Suppression and Enhancement of the *Aspergillus nidulans medusa* Mutation by Altered Dosage of the *bristle* and *stunted* Genes

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ABSTRACT

Asexual reproduction in *Aspergillus nidulans* is characterized by the orderly differentiation of multicellular reproductive structures (conidiophores) and chains of uninucleate conidia (spores). Mutations in the developmental modifier *medusa* (*medA*) result in aberrant conidiophores with branching chains of reiterated reproductive cells (metulae), delayed conidial differentiation and frequent reinitiation of secondary conidiophores. We show that incorrect morphology is in part a consequence of modified *bristle* (*brlA*) and *abacus* (*abaA*) expression, key regulators of the core genetic pathway directing conidial differentiation. First, correct temporal expression of both *brlA* transcripts (*brlA α* and *brlA β*) requires MedAp. Second, MedAp functions as a coactivator required for normal levels of *abaA* expression. Finally, we show that wild-type morphology results from a finely tuned balance in the expression of *brlA*, *medA* and the developmental modifier *stunted* (*stuA*). One extra copy of *brlA* suppresses *medA* mutations and restores normal *abaA* mRNA abundance. In contrast, an extra copy of *stuA* in a *medA*⁻ strain results in an enhanced medusoid phenotype with extensive metulae proliferation and nearly complete absence of conidia. *abaA* and *brlA α* transcription are completely repressed in these strains. In general, low *stuA:brlA* ratios promoted conidiation while high ratios caused proliferation of unicellular sterigmata and inhibited conidiation.

ASEXUAL reproduction in the filamentous fungus *Aspergillus nidulans* requires a shift from highly polarized hyphal growth to growth by budding, and the transition from a multinucleate to a uninucleate state. A precise pattern of cell differentiation events results in the formation of a multicellular conidiophore consisting of an aerial hypha, a multinucleate vesicle and two tiers of uninucleate sterigmata, the metulae and phialides. Chains of conidia are formed by repeated rounds of interstitial budding of the phialides (Figure 1A; CLUTTERBUCK 1969, 1977; MIMS *et al.* 1988; MILLER 1990; TIMBERLAKE 1990). A limited set of regulatory loci controls the coordinated temporal and spatial expression of hundreds of genes required for morphogenesis (CLUTTERBUCK 1969; MARTINELLI 1979; BOYLAN *et al.* 1987; MIRABITO *et al.* 1989; MILLER *et al.* 1992; AGUIRRE 1993). Sequential expression of *bristle* (*brlA*), *abacus* (*abaA*) and *wet-white conidia* (*wetA*) establishes a linear regulatory pathway (*brlA* > *abaA* > *wetA*) required for the transition from vegetative hyphae to conidia (ADAMS *et al.* 1988; MARSHALL and TIMBERLAKE 1991). The *brlA* locus consists of two overlapping transcription units, *brlA α* and *brlA β* , that encode redundant proteins (BrlAp) required to initiate this pathway (HAN *et al.* 1993; PRADE and TIMBERLAKE 1993). It has been proposed that *brlA β* expression plays a predominant role

during initiation and early stages of conidiophore differentiation while *brlA α* is essential for later stages of development (HAN *et al.* 1993; PRADE and TIMBERLAKE 1993). AbAp is required for phialide differentiation while WetAp is required for conidia maturation (CLUTTERBUCK 1969; SEWALL *et al.* 1990; MARSHALL and TIMBERLAKE 1991). BrlAp and AbAp, and probably WetAp, are transcription factors (ADAMS *et al.* 1988; ANDRIANOPOULOS and TIMBERLAKE 1991; BURGLIN 1991; MARSHALL and TIMBERLAKE 1991). Target genes and response elements have been identified for both BrlAp and AbAp (ARAMAYO and TIMBERLAKE 1993; CHANG and TIMBERLAKE 1993; ANDRIANOPOULOS and TIMBERLAKE 1994). *brlA* and *abaA* are themselves target genes. BrlAp activates *abaA* expression. In turn, it is proposed that AbAp binds to multiple sites located upstream of the *brlA α* RNA start site, establishing a positive feedback regulatory loop and ensuring commitment to the developmental program (ADAMS *et al.* 1988; MIRABITO *et al.* 1989; HAN *et al.* 1993; ANDRIANOPOULOS and TIMBERLAKE 1994).

