Y  T  A  F  E
2161 CAAAACTCATGGCGCCATACAGCAAAGATTGGACTGGCCCTGATGCTGCCAATACCGAACGAGAACAAGACCCTAGTGAGCTTACTCGTGAAGATCAGATTTACCTCTATACTGCCTTTG 2280

    L  N  H  Y  W  K  A  R  M  R  A  L  A  L  T  R  S  F  E  P  D  Y  E  H  I  S  A  K  L  R  E  V  V  R  V  S  E  S  L  R
2281 AGTTGAATCACTACTGGAAGGCAAGAATGCGTGCGCTTGCGCTGACTCGGTCTTTTGAGCCAGACTATGAGCATATTTCTGCTAAGCTCAGGGAGGTTGTGCGGGTGAGCGAATCTCTGA 2400

    D  S  V  S  L  M  N  V  L  G  L  I  L  D  I  G  N  F  M  N  D  A  N  K  Q  A  Q  G  F  K  L  S  S  L  A  R  L  G  M  V
2401 GAGATTCTGTTTCCCTGATGAACGTGCTTGGTTTAATCCTAGACATCGGTAACTTTATGAACGACGCCAATAAGCAAGCGCAGGGTTTCAAGCTAAGCTCTTTGGCTCGTCTTGGAATGG 2520

    K  D  D  K  N  E  T  T  F  A  D  L  V  E  R  I  V  R  N  Q  Y  P  E  W  E  D  F  T  E  Q  I  S  G  V  I  G  L  Q  K  L
2521 TCAAGGACGACAAGAATGAGACAACGTTTGCAGATCTTGTTGAACGTATCGTTCGGAACCAGTATCCTGAATGGGAAGACTTTACCGAACAGATCAGCGGAGTTATTGGCCTCCAGAAAC 2640

    N  V  D  Q  L  R  T  D  A  K  K  Y  I  D  N  I  K  N  V  Q  A  S  L  D  A  G  N  L  S  D  P  K  K  F  H  P  Q  V  R  V
2641 TCAATGTCGACCAGCTGCGGACTGATGCAAAGAAGTATATCGATAACATCAAGAATGTGCAGGCGAGCTTGGATGCGGGCAATCTCAGCGACCCGAAAAAGTTCCATCCACAAGTCCGTG 2760

    S  Q  I  T  Q  R  S  M  K  D  A  R  R  K  A  E  Q  M  Q  L  Y  L  E  E  M  L  K  T  Y  D  D  I  M  V  F  Y  G  E  D  N
2761 TCAGCCAGATTACTCAACGCAGTATGAAAGATGCTAGGCGAAAAGCGGAGCAGATGCAGCTTTACCTTGAAGAGATGCTCAAGACCTATGACGATATTATGGTTTTCTACGGCGAAGACA 2880

    T  D  D  G  A  R  R  D  F  F  A  K  L  A  A  F  L  Q  E  W  K  V  C  Y  *
2881 ATACCGATGATGGTGCTCGGCGGGATTTCTTTGCGAAGTTGGCTGCGTTCTTACAGGAGTGGAAGGTATGCTACTAAGCCAGTTTTTCTTGCTTTTTAATGCTAATCGTGATGATAGAAA 3000

3001 TCAAAAGAGAAGAACATCGCCTTGGAGGAAGCTAGAAGGCGCACTGAAGCATCTTTGGCTCGCAAGCGCATCAATGTCGGCCTTGCGAATGGCGCAGGCGCGGCAGGAGATGCCCCAGTT 3120

3121 TCCCCAGCCACAAGCGGAGCCATGGATTCGCTGCTGGAGAAATTACGTGCTGCTGCCCCTCAAGCCAAGGATCAGCGTGACCGTCGCCGCCGTGCGAGATTAAAGGAGCGGCACCAAGTT 3240

3241 CGAGTTGCCTCAGGGCAGAAGATCCCAGATCTCGAAGGCGCCGAGGCCCCGGGCAGTGGCGGACAGAACAGAGGTGCCACTGACACAAATGCTACCGACTCTAGCCTATTGAGTCCCACT 3360

3361 ATCCAGGAACCTGAAGGTGGCAGCTCACCAATTGCCTCGCAAAGTGAAGATGTTGCCGACCGAGCAGCTAGCATGCTACAAGATATGTTAAGAAACAGTCCTGACCCAGAACGCACGCGG 3480

3481 AGGCGTAGAGAAAGTGCAGAAGAGGAACGAAGGAAGCGCCGGTTAAGGAGACGCAATGGAGCCACAAGTGGAAGCAAGGACAGCAATGATACCACACCTTTGTCCCCTGTGACAGAACCA 3600

3601 ACTTCGACCCAGGGCGAGTCAGCAGAACCGGAAAATTTGTCACTTTCTAGCCCTCCAAATGGAGAAGACCCGACCCTAAACCCTCCGACTATTGTCTTATCATCTGATGCTTCTGACACA 3720

3721 CCTGATGATGAACATCGCCCCTCGACCAGCTAGCTGTTTCTCAAGCAAAGGAAAAAGCGACGCACACACCGACCTATTACCCATTTCCTTTCCCTAATATAATGTTTGAATAGACTACAT 3840

