The Neurospora *rca-1* Gene Complements an Aspergillus *flbD* Sporulation Mutant but Has No Identifiable Role in Neurospora Sporulation

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ABSTRACT

The *Aspergillus nidulans flbD* gene encodes a protein with a Myb-like DNA-binding domain that is proposed to act in concert with other developmental regulators to control initiation of conidiophore development. We have identified a *Neurospora crassa* gene called *rca-1* (regulator of conidiation in Aspergillus) based on its sequence similarity to *flbD*. We found that *N. crassa rca-1* can complement the conidiation defect of an *A. nidulans flbD* mutant and that induced expression of *rca-1* caused conidiation in submerged *A. nidulans* cultures just as was previously observed for overexpression of *flbD*. Thus, the *N. crassa* gene appears to be a functional homologue of *A. nidulans flbD* and this is the first demonstration of functional complementation of an *A. nidulans* sporulation defect using a gene from an evolutionarily distant fungus. However, deletion of the *rca-1* gene in *N. crassa* had no major effect on growth rate, macroconidiation, microconidiation, or ascospore formation. The only phenotype displayed by the *rca-1* mutant was straight or counterclockwise hyphal growth rather than the clockwise spiral growth observed for wild type. Thus, if *rca-1* is involved in *N. crassa* development, its role is subtle or redundant.

NEUROSPORA crassa and *Aspergillus nidulans* are filamentous fungi that provide simple genetic systems for the molecular genetic examination of fungal multicellular development. *N. crassa* produces two types of asexual spores; multinucleate macroconidia that are spherical to barrel-shaped and 5 to 10 μm in diameter, and uninucleate microconidia that are pyriform to spherical in shape and 2 to 3 μm in diameter (Springer 1993). During macroconidiation, *N. crassa* produces a conidiophore that is composed of simple aerial hyphae that branch symmetrically. In synchronous cultures, the growth mode of these elongating aerial hyphae switches from the filamentous form to a budding growth form about 8 hr after induction of development, leading to the production of proconidial chains. These chains of cells then separate by cleavage of a double cell wall layer that is deposited between the cells, releasing the macroconidia. In contrast, microconidia are produced from simple branched structures called microconidiophores. Microconidiophores are most often overlooked in culture because the massive canopy of macroconidiophores masks the microconidiophores that form in older cultures. The microconidiophores include specialized cells called phialides that can bud repeatedly to release chains of spores (Rossier *et al.* 1977; Springer 1993).

A. nidulans conidiation proceeds through formation of phialides that bud repeatedly to produce long chains of spores that are spherical in shape and ~2 μm in diameter. However, the process differs from *N. crassa* conidiation in that the spore-producing *A. nidulans* phialides are elaborated on highly organized conidiophores comprised of multiple cell types (Adams 1995). *A. nidulans* conidiophore development begins with formation of the stalk cell which is a specialized aerial hypha. The swollen tip of the stalk cell is called the vesicle and multiple buds are formed on the vesicle surface to produce a layer of cells called metulae which in turn bud to give rise to the phialide cells. Conidiation in a growing *A. nidulans* colony is governed by a well-defined genetic program that controls expression of a set of regulatory genes required for morphogenesis of the conidiophore (Adams 1995). The central regulators of conidiophore morphogenesis are *brlA*, *abaA*, and *wetA* and induction of development depends on activation of *brlA* (Adams 1995). *brlA* activates expression of *abaA*, which in turn stimulates both *brlA* expression to higher levels and activates *wetA* (Mirabito *et al.* 1989). Genes involved in activation of *brlA* were identified in screens for aconidial mutants with reduced *brlA* expression and include *fluG*, *flbA*, *flbB*, *flbC*, *flbD*, and *flbE* (Wieser *et al.* 1994). Mutations in any of the four genes *flbB*, *flbC*, *flbD*, or *flbE* result in colonies with similar phenotypes that are characterized by abundant aerial hyphae formation and some conidiation, but only after a substantial delay (Wieser *et al.* 1994). *flbD* has been characterized and shown to encode a 314 amino acid protein with two repeats of the DNA binding domain found in the Myb class of transcription factors (Wieser and Adams 1995). In plants, Myb-related proteins regulate a variety

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of activities related to morphogenesis, including tissue-specific production of anthrocyanin pigments (Paz-Ares *et al.* 1987) and trichome differentiation (Oppenheimer *et al.* 1991). Induced expression of *flbD* under control of the *alcA* promoter caused *A. nidulans* to conidiate in liquid-grown cultures where conidiation is normally suppressed, leading to the proposal that *flbD* regulates *brlA* expression and conidiophore morphogenesis.

Much less is known about the genes controlling macroconidiation in *N. crassa* (Springer and Yanofsky 1989). Several mutants are known that specifically interfere with macroconidiation. *acon-2* (aconidiate) and *fld* (fluffyoid) mutants are arrested during macroconidiation at the earliest stages of development. *acon-3* and *fl* (fluffy) mutants are blocked in the budding stage of macroconidiation. *csp-1* (conidial separation) and *csp-2* are blocked at the latest stage of development, release of macroconidia. Of these, only the *fl* gene has been cloned (L. A. Bailey and D. J. Ebbole, unpublished results).

Here, we attempt to address the question of whether ascomycetous fungi as distantly related as *A. nidulans* and *N. crassa* share common genetic strategies for controlling asexual sporulation. We used a number of the known regulators of conidiation in *A. nidulans* as heterologous probes for hybridization experiments in *N. crassa*. *flbD* was the only gene that was found to cross-hybridize with *N. crassa* genomic DNA and we cloned and characterized an *N. crassa* gene with sequence similarity to *A. nidulans flbD*. The gene, *rca-1* (regulator of conidiation in Aspergillus), functionally complemented an *A. nidulans flbD* mutant. However, no major role for the gene in regulation of conidiation was observed in *N. crassa*. A subtle but readily scorable phenotype of *rca-1* mutants was a reduction in clockwise axial growth of hyphae in young colonies.

MATERIALS AND METHODS

Fungal strains, growth conditions, and genetic techniques: Restriction fragment length polymorphism (RFLP) mapping strains of *N. crassa* were obtained from the Fungal Genetics Stock Center (FGSC; Kansas City, KS). The *rca-1* deletion mutant WC1 was crossed to the *pe, fl* strain FGSC 5511 to generate *pe, fl* and *pe, fl*; Δ*rca-1* progeny that were examined for microconidiation and spiral growth. All other fungal strains used in this study are listed in Table 1. *N. crassa* strains were maintained by standard procedures (Davis and de Serres 1970) on Vogel's minimal medium or supplemented media as indicated. Standard genetic techniques (Davis and de Serres 1970; Pontecorvo *et al.* 1953) and protoplast transformation procedures (Yelton *et al.* 1984; Vollmer and Yanofsky 1986) were used. All *A. nidulans* strains were grown at 37° in minimal medium (Käfer 1977) or complete medium (Lee and Adams 1994a). Spiral growth of *N. crassa* was examined as previously described (Yamashiro *et al.* 1996).

For induced expression in *A. nidulans*, *alcA(p)* fusions were induced by inoculating conidia (1×10^6 conidia/ml) into minimal medium containing 50 mM glucose and shaking at 300 rpm for 14 hr. Cells were harvested by centrifugation, washed with minimal medium without glucose, and transferred to medium containing 100 mM L-threonine for induction. Samples were harvested for microscopic examination and photography 14 hr after the shift to threonine medium.