Beside the core regulatory pathway, normal patterns of cell differentiation require the developmental modifier genes *stunted* (*stuA*) and *medusa* (*medA*) (CLUTTERBUCK 1969; MILLER 1990; MILLER *et al.* 1992). *stuA* null mutants have extremely shortened aerial hyphae and differentiate only a few conidia directly from buds formed on the conidiophore vesicle. In some cases, single conidia develop from intermediate cell types resembling aberrant phialides. StuAp is a member of a family

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FIGURE 1.—Differential interference contrast micrographs of strains with the wild-type, *medusa*, suppressed or enhanced medusoid phenotypes. Size of metulae and phialide are 6 μm and conidia 3 μm . Insets are enlargements of branched chains of reiterated metulae. (A) FGSC 26 (wild type). (B) FGSC 586 (*medA15*). (C) UI 11-T1.3 (suppressed). (D) UI 52-KA6.8A (enhanced). CV, conidiophore vesicle; M, metulae; P, phialide; C, conidia; SC, secondary or reiterated conidiophore.

of fungal DNA-binding proteins essential for progression through the cell cycle or for morphogenesis (LOWNDES *et al.* 1992; MILLER *et al.* 1992; PRIMIG *et al.* 1992; CALIGIURI and BEACH 1993; KOCH *et al.* 1993; GIMENO and FINK 1994; ZHU *et al.* 1994). *StuA*p modulates expression of the core regulatory pathway by spatially restricting *brlA* expression to the periphery of the conidiophore vesicle, metulae, phialides and immature conidia and *abaA* expression to metulae, phialides and immature conidia (MILLER *et al.* 1992; AGUIRRE 1993). In contrast to *stuA*[−] strains, *medA* mutants have delayed differentiation of phialides and conidia, resulting in proliferation of branching chains of sterigmata. The development of secondary conidiophores is also a characteristic of *medA* mutants (Figure 1B; CLUTTERBUCK 1969; MARTINELLI 1979). The *medA* mutant phenotype is very similar to that of *abaA* null mutants and some hypomorphic *brlA* mutants (CLUTTERBUCK 1969, 1977; MARTINELLI 1979; SEWALL *et al.* 1990; PRADE and TIMBERLAKE 1993). Analysis of double mutants led MARTI-

NELLI (1979) to suggest complex interactions, particularly between *brlA* and *medA*.

We are interested in the molecular mechanisms by which developmental modifier genes establish correct patterns of cell differentiation in *A. nidulans*. In this report, we show that *medA* function effects expression of the core regulatory pathway at several points during development. First, *MedA*p represses premature expression of both *brlA* transcripts during early development and downregulates *brlA*b during later stages. Second, *MedA*p is required as a coactivator of *abaA* expression. Our results suggest that delayed phialide differentiation in *medA*[−] mutants is most likely a consequence of insufficient *abaA* expression. We have found that altering the relative gene dosages of *brlA*, *medA* and *stuA* has a significant effect upon conidiophore morphology. Altered patterns of cell differentiation were associated with increased or decreased *brlA* and *abaA* expression. These results provide a molecular framework for genetic interactions that take place during conidiation

TABLE 1

Aspergillus nidulans strains used in this study

Strain	Genotype	Source
AJC 48	<i>medA26, biA1</i>	A
FGSC 26	<i>biA1</i>	B
FGSC 586	<i>medA15, biA1</i>	B
GO256	<i>stuA1, biA1</i>	A
JLM 004	<i>pabaA1; brlA1, trpC 801</i>	C
UI 11	<i>medA15, pabaA1, γA2; trpC 801</i>	This study
UI 25	<i>medA26, pabaA1, γA2; trpC 801</i>	This study
UI 29	<i>biA1; brlA42^{ts}, trpC 801</i>	This study
UI 30	<i>biA1; wA2; metH2, argB2; brlA42^{ts}</i>	This study
UI 41	<i>medA26, riboA1, anA1, pabaA1, γA2, ade20, biA1; trpC 801</i>	This study
UI 52	<i>medA26, pabaA1, biA1; argB2</i>	This study
UI 53	<i>medA15, pabaA1, biA1; argB2; metG2</i>	This study

All strains are *veA1*. References are: A, A. J. CLUTTERBUCK, Institute of Genetics, University of Glasgow; B, Fungal Genetics Stock Center, Department of Microbiology, University of Kansas Medical Center; C, J. L. MOONEY, Department of Genetics, University of Georgia.

between *brlA*, *abaA*, *medA* and *stuA*. Finally, data suggest that the sexual reproductive cycle has requirements for MedAp function that are independent of those involved in conidiation.