3841 GACGTCGTCTCGGAGACCCATCATTATATTATTATAATCTTCGGCCTGTCTACCTCCAACTCCTTACCCGTCATTCACCGTCTCCTTTTTGTTGTTTGTGCTATCCAGCGTGACGGGAC 3960

3961 CAGGTTTGATGTCATGATCATCTTTTCCCCCTGCCCTCTCTCATTAACATATCTTGCATCTTCCCGTATAATT 4033
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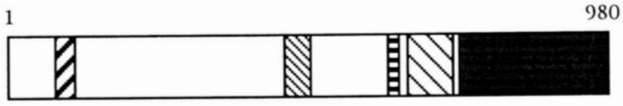
FIGURE 5.—DNA sequence of the region fused to the *alcA(p)* in TTAP22. The DNA sequence of the *figA* gene fused to the *alcA(p)* in the FIG mutant strain TTAP22 and the predicted amino acid sequence for the putative FigA polypeptide are shown. The GenBank accession number for the sequence is L36341.



Position	Codons	% Identity	% Similarity
76 - 109	33	32	47
454 - 498	44	46	48
621 - 641	20	38	76
650 - 728	78	35	58
737 - 977	240	42	64

FIGURE 6.—FigA is related to BNI1. Regions conserved between FigA and the C-terminal half of BNI1 are illustrated (filled boxes). The most highly conserved region extends from codons 737 to 977 of FigA and is 42% identical to BNI1 sequence. The region from 454 to 498 is 60% proline.

growth was inhibited severely in the other 52 mutant strains (designated FIG) when grown under the same conditions, little or no *brlA* mRNA was detected. This result supports the idea that *brlA* activation is not a consequence of nonspecific growth inhibition but instead results from specific activation of development.

This screen for potential developmental regulators differs from other, more traditional genetic studies of *A. nidulans* development in at least two important ways. First, it lacks the usual assumptions about the null phenotype. Genes such as *brlA* and *abaA*, and various fluffy mutant loci were all identified by screening for mutants that did not develop normally and had little or no vegetative growth defects (CLUTTERBUCK 1969; DORN 1970; MARTINELLI and CLUTTERBUCK 1971; YAGER *et al.* 1982; WIESER *et al.* 1994). In many cases, genes identified as being required for conidiophore development have had the opposite effect when overexpressed—they caused inappropriate developmental activation and growth inhibition (ADAMS 1988; MIRABITO *et al.* 1989; LEE and ADAMS 1994; B. LEE, J. WIESER, J. FONDON and T. ADAMS, unpublished data). This has been taken as evidence that the gene in question encodes a developmental regulator because its expression is both necessary and sufficient for normal conidiophore development. Because isolation of the FAB genes was based on their overexpression phenotypes, it will be particularly interesting to observe the loss of function phenotype. This has the potential to identify new mutant phenotypes that would have been ignored in previous screens. For instance, genes regulating aspects of both growth and development would likely be culled in a screen for loss of function mutants that specifically alter development. It is also possible that FAB mutants could identify genes encoding redundant developmental activators. In this case, loss of function mutations would have no observable phenotype. Thus, redundant developmental regulators would never be identified by conventional genetic approaches but might turn up in our overexpression screen because activating mutations in redundant regulatory genes could cause development.

The second difference between the approach described here and more traditional genetic screens is that because generation of a mutant phenotype requires integration of a plasmid containing the *alcA(p)* into the genome, the DNA region associated with the phenotype of interest is "tagged" by plasmid sequences and easily recovered. We have recovered plasmids from three of the four FAB mutants in which *alcA(p)*-induction not only resulted in growth inhibition and accumulation of *brlA* mRNA but also caused sporulation from hyphal tips (Figure 3) under conditions that normally suppress conidial development. We then determined the partial DNA sequence of the region placed under *alcA(p)* control and found that in one of the sporulating FAB mutant strains (TTAP721) the sequence corresponded to the *brlA* gene itself. This result confirms the potential value for this approach in identifying developmental regulators. Because the sequences fused to the *alcA(p)* in the other sporulating FAB mutants are not related to any known Aspergillus developmental regulators, we predict that these regions likely encode previously unidentified early developmental regulators that function before *brlA* in activating development.

Although hyphal abnormalities including swelling and vacuolation accompanied growth inhibition and *brlA* mRNA accumulation in each of the other 10 FAB mutants, no spores were produced (Figure 3; data not shown). This result is reminiscent of the changes observed after *alcA(p)*-induced expression of *abaA* in vegetative hyphae (MIRABITO *et al.* 1989). *abaA* normally functions after *brlA* in development and is activated in response to *brlA* expression (MARTINELLI 1979; ADAMS *et al.* 1988). Induced expression of *abaA* in vegetative hyphae, however, results in *brlA* transcript accumulation to levels similar to those observed after *alcA(p)*-induced *brlA* expression but does not cause sporulation. This has been interpreted to mean that *brlA* and *abaA* encode reciprocal inducers with the *abaA* product functioning as a positive feedback activator of *brlA* expression (MIRABITO *et al.* 1989; TIMBERLAKE 1990). The order of expression is important and *brlA* activation must precede *abaA* activation for development to conclude in spore formation. Thus, nonsporulating FAB mutants could result from regulated overexpression of developmental regulators that normally function after *brlA* in development but, like *abaA*, contribute to positive feedback maintenance of *brlA* expression as development proceeds.