Growth rates of the *rca-1* and wild-type *N. crassa* strains were measured in race tubes as described (White and Woodward 1995). Macroconidiation of the *rca-1* mutant grown on several media was compared with that of the wild-type strain. Macroconidial yield was also tested for cultures grown at 34° and 25° and cultures grown in constant light and constant dark. The number of macroconidia were quantitated from cultures

TABLE 1

Strains used in this study

Strain	Description or genotype	Source
N. crassa		
74-OR23-1A	mating type *A*	FGSC
ORS6*a*	mating type *a*	FGSC
FGSC7023	*a*; *fld*, delayed conidiation	FGSC
FGSC5511	*a*; *pe, fl*	FGSC
WC1	Δ*rca-1* transformant	This study
WC11-WC15	Δ*rca-1* progeny of WC1 × ORS6a	This study
DE41-DE45	*pe, fl*; Δ*rca-1* progency of WC1 × FGSC5511	This study
DE46-DE50	*pe,fl*; Δ*rca-1* progeny of WC1 × FGSC5511	This study
WC21	Δ*rca-1*; *fld* progeny of WC1 × *fld*	This study
A. nidulans		
FGSC237	*pabaA1, yA2*; *trpC801, veA1*	FGSC
TJW29.2	*pabaA1, yA2*; *trpC::alcA*(*p*)*::flbD, veA1*	Wieser and Adams (1995)
RBN070	*biA1*; *argB2*; *flbD14, veA1*	Wieser *et al.* (1994)
RJW150	*yA1, pabaA1, flbA 100*; *argB2*; *veA1*	This study
TWC41	*pabaA1, yA2*; *trpC::alcA*(*p*)*::rca-1, veA1*	This study
TWC3.1	*biA1*; *flbD14, veA1*; *rca-1*	This study
TX15:E2	*biA1*; *flbD14, veA1*; *rca-1*	This study

FGSC, Fungal Genetics Stock Center.

grown in 16 × 150 mm slants (6 ml medium), 125 ml flasks (50 ml medium), and 150 mm × 300 mm bottles (50 ml medium). For synchronous macroconidiation studies, mycelial pads were harvested by filtration from overnight cultures grown in minimal medium. Filter papers were placed onto minimal agar medium with the mycelial pads exposed to air. Strains were induced to produce microconidia as described (Pandit and Maheshwari 1993). Colonies of *rca-1* mutant and wild-type strains were examined with a stereomicroscope at ×70 magnification to examine production of microconidiophores. *pe*, *fl* strains were point inoculated in the center of 7-cm petri dishes and microconidia were harvested after 7 days of growth. Microconidial yield was then quantitated by counting with a hemacytometer.

The location of *rca-1* on the *N. crassa* genetic map was determined using the standard RFLP mapping strains (Metzenberg and Groteluesch en 1995). A polymorphism was detected using cosmid X15:E2 as a probe after digestion of chromosomal DNA of the parent strains with *Sal*I. Analysis of the RFLP pattern of *Sal*I-digested progeny DNA was used to determine the genetic location of *rca-1* by comparison to RFLP patterns of known markers.

Nucleic acid procedures: Heterologous hybridization using *A. nidulans* genes was performed at 42°. Following overnight hybridization, blots were washed with 2× SSC (0.3 M sodium chloride, 0.03 M sodium citrate), 0.1% sodium dodecyl sulfate at room temperature for 15 min and then washed once with the same solution at 50° for 30 min.

The locations of degenerate primers based upon conserved amino acid sequences are shown in Figure 1. The primer sequences are 5′-GGNCCNTGG[A/G][T/C]NCCNGA[A/G]GA[T/C]CA-3′ and 5′-[A/G]TTCCA[A/G/C][C/T][A/G][A/G]TT[C/T]TTNA[C/T]NGC[A/G]TT[A/G]TC-3′. The PCR amplification employed the "Touchdown" PCR strategy (Roux 1994). The annealing temperature decreased by 1° from 55° to 37° every 3 cycles followed by 20 cycles at 37°. Amplification was performed in a Perkin-Elmer DNA Thermal Cycler (Perkin-Elmer Corp., Norwalk, CT).

An *N. crassa* cosmid library (Orbach and Sachs 1991) and a cDNA library constructed by Dr. R. H. Garrett were screened using the PCR product. Several positive cosmid clones were identified and one (X15:E2) was chosen for further analysis. A 9.5-kb *Bam*HI fragment containing *rca-1* was subcloned into pBluescript SK$^-$ to produce plasmid pWC1. Cosmid X15:E2 was cotransformed with pSALargB into RBN070, an *A. nidulans argB*$^-$ *flbD*$^-$ strain, to produce strain TX15:E2. The 9.5-kb *Bam*HI fragment was also cloned into pPK1 (Wieser and Adams 1995) to create pWC3 and used for transformation of RBN070 (to generate TWC3.1) and an *A. nidulans argB*$^-$ *flbA*$^-$ mutant, RJW150 (J. Wieser and T. H. Adams, unpublished data). A cDNA clone was identified by plaque hybridization (pWC2).

DNA sequence analysis was carried out using SequiTherm Cycle Sequencing Kits (Epicentre Technologies Corp., Madison, WI). DNA sequences of genomic and cDNA clones were confirmed on both strands. The nucleotide sequence was analyzed using the BESTFIT (Devereux *et al.* 1984) program and the databases were searched using the BLAST search algorithm (Altschul *et al.* 1990). The DNA sequence of *rca-1* has been deposited in GenBank with accession AF006202.

RNA was isolated from synchronously developing cultures as described (Sachs and Yanofsky 1991). RNA blots were hybridized with gel-purified insert of pWC2 or conidiation-specific genes (Sachs and Yanofsky 1991). Probes were radiolabeled by the random priming method (Sambrook *et al.* 1989).

In order to delete *rca-1* from the *N. crassa* genome, pWC1 was digested with *Mlu*I, digested with exonuclease III, and treated with S1 nuclease to create blunt ends. The 1.5-kb *Hpa*I fragment from pCB1004 (Carroll *et al.* 1994) was added and the mixture was ligated and transformed into *E. coli*. Several colonies were screened and pWC5 was selected for further analysis. The 5′- and 3′-deletion end points of the fragment in pWC5 were determined by sequencing. pWC5 was used directly for transformation of *N. crassa* 74-OR23-1*A*. Transformants were screened for resistance to hygromycin following serial passage from conidia and 34 independent stable transformants were recovered. The presence of *rca-1* sequences was screened by amplification of DNA directly from conidia (Xu and Hamer 1995) with *rca-1* internal primers. Transformants that failed to yield an *rca-1* amplification product were further characterized by Southern blot analysis and one isolate was identified that contained a single copy replacement of *rca-1* with the *hygB* gene. The Δ*rca-1* strain was backcrossed to ORS6*a* and hygromycin-resistant progeny were examined by Southern blot analysis to verify the gene replacement event. The *Bam*HI fragment of pWC5 was cloned into pPK1 to generate pWC6. pWC6 was used as a control plasmid for transformation experiments with *A. nidulans flbD* mutants.

To express *rca-1* in *A. nidulans*, an *alcA(p)::rca-1* fusion was prepared by inserting the cDNA insert from pWC2 into pBN55 (Lee and Adams 1996) to create pWC13. pWC13 was transformed into *A. nidulans* FGSC237 to produce strain TWC41.

Microscopy and photography: Photomicrographs of hyphal development were taken with an Olympus BH2 microscope (Olympus Corp., Lake Success, NY) using differential interference contrast optics. The micrographs of whole colonies were obtained using an Olympus SZ-11 stereomicroscope.