MATERIALS AND METHODS

A. *nidulans* strains, media, genetic manipulations and transformation: Table 1 describes strains used in this study. Media was prepared following the recipes described by KÄFER (1977) and MILLER *et al.* (1991). Standard genetic manipulations were used (PONTECORVO *et al.* 1953; CLUTTERBUCK 1974). DNA-mediated transformation was performed as described previously by YELTON *et al.* (1984) and MILLER *et al.* (1985). Cosmids were recovered from *A. nidulans* transformants using methods of MILLER *et al.* 1991. Complementing DNA sequences within these cosmids were localized as described previously (MILLER *et al.* 1991).

Synchronous conidiation: Procedures for the induction of synchronously conidiating *A. nidulans* cultures is described in MILLER *et al.* 1992.

Determination of conidial yields: Conidial stocks were collected in 0.01% Tween 80 using a glass rod, filtered through four layers of Miracloth and stored for 10 days at 4° to ensure that all hyphal fragments were removed or were no longer viable. The concentration of viable conidia was determined and each stock diluted so that ~20 conidia were used for centerpoint inoculation. After 4 days at 37°, the area of a conidiating colony was determined from three separate measurements of the colony radius. The colony was cut from the plate and dounced 10 times in sterile 0.01% Tween 80. This homogenous solution was filtered through two layers of Miracloth to remove cell debris and agar. The filtered conidia were brought to a final volume of 6 ml, serial dilutions made and the concentration of conidia determined using a hemacytometer. Conidial yield (conidia/square centimeter) was determined in triplicate for each strain tested. Standard deviations were 5–20% of the mean for the different strains tested. For the control strain FGSC 26, conidial yields were 2.9×10^8 conidia/cm² and 2.2×10^8 conidia/cm² at 37 and 25°, respectively.

TABLE 2

Increased *brlA* gene dosage suppresses *medA*[−] mutations

Strain	Allele	Conidial yields	
		37°	25°
FGSC 26	Wild type	100	100
UI 11-JW2.2	<i>medA15</i>	13	0.25
UI 11-T1.1,2,3,4,5,6	<i>medA15</i>	65 (45–103)	ND
UI 11-T1.5	<i>medA15</i>	103	17
UI 11-RG.11.5	<i>medA15</i>	90	27
UI 11-RG.11.6	<i>medA15</i>	97	ND
UI 25-JW2.2	<i>medA26</i>	5.2	0.14
UI 25-T1.1,4,7,8,10	<i>medA26</i>	63 (45–93)	ND
UI 25-T1.4	<i>medA26</i>	69	1.6
UI 25-RG.11.8	<i>medA26</i>	52	1.0

Strains designated JW2 were transformed with the control plasmid pJW2 that contains only the *trpC*⁺ gene. Strains with the designation T1 were obtained by transformation of either UI 11 or UI 25 with cosmid Cos T1C. Strains designated RG.11 carry single copies of a plasmid containing the isolated *brlA* gene (obtained from T. H. Adams, Texas A&M University). Strains were grown at 37 and 25° and conidial yields (expressed as percent wild type) determined as described in MATERIALS AND METHODS. The means of the conidial yields and their range, in parenthesis, is shown for several of the Cos T1 transformants. ND, not determined.

Nucleic acid isolation and manipulations: Standard methods for construction, maintenance and isolation of recombinant plasmids were used (SAMBROOK *et al.* 1989). DNA and RNA was isolated from *A. nidulans* as previously described (MILLER *et al.* 1985, 1987). Methods for Northern and Southern blot analysis have been described previously (MILLER *et al.* 1991). The sequence of complementing DNA fragments was determined by subcloning fragments into pBluescript KS (+/−) phagemids (Stratagene Cloning Systems, La Jolla, CA) and sequencing single-stranded DNA with Sequenase (U.S. Biochemical Corp., Cleveland, OH).

RESULTS

***medA* mutants with extra copies of *brlA* have normal conidiophore morphology:** The wild-type *medA* gene was cloned by mutational complementation of the *medA26* strain, UI 25, with the pKBY2 cosmid library (YELTON *et al.* 1985). A complementing cosmid, CosT1, was recovered from a transformant with wild-type conidiophore morphology. CosT1 was able to complement at high frequency both the *medA26* and *medA15* alleles (strains UI 25 and UI 11, respectively). Southern blot analysis (data not shown) indicated that all transformants with wild-type conidiophore morphology carried at least one copy of CosT1. UI 11-CosT1 transformants had conidial yields (conidia/square centimeter) that ranged from 45–100% of wild type (mean = 65%). Variation was dependent upon the particular transformant, possibly due to integration at different genomic positions (Table 2). UI 25-CosT1 transformants also had conidial yields of 45–93%

(mean = 63%). UI 11 and UI 25 transformants with near wild-type conidial yields had conidiophore morphologies similar to wild type (Figure 1A). CosT1 transformants with partially restored conidial yields had 3–4 tiers of metulae, but no secondary conidiophores. UI 11-T1.3, with a conidial yield 48% of wild type, is an example of this type of transformant (Figure 1C). Transformants with control cosmids or plasmids had conidiophore morphologies and conidial yields similar to the original *medA15* and *medA26* strains (Figure 1B, Table 2).