Although our main interest is to identify genes controlling entry into the asexual sporulation pathway, the regulated overexpression of random DNA fragments

has the potential to identify genes involved in other processes. Many different genes with known and unknown functions have been demonstrated to block growth in Aspergillus or in *S. cerevisiae* when expressed at high levels (ROSE and FINK 1987; ADAMS *et al.* 1988; HURT 1988; BURKE *et al.* 1989; MIRABITO *et al.* 1989; OSMANI *et al.* 1988a,b; LIU *et al.* 1992). Along with developmental activators, these include genes encoding cytoskeletal components like actin and tubulin, kinases and phosphatases and proteins involved in movement of other proteins into the nucleus. It is likely that some of the FIG mutants we have identified result from overexpression of analogous genes but others may identify distinct functions. In addition, because our library was constructed using randomly cloned genomic DNA fragments rather than directionally cloned cDNAs, growth inhibition might result from antisense expression causing repression of required genes rather than overexpression of a protein.

We recovered plasmids from two different FIG mutants (TTAP22 and TTAP79) having distinct phenotypes and characterized the genomic DNA fragments inserted next to the *alcA(p)*. TTAP22 produced highly branched, deformed and thickened hyphae when grown under *alcA(p)*-inducing conditions (Figure 3). We found that the DNA fragment next to the *alcA(p)* in TTAP22 hybridized to a large (>7 kb) RNA in wild-type cells but *alcA(p)*-induction resulted in overexpression of a 3.5-kb transcript corresponding to the 3′ half of the wild-type RNA (data not shown). DNA sequence analysis of this region showed the shortened transcript is predicted to encode a 980 amino acid polypeptide with significant similarity to the C-terminal half of the predicted 1953 amino acid *S. cerevisiae BNI1* gene product (Figure 5). *BNI1* was isolated based on the synthetic lethality of *bni1* mutations in a *cdc12* mutant strain (HAARER and PRINGLE 1987) suggesting that it is in some way involved in yeast bud neck formation (H. FARES and J. PRINGLE, personal communication). Both haploid and homozygous diploid *bni1* deletion mutant strains form wider than normal bud necks, and the diploid *bni1* mutants exhibit a random budding pattern. We tentatively have called the gene identified by the *A. nidulans* overexpression mutation *figA* and propose that *alcA(p)*-directed transcription results in overexpression of an N-terminal deleted form of the *A. nidulans BNI1* homolog. In this respect, the increased branching and swollen hyphae observed in TTAP22 could be viewed as analogous morphological changes to those observed for Saccharomyces *bni1* mutants. This raises the possibility that the overexpression phenotype observed in TTAP22 results from expression of a partial protein that interferes with the activity of the wild-type protein in a dominant negative fashion (HERSKOWITZ 1987). We have attempted to test this hypothesis by disrupting the wild-type *figA* gene but to date have been unable to recover viable homokaryotic transformants having the predicted integration event in *figA*. The second FIG mutant analyzed, TTAP79, had a nongerminating phenotype on *alcA(p)*-inducing media. No significant similarities were observed between sequences overexpressed in this strain and any known genes.

The potential uses for the strategy of overexpressing random genomic sequences by integration of a tightly regulated promoter [such as *alcA(p)*] into the genomes of filamentous fungi should not be limited to identification of conditional growth-inhibition mutants. For instance, in *S. cerevisiae* it has been observed that overexpressing unique gene products often can substitute for, or bypass, the function of a mutant gene product. This was the case in analyzing *sst2*$^-$ *S. cerevisiae* mutants, for example, where it was demonstrated that overexpressing genes encoding components of the mating pheromone signaling pathway, including *SCG1*, which encodes the α subunit of a trimeric G-protein required for mating, suppress the *sst2*$^-$ mutant defect (DIETZEL and KURJAN 1987). Such overexpression suppressors in *S. cerevisiae* often can be identified by simply transforming the mutant of interest with a multicopy plasmid library and screening for strains with the desired phenotype. However, the lack of replicating plasmid vectors necessitates an alternate strategy in filamentous fungi. This type of analysis could be facilitated in filamentous fungi through random integration of an inducible promoter into the genome.

We thank THU NGUYEN for technical assistance, Dr. JOHN PRINGLE and HANNA FARES for communicating unpublished results and Dr. MIKE PLAMANN, Dr. DEBBY SIEGELE, Dr. RODOLFO ARAMAYO and our colleagues in the laboratory for critically evaluating the manuscript and for many helpful discussions. This work was supported by National Institute of General Medical Science grant GM-45252 and Texas Advance Research Program grant 010366-79 to T.H.A.

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Communicating editor: R. H. DAVIS