RESULTS

Identification and cloning of an *N. crassa flbD* homolog: We examined the possibility that *N. crassa* had homologs of the *A. nidulans* developmental regulatory genes *fluG*, *flbA*, *flbC*, *flbD*, *brlA*, and *abaA* by probing *N. crassa* genomic DNA with gene-specific fragments under low stringency hybridization conditions (see materials and methods). The only reproducible signal detected was with a *flbD* gene-specific probe although the hybridization signal was weak. Because the N-terminal portion of *A. nidulans* FlbD shares significant identity with the DNA-binding domain of the Myb family of transcription factors (Wieser and Adams 1995) we attempted to isolate an *N. crassa flbD*-like gene by PCR amplification using degenerate oligonucleotide primers based on conserved residues between FlbD and the DNA-binding domain in other Myb-like proteins (Figure 1). Direct sequencing of the PCR product indicated that the amplified sequence was unique and had sequence similarity to Myb-like transcription factors (Figure 2). This PCR product was also used as a hybridization probe to identify cosmid X15:E2 from a cosmid library of *N. crassa* genomic DNA. The cosmid clone was used as a hybridization probe with a standard set of RFLP mapping strains (Metzenberg and Groteluesch en 1995) to map the gene to the right arm of *N. crassa* chromosome V in the 20 map unit region between *leu-5* and *al-3* (Perkins *et al.* 1982). This map location is not near any known genes that specifically influence conidiation.

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CGATAAACGATTTGTAAGGGGCTGAGAAGTGGACTGCCTGGTATGCGGAATCCCACCTTGTACTATGTAC
ACCAATCGACCCCTGGCTGAGACGCGACCAGAAGTGGAAATCCGTTCTTCCAAACGGGGGGAGCTCCAGT
CATGGACTAGCACTTGGGTACCTGCAAGTGAAATACGTGGGCTAGCCGATATCCAGCAGGCTAGTACACTT
CAGTCCTGGTAAGTTCGTTAGCTTGGTACAAACTGGGGTTTTGAACAGGGGAGGAAGCAGGGGATAAAAC
CAATGATTTCGATCTGAAGCCAGTATAAAGCGGGTTTGTGTGTGTGTGCTGGAACACCTTGAGATCAGAC
AATTTCAGCCAATAAGAAGTCGTCAATAGACCTGTTCCGCCTTGCAATTCCCCCCTTCAGAGAAAATATG
GGGCAGGAACCCACGCTAACAAACTTACAGGGACTGATATGTACATACATGACATTTCGGGCCTGCGCCT
TACTTTCTTCTTCCTCGCATCCCGTCATCTACTAGCGTCCGTCGGTGGCATTAGACCGTCTGAGAACATC
ATCCCACACACCTTCAGATCGGCCAGTCATCTTATAAACCTCGTTCCCTTCTAACTACTTTATCTTCCCC
CCCATCCCCAACCTGCATTCCCGTCCACCTTCAACCACCCATCTCTACCATCAAACATCTGTTACAATCA
TCGACATCCACCACGACTCGACATCAACAAAGCTCCATTGAAGGATAACATCATTAGTCTTCAAGGCAAC

CAAGACCAAAACCGTCGTTCACCTTATCGACATTTTCGATCATCATGTCTAACATGCCCGACCAACGGAG
                                                M  S  N  M  P  D  Q  R  R
AGGTCCTTGGTCAGCAGGGGAGGATCAGCGACTCATCAAGTTGGTCAAGGACCTAGGACCGGGAAACTGG
 G  P  W  S  A  G  E  D  Q  R  L  I  K  L  V  K  D  L  G  P  G  N  W
GTCAACGTTGCCCGCATACTCGGAACCAGAACCCCCAAGCAGTGCCGAGAGCGATGGCACCAAAACCTGA
V  N  V  A  R  I  L  G  T  R  T  P  K  Q  C  R  E  R  W  H  Q  N  L  K
AGCCAGGGCTGAACCACGGACCTATGACACAGGAAGAGGCCGCTATCATCGTTAGAGAGGTAGATCTGAA
  P  G  L  N  H  G  P  M  T  Q  E  E  A  A  I  I  V  R  E  V  D  L  K
GGGGCCTCGCTGGGCAGACATTGCCAGGAAGCTCCAGGGCCGCAGCGACAACGCAGTCAAGAACTATTGG
 G  P  R  W  A  D  I  A  R  K  L  Q  G  R  S  D  N  A  V  K  N  Y  W
AACGGCCTCAACAACCGCAAGAAGAACCAGCTTCGGAGACAGAGTGCACCAAGACGCGTTTCTGCCTCCG
N  G  L  N  N  R  K  K  N  Q  L  R  R  Q  S  A  P  R  R  V  S  A  S  D
ATGTTCTAAGAAGCAGCCCCGGTCAACTGCCGAGAGCGCACATGCAGCGCCCATCAGATATCCTCAGGAC
  V  L  R  S  S  P  G  Q  L  P  R  A  H  M  Q  R  P  S  D  I  L  R  T
ATCTACACGGGATCTAGGAGGCCATCCAGTCCCAGTAGCTTCAACGACAGCCTTCATCACCGCGTCCATG
 S  T  R  D  L  G  G  H  P  V  P  V  A  S  T  T  A  F  I  T  A  S  M
AGTCCATCGAGTGGTTCCCTTCTAGGCCACAGCAGCAGCAACAGCAACAGGCTCGATGCAC
S  P  S  S  G  S  L  L  G  H  S  S  S  S  N  S  N  S  N  R  L  D  A  L
TACACCCTTCACATCGTTTTACCCTCCATCACATGCCTTCCCCACTACCGATGGATATCAAGATGGAAGC
  H  P  S  H  R  F  T  L  H  H  M  P  S  P  L  P  M  D  I  K  M  E  A
GATGGCCCAACTGGAAGCACTCTGCGGCTCTTGACACCACCCACATCAAGGCGACTTGCCCCATTCAGCA
 M  A  Q  L  E  A  L  C  G  S  *

CCTTGCCCTCTCCTACTACCTCATCCATCGGAGATTTGGATGAGTTTGATCGCCGACAGCTAGCAGCATT
CGCACCACCTGCGGCCTACCGTCGTCCCAGTCTTCCTTTCCCTCAGCAATCATCAATGGGATACTTGCCT
TCTGCCACCGAGTACCTTCCCACGGCACCTAGCTCGCCTATTGCCTTGATTCAACGAGACGAGAAAAACC
ATGCTAGGATTCCTGTCACAAGCCTCCTGTGCTCTTGAACAGGTGGAACAAAATACCAACTATCTCCCAC
CTACGAAGATCATAATAACCCGAAAGCCGAAGTAGCCGGGGGCGTGTCGAACTGGTCATAATCACATATG
GTAAGTTTTCCTGATGACGATGCCCTCCTCAAACACACATGACGCTTACATAGTACATCAGATCAGAACA
GCGTACAGCATCAATCCTGAGCTTGGTGAAATCACCACACTCATCTTGTCCTATCAAAATAAAGGGATTG
CAACTATCCTTATAGCGTAATTAAAAGCAGACAGAAGGAGCTGAAAATTGGGTTGGGCATGGCGGATGGA
TAGAAATACGTTGCTCACGCCCCGCACTACCCACAATCAAGGAGTCAGTCGGTGGTTTTCTGTTCTTCTT
TCCTCTTTTCTTGTATCAAGGGTCGGATGGGCAGACGGAAGAGGTCTTTTTTTTTTACTGTTGAGATACC
CCCCACATCAGCAGATGCTTTGCGGCACGCTGTTTTTCAAAAAATCAAAGAAATATTAATCGGTCAAATC
AAATTACAGTGGCAATCTTCCCTTGCTTGTTCTACTTTGTGCCTACCTGAGTTCAACAGTAGAAAGTGAA
CATAAATGCGTGTCGATGTAGCCGACGAGCTGATAAAGCTCACGACATCGCATAAGGTACCAAGAGTCTT
CTTAACTTGGGGTCGTTCAGGGCCAATGACTACAATCGTTACCAGCATAC
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Figure 1.—The DNA sequence of the *rca-1* genomic region and the predicted RCA-1 amino acid sequence. The Myb-like DNA binding domain is underlined. The ends of the cDNA clone (pWC2) are indicated by large dots above the nucleotides. The 5′ and 3′ deletion junctions produced by exonuclease III digestion from the *Mlu*I site (bold type) are indicated by boxed nucleotides. The hygromycin phosphotransferase gene replaces the deleted region in the gene replacement vector pWC5. The locations of the degenerate oligonucleotides used for amplification are shown by dashed underlines.