CosT1 hybridized to two developmentally expressed transcripts present in RNAs isolated from synchronously conidiating cultures of a wild-type strain. These RNAs were expressed from the same gene and closely mimicked the two *brlA* transcripts in both size and pattern of expression (Figure 2A). Detailed transcriptional mapping and DNA sequence analysis confirmed that the CosT1 insert contained the *brlA* gene. It was possible that an undetected gene present within the CosT1 insert, and not *brlA*, suppressed the *medA* mutation. Therefore, we obtained an independently isolated plasmid, pRG.11, containing a 10.4-kb *SstI* fragment (Figure 3). This *SstI* fragment includes the *brlA α* and *brlA β* transcriptional units and 3.5 kb of 5' DNA sequences required for correct *brlA* regulation (AGUIRRE 1993; HAN *et al.* 1993; PRADE and TIMBERLAKE 1993; T. H. ADAMS, unpublished data). A plasmid, pRPO5, containing this *SstI* fragment has been described previously (PRADE and TIMBERLAKE 1993). pRG.11 contains a fragment of the wild-type *trpC* gene capable of complementing the *trpC801* mutation. Therefore, integration of the transforming plasmid can be targeted to the *trpC* locus. UI 11 (*medA15*) transformants with a single copy of pRG.11 had wild-type conidiophore morphology and conidial yields that were essentially wild type; 90–97% (Table 2). However, a single targeted copy of pRG.11 was able to restore conidial yields to only ~50% wild-type levels in the *medA26* strain UI 25 (Table 2).

The 4.0-kb *SstII* fragment containing the *brlA α /* $\beta transcriptional unit, but only 2.1 kb of essential upstream regulatory sequences, could only partially suppress *medA* mutations (Figure 3). Transformant conidiophores had a morphology intermediate between wild type and medusoid (not shown). However, the 4-kb *SstI/KpnI* fragment that includes the 5' flanking DNA sequences and truncated *brlA* ORF was unable to suppress *medA* mutations (Figure 3). The 4.4-kb *SalI/SstI* fragment that includes the 3' end of the *brlA α /* β transcriptional unit and 3' flanking DNA sequences gave the same result. Therefore, for the asexual reproductive cycle, complete suppression of *medA* lesions requires an additional copy of the *brlA* gene that includes most, or all, of 5' flanking sequences required for correct spatiotemporal regulation. In contrast, we never observed that extra copies of *brlA* suppressed the sexual reproductive defect of a *medA* mutant. Suppressed$

FIGURE 2.—Expression of *brlA α* , *brlA β* and *abaA* in strains with the wild-type, *medusa*, suppressed or enhanced medusoid phenotypes. Poly(A)⁺ RNAs from developmentally induced cultures were screened with probes specific for the *brlA α /* β , *abaA* and *argB* transcripts. *argB* was used as a constitutively expressed control for balancing RNA samples. (A) Expression of *brlA α /* β during development of wild-type and *medA26* strains. RNAs were isolated from tissue 0, 2.5, 5, 7.5, 10, 12.5 and 14 hrs post induction (PI). Conidiophore vesicles and metulae initials are observed at 5 hr; metulae and phialide by 7.5 hr; immature, unpigmented conidia by 10 hr and mature, pigmented conidia by 12.5 hr. (B and C) Odd lanes, 0 hr undifferentiated hyphae; even lanes, 14 hr. PI cultures with fully developed conidiophores and mature conidia. Lanes 1 and 2, wild type; lanes 3 and 4, *medA15*; lanes 5 and 6, *medA26*; lanes 7 and 8, *medA15*-CosT1 (suppressed); lanes 9 and 10, *medA26*-CosT1 (suppressed); lanes 11 and 12, *medA26*-pRG.11 (suppressed). (D) Odd lanes, 0 hr undifferentiated hyphae; even lanes, 14 hr. PI cultures: lanes 1, 2, 7, and 8, wild type; lanes 3, 4, 9, and 10, *medA15*-pK6.8 (enhanced); lanes 5, 6, 11, and 12, *medA26*-pK6.8 (enhanced).

strains still produced only unorganized masses of Hülle cells and did not differentiate cleistothecia or ascogenous tissue. This result is consistent with the observation that *brlA* and *abaA* expression are not required for the sexual cycle (CHAMPE *et al.* 1994).

Suppression of *medA* mutations by extra *brlA* gene dosage is cold sensitive: Conidial yield for the wild-type strain was only slightly effected when grown at 25° instead of 37° (Table 2). In contrast, conidiation in *medA* mutants showed a cold-sensitive response with enhanced proliferation of sterigmata and secondary co-

FIGURE 3.—The 10.4-kb *SstI* fragment from pRG.11. This fragment carries the *brlAα/β* transcription unit and 3.5 kb of upstream DNA sequences required for correct *brlA* expression. This fragment has been described previously as part of pRP05 (PRADE and TIMBERLAKE 1993).

nidiophores at 25°. Conidial yields dropped by an additional two orders of magnitude, to <1.0% of wild-type levels (Table 2). The ability of additional *brlA* dosage to restore wild-type morphology and conidial yield was severely limited at 25° for the *medA15* strain and ineffective for the *medA26* strain.

Increased *brlA* gene dosage in a *medA* mutant restores normal *brlAα*, *brlAβ* and *abaA* RNA abundance: The *medA* mutant phenotype suggests the inability to correctly progress through, or maintain commitment to, the program for conidial differentiation. Therefore it was possible that *medA* mutations modify the expression of *abaA* and/or *brlA*. Poly(A)⁺ RNA was isolated from undifferentiated hyphae and from cultures at various times during synchronously induced development. In the wild-type strain, both *brlA* transcripts were first detected at 7.5 hr post induction (Figure 2A). Conidiophore vesicles and metulae are present at this time. RNA abundance of the *brlAβ* transcript remained constant thereafter, while *brlAα* abundance increased dramatically (fivefold) during conidiation. Similar results have been reported by HAN *et al.* (1993) and PRADE and TIMBERLAKE (1993). In contrast, both *brlA* transcripts were detected 2.5 hr earlier, at 5 hr post induction, in developmental cultures of a *medA26* strain (Figure 2A). Incorrect temporal *brlAα/β* expression did not cause precocious development of conidiophore vesicles or metulae (data not shown). In the *medA* mutant, *brlAβ* RNA abundance continued to increase throughout development. Fully developed cultures had an aberrant *brlAα:brlAβ* ratio where *brlAα* and *brlAβ* abundance were similar. Transformation of either *medA*⁻ strain with an extra copy of the *brlA* gene resulted in increased *brlAα* RNA abundance compared with *brlAβ* in developmental cultures (Figure 2B).

The *abaA* transcript can not be detected in undifferentiated hyphae. *abaA* mRNA abundance increases during the formation of metulae and phialides, then remains constant (BOYLAN *et al.* 1987). We found that *abaA* transcript abundance was greatly reduced in 14-hr developmental cultures from the *medA15* strain when compared with the wild type. The transcript was not detected in the *medA26* strain (Figure 2C). Addition of an extra copy of the *brlA* gene in *medA15* or *medA26*

TABLE 3

Increased *stuA* gene dosage in *medA*⁻ strains results in an enhanced medusoid phenotype

Strain	Allele	Conidial yield
FGSC 26	Wild type	100
AJC 48	<i>medA26</i>	17
UI 52-KA6.8.A	<i>medA26</i>	2
UI 52-KA6.8.B	<i>medA26</i>	3
FGSC 586	<i>medA15</i>	20
UI 53-KA6.8.A	<i>medA15</i>	<1
UI 53-KA6.8.B	<i>medA15</i>	1

The strains UI 52 and UI 53 were transformed with the plasmid pKA6.8 containing the cloned *stuA* gene (MILLER *et al.* 1991; MILLER *et al.* 1992). The resulting transformants (-KA6.8) have a single copy of this plasmid integrated at the *argB* locus.

strains restored *abaA* transcript abundance to levels equal to, or greater than, wild-type levels.