Further analysis of cosmid X15:E2 showed that the putative *flbD*-like gene was located on a 9.5-kb *Bam*HI fragment that was subcloned and used as a template for additional sequencing. Sequencing primers were synthesized based on the sequence of the PCR fragment and additional primers were used to complete the sequence of the 2500-bp region containing *rca-1*. As shown in Figures 1 and 2, this sequence predicts a 229-codon open reading frame that includes a region with high similarity to FlbD and other proteins with Myb-like DNA binding domains. A cDNA clone (pWC2) was also sequenced that initiated 121 nt upstream of the ATG initiation codon and extended 318 nt downstream of the predicted termination codon. The identity of the cDNA and genomic sequence indicates that the gene lacks introns (Figure 1).

Alignment of the putative DNA binding domain sequence of *A. nidulans* FlbD and *N. crassa* RCA-1 revealed 75% similarity and 57% identity when analyzed by BESTFIT alignment (Devereux *et al.* 1984). In contrast, only 42% similarity and 21% identity was observed in the region outside the predicted DNA binding domain (Figure 2). The fungal proteins align most closely with plant Myb-like proteins such as maize C1 and Arabidopsis GL1 (Figure 2; Paz-Ares *et al.* 1987; Oppenheimer *et al.* 1991). RCA-1 has 37% identity to the DNA binding domain region of both of the plant proteins. There is little sequence similarity of the fungal and plant proteins

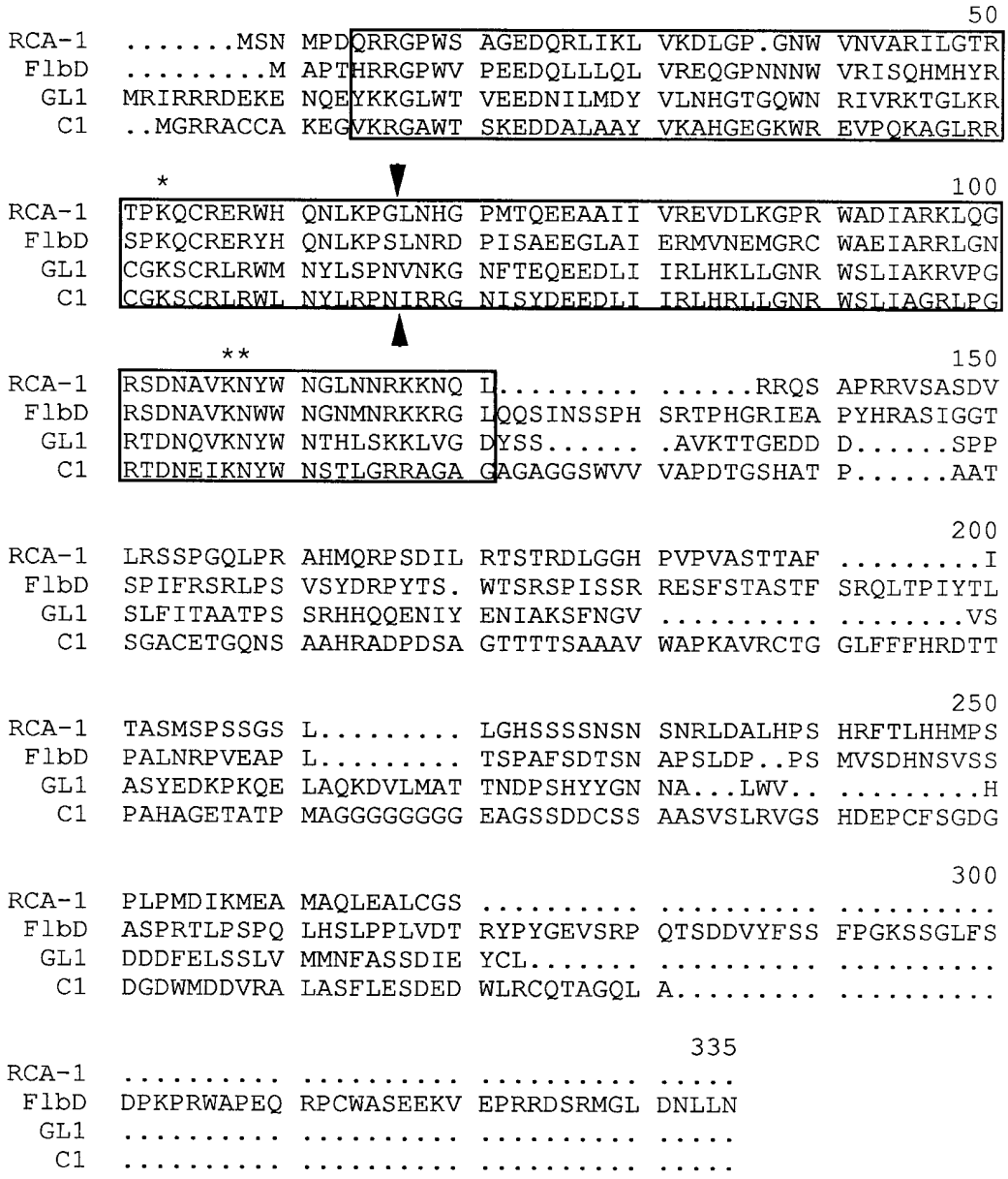

Figure 2.—Comparison of deduced amino acid sequences of *N. crassa* RCA-1, *A. nidulans* FlbD, Arabidopsis GL1, and Maize C1 proteins. The Myb-like DNA binding domains of these proteins are boxed. The conserved residues in c-Myb that make specific contacts with the nucleotides of the AACNG sequence are marked by asterisks. Arrowheads mark the boundary between the imperfect repeats of the Mby-like DNA binding domains.

outside of the DNA binding domain. All of these proteins can be characterized as having two closely related imperfect repeats of Myb-like DNA binding domains followed by 100 to 200 amino acid segments that are hydrophilic and serine/threonine-rich (Figure 2).

***N. crassa rca-1* can complement an *A. nidulans flbD* mutant:** The similarity in the DNA binding domains suggested that RCA-1 and FlbD proteins may be transcription factors that recognize similar DNA sequences. To test this idea, an *A. nidulans flbD* mutant strain was transformed with the *N. crassa rca-1* gene. The 9.5-kb *Bam*HI fragment with the *N. crassa rca-1* gene was subcloned into the *A. nidulans argB*-containing vector pPK1 and both this plasmid (pWC3) and cosmid X15:E2 were used to transform an *A. nidulans flbD* mutant strain. In addition, an internal deletion within the 9.5-kb *Bam*HI fragment was created by exonuclease III digestion to eliminate the *rca-1* coding region (Figure 1) and this plasmid was used in transformation experiments as a control. For each of the constructs containing the intact *rca-1* gene, most of the transformants conidiated like wild type (Figure 3). In contrast, all transformants using the *rca-1* disruption plasmid had the delayed conidiation phenotype normally observed for the *A. nidulans flbD* mutant strain (Wieser and Adams 1995). Southern blot analysis was used to verify that only those *A. nidulans* transformants that displayed a wild-type conidiation phenotype had acquired the *N. crassa rca-1* DNA (data not shown). Finally, to test whether complementation of the *A. nidulans flbD* mutation with *N. crassa rca-1* was specific, we used the same *rca-1* containing plasmids in transformation experiments with an *A. nidulans flbA*