Increased *stuA* gene dosage enhances the medusoid phenotype and represses *abaA* and *brlAα*, but not *brlAβ* expression: *StuAp* directly, or indirectly, represses spatial expression of *brlA* and *abaA* (MILLER *et al.* 1992; AGUIRRE 1993). Because *medA* mutants are already deficient in *abaA* and *brlAα* expression, we tested the effect of increased *stuA* gene dosage upon cell differentiation in a *medA*⁻ strain. pKA6.8 was used to target integration of an extra copy of the *stuA* gene to the *argB* locus of *medA15* or *medA26* strains. pKA6.8 contains the *stuA* transcriptional unit and all 5' flanking DNA sequences required for correct spatiotemporal regulation (MILLER *et al.* 1991, 1992). Transformants had an enhanced medusoid phenotype (Figure 1D). Conidiophores were extremely large, with long branching chains of reiterated "metulae." Enhanced strains are essentially aconidial, having conidial yields ≤1% wild-type levels (Table 3). Although additional copies of *stuA* enhanced metulae reiteration, secondary conidiophore development was suppressed. These strains expressed *brlAβ* at levels similar to the *medA* mutant. However, no *brlAα* transcript was detected. In addition, *abaA* expression was eliminated. Extended exposure of Northern blots did not detect *abaA* transcript in developmental cultures (Figure 2D). These results support earlier observations that at least one function of *StuAp* is to spatially repress both *brlA* and *abaA* expression (MILLER *et al.* 1992; AGUIRRE 1993).

stuA is a complex locus from which two overlapping transcripts (*stuAα* and *stuAβ*) are expressed (MILLER *et al.* 1992). Transformants with plasmid constructs containing internal deletions of either the *stuAα* (*stuAΔα*) or *stuAβ* (*stuAΔβ*) transcription start sites express only the *stuAβ* or *stuAα* mRNAs, respectively (J. WU and B. L. MILLER, unpublished observations). *medA26* strains transformed with the *stuAΔβ* construct had the enhanced medusoid phenotype. Transformation with the *stuAΔα* construct had no effect. This is consistent

TABLE 4
Effect of altered gene dosages upon conidiophore morphology

Strain	Ploidy	Gene dosage				Metulae	Secondary conidiophore
		<i>stuA</i>	<i>brlA</i>	<i>medA</i>	<i>abaA</i>		
FGSC 26	n	1	1	1	1	+	—
GO256	n	0	1	1	1	—	—
AJC 48 or FGSC 586	n	1	1	0	1	+++	+
suppressed	n	1	2	0	1	+	—
enhanced	n	2	1	0	1	++++	—
UI 52-TABA3	n	1	1	0	2	+++	+
UI 30-TKA6.8 (25°)	n	2	1	1	1	+	—
<i>medA15/medA26</i>	2n	2	2	0	2	+++	+
<i>medA⁻/enhanced</i>	2n	3	2	0	2	++++	+
<i>medA⁻/brlA1</i>	2n	2	1	1	2	+	—
<i>medA⁺/medA⁻</i>	2n	2	2	1	2	+	—
<i>medA⁺/enhanced</i>	2n	3	2	1	2	+	—
enhanced/ <i>brlA</i>	2n	3	1	1	2	++	—

with our observation that the *stuA* transcript is developmentally regulated and plays the major role during conidiation (MILLER *et al.* 1992; J. WU and B. L. MILLER, unpublished data).

Altered gene dosage ratios of *brlA*, *medA* and *stuA* control proliferation of sterigmata and secondary conidiophore formation: A suppressed strain has a *stuA:brlA:medA* ratio of 1:2:0, while a strain with enhanced medusoid phenotype has a ratio of 2:1:0. A *brlA42^{ts}* strain was transformed with a plasmid containing a copy of the *stuA* gene. At the permissive temperature, this transformant has a functional *stuA:brlA:medA* ratio of 2:1:1. This strain had wild-type conidiophore morphology (Table 4). A *brlA⁺, medA⁺* strain transformed with an extra copy of *stuA* gave the same result. Therefore, we were unable to detect any effect of increased *stuA* gene dosage in strains with a wild-type *medA* allele. Increased ratios of *stuA:brlA* in a diploid resulted in an enhanced medusoid phenotype, but again, only in the absence of a functional *medA* gene (Table 4). These results suggest that MedAp can modulate the repressive effects of StuAp upon *brlA* and *abaA* expression, allowing normal development. The only exception was a diploid constructed between a *brlA1* null strain and a strain with the enhanced medusoid phenotype. In this diploid, the ratio of *stuA:brlA:medA* was 3:1:1. This strain had an intermediate phenotype similar to that shown in Figure 1C. Three to four tiers of sterigmata were present, but no secondary conidiophores. Therefore, highly elevated levels of *stuA* gene dosage can result in a conidiophore with medusoid characteristics even in the presence of a functional *medA* gene.