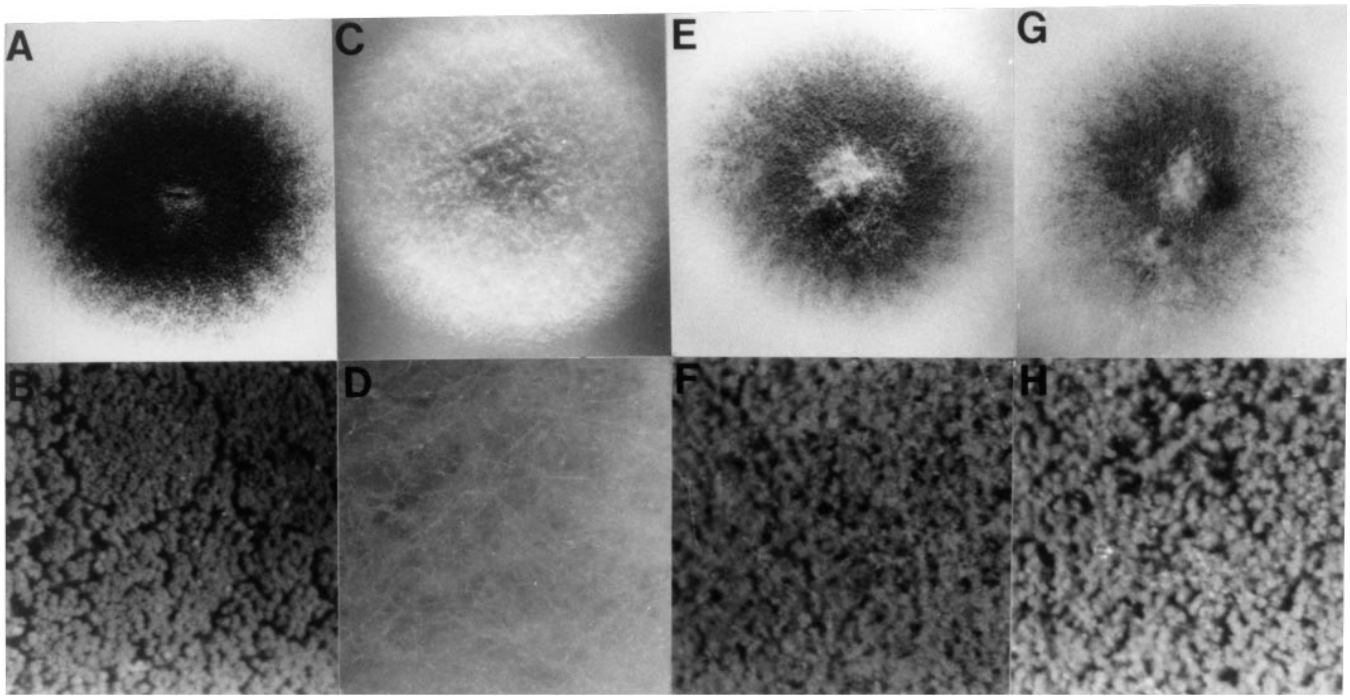

Figure 3.—*N. cassa rca-1* complements the *A. nidulans flbD* mutant. *A. nidulans* wild-type strain (A and B), *flbD* mutant (C and D), and *flbD* mutant cotransformed with cosmid X15:E2 (E and F) or transformed with the 9.5 kb *Bam*HI fragment *rca-1* subclone (G and H) were point inoculated on complete medium and grown for 3 days. B, D, F, and H show a ×30 magnification of the colonies.

mutant strain (Lee and Adams 1994b). All of the transformants had a fluffy autolytic phenotype identical to the *flbA* mutant parent (data not shown).

Wild-type *A. nidulans* does not normally conidiate in submerged liquid culture (Figure 4A) (Timberlake 1990). A striking property of the *A. nidulans flbD* gene is that its overexpression from the highly inducible *alcA* promoter during growth in submerged culture can cause inappropriate conidiation (Figure 4B). The cDNA corresponding to *rca-1* was placed under the control of the *A. nidulans alcA* promoter and the fusion construct was used to transform *A. nidulans.* As observed with the *A. nidulans flbD* gene (Wieser and Adams 1995), overexpression of *rca-1* by induction of the *alcA* promoter activated *A. nidulans* sporulation in liquid culture (Figure 4C).

***N. crassa rca-1* is present throughout the life cycle:** Although the *A. nidulans flbD* gene functions specifically during sporulation and has no apparent role during vegetative growth, it was previously shown that *flbD* mRNA was present at relatively constant levels in vegetatively growing and developing *A. nidulans* cultures (Wieser and Adams 1995). Similarly, we found that *rca-1* mRNA was present in both mycelial and developing cultures of *N. crassa* (Figure 5). However, over a time course of synchronized conidial development, we observed a slight elevation of *rca-1* mRNA accumulation relative to expression of actin mRNA (Figure 5). Finally, the Δ*rca-1* strain (see below) lacked *rca-1* mRNA as measured by northern blot analysis (Figure 5).

***rca-1* disruption has no detectable effect on the sporulation in *N. crassa*:** *N. crassa* wild-type 74-OR23-1*A* was

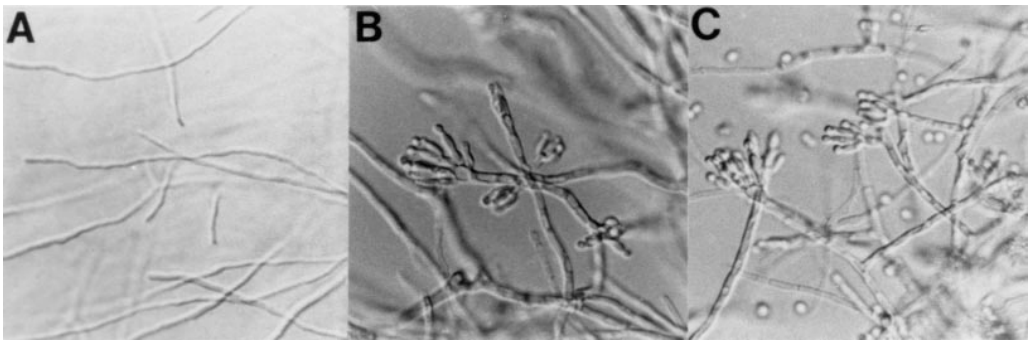

Figure 4.—Expression of *rca-1* in *A. nidulans* using the *alcA* promoter. *A. nidulans* strains TTA11 (panel A; wild type), TJW29.1 [panel B; *alcA(p)::flbD*], and TWC41 [panel C; *alcA(p)::rca-1*] were grown in liquid medium containing glucose for 14 hr and then shifted to liquid minimal medium with threonine and allowed to grow. Photographs were taken 14 hr after the shift.

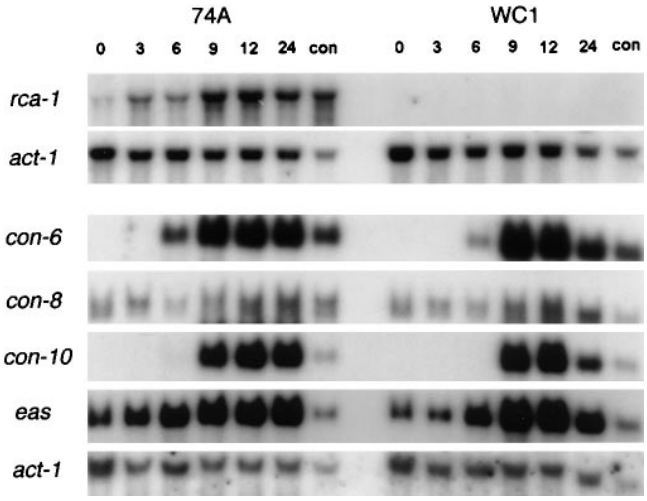

Figure 5.—Northern analysis of *N. crassa* wild-type (WT) strain 74-OR23-1*A* and the Δ*rca-1* strain WC1. Total RNA was extracted from the wild-type and Δ*rca-1* strains at different developmental stages after induction of macroconidiation. Time after induction (hr) is indicated for each lane. RNA from 7-day-old macroconidia (con) was also isolated. The top two panels show a single blot of RNA from WT and Δ*rca-1* strains sequentially probed with *rca-1* and actin (*act-1*). The lower five panels represent a second blot of the same RNA samples probed sequentially with *con-6*, *con-8*, *con-10*, *eas*, and *act-1* clones.

transformed with the plasmid containing the *rca-1* deletion construct, pWC6, and hygromycin-resistant transformants were selected. Primers that could amplify the *rca-1* gene were used to screen transformants for the absence of the endogenous *rca-1* gene from 34 transformants. The resulting candidate strains were further screened by Southern blot analysis and one isolate was identified for which the *rca-1* gene region had been replaced by the hygromycin phosphotransferase gene. This isolate was backcrossed to the wild-type strain, ORS6*a*, and hygromycin-resistant progeny were examined by Southern blot analysis to confirm the gene replacement event (data not shown). The degenerate primers initially used to amplify the *rca-1* gene were again used to test chromosomal DNA from the deletion strain to verify the absence of the *rca-1* DNA binding domain in the genome (data not shown).

A number of conditions were examined in an attempt to discern a phenotype for the Δ*rca-1* mutant. Mycelial growth rate was measured in race tubes with Vogel's minimal agar medium, minimal medium with 2% peptone, or synthetic crossing medium and was always indistinguishable from the parental wild-type strain (not shown). Quantitation of macroconidial yield for Δ*rca-1* (WC1) or wild-type (74-OR23-1*A*) strains grown in flasks for 7 days with: constant light, constant dark, Vogel's minimal medium agar with no added carbon source, minimal agar medium with 1.5% sucrose, minimal medium with 2% peptone, or synthetic crossing medium, did not reveal any striking differences between strains (not shown). The WC1 strain appeared to produce slightly fewer macroconidia on average than the parent strain on synthetic crossing medium. The yields for 74-OR23-1*A* and WC1 were $7.1 \pm 0.3 \times 10^7$ and $5.3 \pm 0.6 \times 10^7$ macroconidia from growth in 125 ml flasks (>95% confidence level). However, the 34% reduction in macroconidia of WC1 relative to 74-OR23-1*A* observed in this experiment did not provide a visual phenotype.

Strain WC1 was backcrossed to wild type and we observed 1:1 segregation of hygromycin resistance. We noted that the behavior of the Δ*rca-1* mutant was similar to that of wild type as either the male or female parent in meiotic crosses. Hygromycin-resistant progeny WC11, WC13, and WC15 were tested for macroconidiation at different times after inoculation of flasks (Table 2). Macroconidiation occurred by 47 hr after inoculation of flasks with 74-OR23-1*A* (wild type) and each of three different Δ*rca-1* progeny examined. By 66 hr after inoculation of flasks, the Δ*rca-1* strains had produced from 36 to 61% of the macroconidia made by wild type (Table 2) and this difference was statistically significant (95% confidence level). However, by 96 hr, macroconidial yields of mutant and wild-type strains were indistinguishable (Table 2). To examine more carefully the possibility that the Δ*rca-1* mutation affected the timing of conidiation, synchronous sporulation was induced (Springer and Yanofsky 1989) and the timing of appearance of the first macroconidiophores was observed to be the same for all strains (not shown). Thus, we conclude that the Δ*rca-1* mutation has no distinguishable effect on the timing of conidiation but may have minor effects on conidial yield. This conclusion was tested further by growing cultures in different containers including 16 × 150 mm tubes or 150 × 300 mm bottles (not shown). In all cases the intrinsic variation of the experiments limited our ability to distinguish whether small variation between strains had any biological significance.

Environmental conditions including starvation in submerged culture have been shown to induce sporulation in wild-type strains (Plesofsky-Vig *et al.* 1983; Springer 1993). We therefore examined macroconidiation by the Δ*rca-1* strain in liquid culture but again saw no apparent change in timing when compared to a wild-type strain grown under the same conditions. We also examined the effect of the Δ*rca-1* mutation on macroconidiation of strains grown in flasks at either 25° or 34° to see if the mutant phenotype could be enhanced by temperature sensitivity, but no significant effect was seen (not shown).

Because we were unable to detect any major differences in macroconidiation between wild type and the Δ*rca-1* mutant, we examined the possibility that a minor developmental phenotype for Δ*rca-1* would be amplified in a strain that already had a partial defect in macroconidiation. We therefore crossed the Δ*rca-1* mutant with a *fluffyoid* (*fld*) mutant strain and examined the prog-

TABLE 2

Timing and extent of macroconidiation by wild-type and Δ*rca-1* strains

Time	74-OR23-14	ORS6*a*	WC11	WC13	WC15
47	0.12 ± 0.06[a]	0.22 ± 0.05	0.051 ± 0.009	0.10 ± 0.03	0.11 ± 0.04
66	3.8 ± 1.0	3.1 ± 0.6	1.7 ± 0.02	1.4 ± 0.1	1.9 ± 0.9
96	9.5 ± 3.7	8.7 ± 1.0	7.2 ± 2.0	7.1 ± 1.4	8.3 ± 0.6

Total yeild of conidia ($\times 10^{-7}$) from flasks of Vogel's minimal medium containing 1.5% sucrose were inoculated and grown at 25° for the time (hr) indicated prior to harvesting.

[a] Standard deviation of triplicate samples.

eny. *fld* mutants are initially aconidial, but following prolonged incubation they do produce reduced numbers of macroconidia (not shown). Approximately 50% of the progeny were initially aconidial as expected for *fld* mutants and half of these were hygromycin resistant, indicating that they carried the Δ*rca-1* construct. We were unable to distinguish any phenotypic differences in the extent or timing of macroconidiation in *fld* and Δ*rca-1; fld* strains.

We also tested the possibility that the *rca-1* deletion could affect the expression of genes known to be activated during macroconidiation. As shown in Figure 5, we examined the timing and level of expression of *eas*, *con-6*, *con-8*, and *con-10* in wild type and in the Δ*rca-1* mutant. In every case, transcript levels and the timing of their expression were unaltered in the Δ*rca-1* mutant strain.

We next examined the effect of deleting *rca-1* on formation of the second *N. crassa* asexual spore type, the microconidium, and found that the Δ*rca-1* strain was capable of producing microconidia. However, because of the difficulty in obtaining synchronous production of large quantities of microconidia we could not distinguish whether or not the *rca-1* deletion had any subtle effects on the timing or extent of microconidiation. We therefore crossed Δ*rca-1* into the *pe*, *fl* (*peach*, *fluffy*) genetic background. *pe*, *fl* strains do not produce macroconidia and the *pe* mutant allele enhances microconidiation. Of 50 progeny examined, we observed 26 *fl* strains and 19 of these produced abundant microconidia, consistent with the known linkage of *fl* and *pe*. We found 9/19 *pe*, *fl* progeny to be hygromycin resistant and therefore carry Δ*rca-1*. Five *pe*, *fl* strains (DE41–DE45) and 5 *pe*, *fl*; Δ*rca-1* strains (DE46–DE50) were examined for microconidiation and microconidia production. No visual difference in the timing of microconidiophore production was observed. After 7 days of growth on synthetic cross agar in 7-cm-diameter petri plates, microconidia were harvested for quantitation. Yields ranged from 6.4×10^8 microconidia/plate to 3.2×10^9 microconidia/plate with no clear correlation of yield to genotype. The combined *rca-1*$^+$ isolates averaged 1.0×10^9 microconidia/plate and Δ*rca-1* strains averaged 1.7×10^9 microconidia/plate.