We have described the effect of altered *brlA*, *medA* and *stuA* gene dosage upon *abaA* expression. We tested the possibility that increased *abaA* gene dosage might also suppress, or compensate for, loss of *medA* function. The plasmid pABA3 contains an insert with the *abaA* transcription unit and upstream sequences required for

correct developmental expression (BOYLAN *et al.* 1987; ADAMS and TIMBERLAKE 1990). Transformation of the *A. nidulans* strain UI 52 with pABA3 did not result in suppression of the *medA26* mutation (Table 4)

DISCUSSION

Multicellular development during conidiation is associated with the establishment of spatial and temporal gradients in *brlA*, *abaA*, *stuA* and *medA* expression (BOYLAN *et al.* 1987; ADAMS and TIMBERLAKE 1990; MILLER *et al.* 1992; AGUIRRE 1993; K. Y. MILLER and B. L. MILLER, unpublished results). However, overexpression of *brlA* in submerged vegetative hyphae activates the core developmental pathway in the absence of normal spatiotemporal regulatory mechanisms. Vegetative growth ceases and conidia differentiate directly from hyphal tips (ADAMS *et al.* 1988). By contrast, overexpression of *abaA* causes cessation of vegetative growth and extensive vacuolization, but no conidial differentiation (MIRABITO *et al.* 1989). These results demonstrate that development of multicellular structures in *A. nidulans* must require modulation of *brlA* and *abaA* expression by additional genetic loci. Indeed, incorrect morphology in *medA* mutants was associated with the aberrant expression of both *brlA* and *abaA*. Furthermore, we found that variations in conidiophore structure caused by altered gene dosages of *brlA*, *medA* and *stuA* were also associated with modified *brlA*, *brlA* and *abaA* expression. Taken together, these results support prior observations and provide new insights into molecular mechanisms required to establish proper patterns of cell differentiation.

Poorly understood environmental and developmental signals initiate conidiation (AXELROD *et al.* 1973; MOONEY and YAGER 1990; LEE and ADAMS 1994; SKROMNE *et al.* 1995). HAN *et al.* (1993) have proposed that induction includes translational regulatory mechanisms that allow *brlA* expression that, in turn, activates *abaA* tran-

FIGURE 4.—A model for multicellular development during the *A. nidulans* asexual cycle. See DISCUSSION for details. *stuA* transcription occurs coincidentally with the onset of developmental competence and therefore prior to induction (MILLER *et al.* 1991). Transcription of *medA* occurs immediately after induction (K. Y. MILLER and B. L. MILLER, unpublished data). I, unknown induction mechanisms; X, proposed repressor; xxx, repression; →, activation. Dashed lines in B indicate that inductive signals are beginning to override MedAp repression. Thick lines in panels C and D indicate that the feedback regulatory loop is predominately between *brlAα* and *abaA*.

scription. AbaAp establishes a positive feedback regulatory loop that activates *brlAα* transcription (Figure 4, B and C). These authors suggest that additional mechanisms may also activate *brlAα* transcription.

StuAp and MedAp apparently function to establish proper spatiotemporal gradients in the amplification of *brlA* and *abaA* expression during conidiophore development (MILLER *et al.* 1992; AGUIRRE 1993). *stuA* null mutants lack sterigmata and express *brlA* and *abaA* throughout the foot cell, aerial hypha and conidiophore vesicle. By contrast, we find that increased *stuA* dosage in a *medA*⁻ strain results in the absence of detectable *brlAα* expression. This is associated with an enhanced proliferation of metulae and the loss of phialide and conidial differentiation. This enhanced medusoid phenotype could be explained by specific requirements for *brlAα* during phialide and conidia differentiation. Alternatively, the complete absence of detectable *abaA* expression in enhanced strains would also be expected to block phialide differentiation. These results suggest that StuAp func-

tions as a repressor of *brlAα*, and possibly *abaA*, expression in somatic hyphae and during early conidiophore development (Figure 4, A and B). StuAp response elements are present upstream of the *abaA* and *brlAα*, but not *brlAβ*, transcriptional start sites; suggesting a direct interaction (J. DUTTON and B. L. MILLER, unpublished observations). Extra *stuA* dosage did not effect *brlAβ* expression in a *medA* mutant. This result supports the conclusions of PRADE and TIMBERLAKE (1993) that *brlAβ* expression alone is sufficient for normal conidiophore stalk, vesicle and metulae development.