Δ*rca-1* mutants are altered in spiral growth: During our characterization of the Δ*rca-1* mutants we noticed that they did not have the pronounced clockwise spiral growth pattern of typical *N. crassa* colonies during the first 24–36 hr of growth (Figure 6). In fact, the mutants appeared in many cases to display counterclockwise spiral growth (Figure 6). The spiral growth phenotype segregated completely with the hygromycin resistance marker at the *rca-1* locus. This phenotype was easily scored among the *pe*, *fl* progeny described above and several *pe*$^+$, *fl*$^+$; Δ*rca-1* strains tested. Although an *A. nidulans* strain (A17) was previously reported to have clockwise spiral growth (Trinci *et al.* 1979), we were unable to detect significant and consistent spiral growth of the *A. nidulans* FGSC 26 wild-type strain. No difference in the growth of hyphae following germination of conidia was observed during comparison of the wild-type and *flbD* mutant strains.

DISCUSSION

Extensive genetic analysis combined with gene characterization has identified several genes in *A. nidulans* that control conidiation (Adams 1995). We sought homologs of these genes in *N. crassa* to examine the possibility that these distantly related ascomycetous fungi use common regulatory mechanisms to control asexual sporulation. The only *A. nidulans* gene for which we successfully identified a related *N. crassa* gene was *flbD*. The role of *flbD* in *A. nidulans* conidiation has been established by the observations that *flbD* mutants are delayed in conidiation and that forced overexpression of *flbD* induces development at inappropriate times (Wieser *et al.* 1994; Wieser and Adams 1995). *flbD* mutations have no effect on the mycelial growth of *A. nidulans*, suggesting that *flbD* functions in a conidiation-specific manner. Remarkably, the *N. crassa rca-1* gene appears to be functionally equivalent to *flbD* in *A. nidulans* in that *rca-1* restored conidiation to the *flbD* mutant strain and forced expression of *rca-1*-induced *A. nidulans* development in submerged culture.

In *N. crassa*, there was no discernible effect of the *rca-1* deletion on growth rate, fertility, and microconidiation. Under conditions of synchronous induction of conidiation we did not observe a delay in conidiation-specific gene expression or morphogenesis. There was an over-

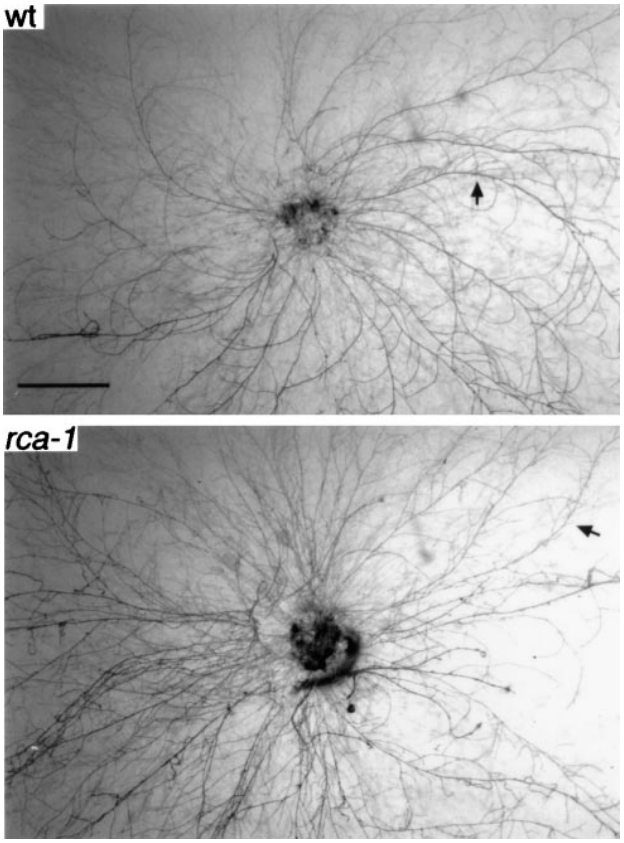

Figure 6.—Spiral growth in wild-type and *rca-1* mutants. Strains were grown on synthetic cross medium containing 1% glucose and inoculated with small blocks of agar. Growth was for 16 hr. Arrows indicate a primary hypha growing along the surface that displays clockwise (wt) or counterclockwise (*rca-1*) spiral growth. Bar, 1 mm.

all tendency with cultures grown in flasks for Δ*rca-1* strains to produce somewhat fewer conidia than wild-type strains. However, because conidial yields were somewhat variable it was difficult to detect a statistically significant difference between conidial yields. A much more extensive analysis of conidia production by several Δ*rca-1* and wild-type strains will be needed to determine whether Δ*rca-1* mutants have a slight reduction in conidial yield. At this time we cannot exclude the possibility that *rca-1* has a subtle role in *N. crassa* macroconidiation but it is clearly not as important to this phase of the life cycle as *flbD* is to conidiation by *A. nidulans*. The lack of any major phenotypic consequence of deleting *rca-1* might be explained if *N. crassa* possesses a *rca-1* homolog that can compensate for the loss of *rca-1*. However, we have been unable to detect a second copy of the gene by probing with *rca-1* in Southern blots or by PCR amplification of genomic DNA isolated from the Δ*rca-1* mutant using the degenerate oligonucleotide primers used to initially amplify *rca-1* from wild type.

Epistasis analysis of *A. nidulans flbD* with other developmental mutants suggests that *flbD* participates in one of two independent pathways that are both needed to efficiently activate *brlA* expression and development. *flbE*, *flbD*, and *flbB* form one pathway, and *flbC* represents an independent pathway for *brlA* activation (Wieser *et al.* 1994). It is possible that a similar set of pathways exist in *N. crassa* but that they have greater redundancy so that loss of the *flbD* homolog, *rca-1*, is largely compensated for by the activity of a *flbC* homolog. Alternatively, *rca-1* has no specific role in controlling development in *N. crassa*, but during the evolution of the regulatory circuit governing conidiation, *A. nidulans* recruited this Myb-like transcription factor to regulate conidiation-specific events.

It is important to recognize that *flbD* and *rca-1* mRNAs are each present in both mycelial and developing cultures (Wieser and Adams 1995), although (ironically) *rca-1* accumulates to higher levels during the course of macroconidiation. Given that *flbD* is required specifically during sporulation, this result has led to the proposal (Wieser and Adams 1995) that as with *myb*-related genes in other organisms, an important aspect of *flbD* regulation is probably post-transcriptional (Luscher and Eisenman 1990; Myrset *et al.* 1993). In this sense, it is particularly intriguing that the similarity between RCA-1 and FlbD is limited primarily to the putative DNA-binding domain at the N termini of both proteins. The amino acid residues implicated in specific interactions of c-Myb with DNA are conserved in most Myb-like proteins (Ogata *et al.* 1992; Myrset *et al.* 1993; Ogata *et al.* 1994), including *rca-1* and *flbD*, making it likely that FlbD and RCA-1 bind to similar sequences that resemble the AACNG c-Myb binding site. If the site for post-translational control of FlbD/RCA-1 is within the DNA-binding domain, one possible mechanism for regulating their activity in controlling sporulation is through changes in the environment. Aspergillus does not normally conidiate unless hyphae are exposed to an air interface (Adams 1995). It has been shown that one mechanism for controlling c-Myb binding involves a redox-regulated conformational change within the DNA-binding domain (Myrset *et al.* 1993; Ogata *et al.* 1994). This redox-sensing mechanism could provide a means of changing the activity of both FlbD and RCA-1 as hyphae are exposed to air. However, it is important to recognize that the DNA-binding activity of c-Myb is inactivated by oxidative environments (Myrset *et al.* 1993; Ogata *et al.* 1994), and the condition under which FlbD activity is needed for development is presumably oxidative. Alternatively, regulation of FlbD/RCA-1 activity could be through as-yet-uncharacterized residues in the C terminus, or posttranslational modification of FlbD may not be necessary for function. In any case, it is likely that we can learn more about the role of FlbD and its regulation by investigating the ability of RCA-1 to activate *A. nidulans* sporulation. FlbD and RCA-1 are most similar to the Myb-like proteins in plants that are commonly found to be involved in tissue-specific gene expression and morphogenesis. For example, maize C1

is required for production of anthocyanin pigment in certain tissues (Paz-Ares *et al.* 1987) and Arabidopsis GL1 is involved in leaf trichome differentiation (Oppenheimer *et al.* 1991). It appears that similar roles in tissue-specific gene expression and development occur in fungi given the phenotypes of the *flbD* and *rca-1* mutants.