MILLER *et al.* (1992) showed that developmental induction results in a *brlA*-dependent spatiotemporal gradient in *stuA* expression that is highest in the conidiophore vesicle, metulae and phialides, but absent in immature conidia. We propose that a feedback loop is established in which StuAp functions to downregulate *brlAα*, and possibly *abaA* expression during mid development (Figure 4C). While the exact mechanisms remain unclear, the action of StuAp allows uninuclear, yeast-like cell divisions to continue. It is notable that

gradients in *brlA* expression are established, in part, by competing positive and negative feedback loops between *brlA* and *abaA* and *stuA*, respectively. During late development, an unknown factor(s) *X* blocks *stuA* expression (Figure 4D). We propose that the absence of *stuA* expression allows maximum *brlA* and *abaA* function and the differentiation of G1 arrested conidia (Figure 4D).

Similar to *stuA*, we have found that MedAp modulates the core regulatory pathway at several levels. First, MedAp represses both *brlA α* and *brlA β* expression during the earliest stages of development (Figure 4B). The effect upon *brlA α* expression may be indirect. Repression of *brlA β* expression by MedAp would block the activation of *abaA* expression and the feedback loop proposed by HAN *et al.* (1993) and MIRABITO *et al.* (1989). CLUTTERBUCK (1969) has shown that *brlA* function is required for the transition from polar to apolar growth during conidiophore morphogenesis. Also, *brlA* expression is detected in the wild-type conidiophore vesicle (AGUIRRE 1993). Therefore, we propose that regulatory mechanisms activating *brlA* expression must begin to override MedAp-mediated repression during the transition from early to middevelopment (indicated by dashed lines in Figure 4B). During later stages of development, MedAp modulates *brlA* function by downregulating *brlA β* expression (Figure 4, B and C). This is based upon the observation that, once development is induced, *medA* mutants overexpress *brlA β* and have altered *brlA α / β* ratios (Figure 2, A and B).

Both overexpression and premature expression of *brlA* in *medA* mutants should promote precocious development and conidiation (ADAMS *et al.* 1988; HAN *et al.* 1993). However, the timing of conidiophore vesicle and metulae formation in a *medA* mutant is the same as the wild type, while aberrant metulae proliferation causes delayed conidial differentiation. A possible explanation for this inconsistency is that MedAp appears to also function as a coactivator with BrlAp to provide maximum *abaA* expression (Figure 4, C and D). Greatly reduced levels of AbaAp in *medA* mutants would be expected to disrupt the expression of AbaAp target genes essential for phialide and conidium differentiation. Consistent with this explanation, the hypomorphic *brlA42^{is}* mutant does not express detectable levels of *abaA* mRNA and fails to differentiate phialides under nonpermissive conditions (R. PRADE, unpublished data). The phenotypic similarity of *abaA*, *medA* and *brlA42^{is}* mutants may be attributed, at least in part, to deficient *abaA* expression and a resulting block in the metulae to phialide transition.

We found that introduction of one extra copy of the *brlA* gene suppresses the *medA* mutation, restoring both *abaA* expression and wild-type conidiophore morphology. Presumably, additional *brlA* gene dosage results in increased BrlAp concentration that, in turn, alleviates the need for MedAp. BrlAp and MedAp may physically

interact with each other. This is suggested by observations that MedAp is a coactivator of some BrlAp target genes and the suppression of *medA* mutations by extra copies of *brlA* is cold sensitive. MedAp may be stabilizing protein-protein interactions, perhaps in a transcriptional complex that includes BrlAp. Indeed, we have been able to demonstrate physical interaction between BrlAp and MedAp and have mapped amino acid domains required for this interaction (K. Y. MILLER and B. L. MILLER, unpublished data). The exact molecular details of MedAp function remain unknown. Mechanisms that allow MedAp to act as both a repressor and an activator may involve concentration dependence or interactions with uncharacterized cell-specific factors.

In conclusion, conidiophore morphogenesis requires a finely tuned balance in the spatiotemporal gradients of products from at least four genes: *abaA*, *brlA*, *medA* and *stuA*. Different classes of cell-specific target genes are likely to possess varying numbers and combinations of response elements for these regulatory proteins. Therefore, small changes in the concentrations of the AbaAp, BrlAp, MedAp and StuAp would be expected to have profound effects upon cell pattern formation. In this respect, development in *A. nidulans* is reminiscent of the regulation of pattern formation in *Drosophila* by overlapping gradients of numerous regulatory factors (ST. JOHNSTON and NUSSLEIN-VOLHARD 1992). Most interesting is the observation that, although increased *brlA* gene dosage can compensate for the loss of *medA* expression during the conidiation, it cannot do so during the sexual reproductive cycle. This suggests MedAp functions required for asexual and sexual reproduction are independent and may involve novel classes of target genes. Mechanisms that direct MedAp functions toward asexual or sexual reproduction remain to be elucidated.

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