The only readily detectable phenotype in *N. crassa rca-1* mutants is an alteration in spiral growth. Spiral growth has been observed in the colonies of many fungi (Madelin *et al.* 1978). In both *N. crassa* and *A. nidulans* clockwise spiral growth has been noted for young colonies (Trinci *et al.* 1979). It has been postulated that the hyphal tip rotates about its axis in a clockwise direction with respect to the subapical regions of the hypha (Madelin *et al.* 1978). Because the subapical regions are fixed to the substrate surface, the rotation of the tip causes the hyphae to curve as they grow across the substrate. This phenomenon could be subject to many environmental factors including nutrients, pH, and signals from other nearby hyphae. *rca-1* mutants may be perturbed in some subtle process that influences events at the hyphal tip. Interestingly, another regulatory gene of *N. crassa*, *rco-1*, has reversed spiral growth (Yamashiro *et al.* 1996). RCO-1 has sequence similarity to Tup1p, a protein involved in repression of many genes in *Saccharomyces cerevisiae* (Yamashiro *et al.* 1996). Tup1p is involved in cell type-specific expression in *S. cerevisiae* and this also appears to be a role for RCO-1 (Yamashiro *et al.* 1996). One role for *rco-1* may be to influence a pathway involving *rca-1* that affects hyphal tip growth. Further examination of *rca-1*, *rco-1* double mutants will be needed to explore this possibility. We could not detect any difference between *A. nidulans flbD*$^-$ and wild-type strains that would suggest that FlbD influences orientation of hyphal tip growth in *A. nidulans.*

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LITERATURE CITED

Adams, T. H., 1995 Asexual sporulation in higher fungi, pp. 367–382 in *The Growing Fungus*, edited by N. A. R. Gow and G. M. Gadd. Chapman & Hall, London.

Altschul, S. F., W. Gish, W. Miller, E. W. Myers and D. J. Lipman, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

Carroll, A. M., J. A. Sweigard and B. Valent, 1994 Improved vectors for selecting resistance to hygromycin. Fungal Genetics Newsl. **41:** 22.

Davis, R. H., and F. J. de Serres, 1970 Genetic and microbiological research techniques for *Neurospora crassa*. Methods Enzymol. **17A:** 79–143.

Devereux, J., P. L. Haeberli and O. Smithies, 1984 A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12:** 387–395.

Käfer, E., 1977 Meiotic and mitotic recombination in *Aspergillus* and its chromosomal aberrations. Adv. Genet. **19:** 33–131.

Lee, B. N., and T. H. Adams, 1994a The *Aspergillus nidulans fluG* gene is required for production of an extracellular developmental signal and is related to prokaryotic glutamine synthetase I. Genes Dev. **8:** 641–651.

Lee, B. N., and T. H. Adams, 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.

Lee, B. N., and T. H. Adams, 1996 *fluG* and *flbA* function interdependently to initiate conidiophore development in *Aspergillus nidulans* through *brlA*β activation. EMBO J. **15:** 299–309.

Luscher, B., and R. N. Eisenman, 1990 New light on Myc and Myb. Part II. Myb. Genes Dev. **4:** 2235–2241.

Madelin, M. F., D. K. Toomer and J. Ryan, 1978 Spiral growth of fungus colonies. J. Gen. Microbiol. **106:** 73–80.

Metzenberg, R. L., and J. Grotelueschen, 1995 Restriction polymorphism maps of *Neurospora crassa*: update. Fungal Genetics Newsl. **42:** 82–90.

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.

Myrset, A. H., A. Bostad, N. Jamin, P.-N. Lirsac, F. Toma *et al.*, 1993 DNA and redox state induced conformational changes in the DNA-binding domain of the Myb oncoprotein. EMBO J. **12:** 4625–4633.

Ogata, K., H. Hojo, S. Aimoto, T. Nakai, H. Nakamura *et al.*, 1992 Solution structure of a DNA-binding unit of Myb: a helix-turn-helix-related motif with conserved tryptophans forming a hydrophobic core. Proc. Natl. Acad. Sci. USA **89:** 6428–6432.

Ogata, K., S. Morikawa, H. Nakamura, A. Sekikawa, T. Inoue *et al.*, 1994 Solution structure of a specific DNA complex of the Myb DNA-binding domain with cooperative recognition helices. Cell **79:** 639–648.

Oppenheimer, D. G., P. L. Herman, S. Svakumaran, 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.

Orbach, M. J., and M. S. Sachs, 1991 The Orbach/Sachs cosmid library of *N. crassa* DNA sequences (pMOcosX). Fungal Genetics Newsl. **38:** 97.

Pandit, A., and R. Maheshwari, 1993 A simple method of obtaining pure microconidia in *Neurospora crassa*. Fungal Genetics Newsl. **40:** 64–65.

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 similarities to transcriptional activators. EMBO J. **6:** 3553–3558.

Perkins, D. D., A. Radford, D. Newmeyer and M. Bjorkman, 1982 Chromosomal loci of *Neurospora crassa*. Microbiol. Rev. **46:** 426–570.

Plesofsky-Vig, N., D. Light and R. Brambl, 1983 Paedogenetic conidiation in *Neurospora crassa*. Exp. Mycol. **7:** 283–286.

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.

Rossier, C., T.-C. Ton-That and G. Turian, 1977 Microcyclic microconidiation in *Neurospora crassa*. Exp. Mycol. **1:** 52–62.

Roux, K. H., 1994 Using mismatched primer-template pairs in touchdown PCR. BioTechniques **16:** 812–814.

Sachs, M. S., and C. Yanofsky, 1991 Developmental analysis of mRNA levels for genes involved in conidiation and amino acid biosynthesis in *Neurospora crassa*. Dev. Biol. **148:** 117–128.

Sambrook, J., E. F. Fritsch and T. Maniatis, 1989 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Springer, M. L., 1993 Genetic control of fungal differentiation: the three sporulation pathways of *Neurospora crassa*. BioEssays **15:** 365–374.

Springer, M. L., and C. Yanofsky, 1989 A morphological and genetic analysis of conidiophore development in *Neurospora crassa*. Genes Dev. **3:** 559–571.

Timberlake, W. E., 1990 Molecular genetics of Aspergillus development. Annu. Rev. Genet. **24:** 5–36.

Trinci, A. P. J., P. T. Saunders, R. Gosrani and K. A. S. Campbell, 1979 Spiral growth of mycelial and reproductive hyphae. Trans. Br. Mycol. Soc. **73:** 283–293.

Vollmer, S. J., and C. Yanofsky, 1986 Efficient cloning of genes of *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **83:** 4869–4873.

White, B., and D. Woodward, 1995 A simple method for making disposable race tubes. Fungal Genetics Newsl. **42:** 79.

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

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

Xu, J.-R., and J. E. Hamer, 1995 Assessment of *Magnaporthe grisea* mating type by spore PCR. Fungal Genetics Newsl. **42:** 80.

Yamashiro, C. T., D. J. Ebbole, B.-U. Lee, R. E. Brown, C. Bourland, L. Madi and C. Yanofsky, 1996 Characterization of *rco-1* of *Neurospora crassa*, a pleiotropic gene affecting growth and development that encodes a homolog of Tup1 of *Saccharomyces cerevisiae.* Mol. Cell. Biol. **16:** 6218–6228.

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.

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