Enhancers of Conidiation Mutants in *Aspergillus nidulans*

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ABSTRACT

Mutants at a number of loci, designated *sthenyo*, have been isolated as enhancers of the oligoconidial mutations at the *medA* locus. Two loci have been mapped: *sthA* on linkage group I, and *sthB* on linkage group V. Two probable alleles have been identified at each locus but two further mutants were unlinked to either *sthA* or *sthB*. Neither *sthA* nor *sthB* mutants have conspicuous effects on morphology on their own, nor could the *sthA1 sthB2* double mutant be distinguished from wild type. Mutants at both loci also interact with the temperature-sensitive *brlA42* mutant at the permissive temperature to give a phenotype described as "Abacoid." *sthA1* also induces a slight modification of the phenotype of an *abaA* mutant. We conclude that *sthenyo* genes act mainly at the phialide stage of conidiation. We also describe the isolation of new *medA* mutants arising spontaneously as outgrowths on *brlA42* colonies.

A SEXUAL sporulation (conidiation) in the filamentous fungus *Aspergillus nidulans* has been the subject of intensive genetic and molecular analysis [reviewed by TIMBERLAKE (1991a,b) and CLUTTERBUCK and TIMBERLAKE (1992)]. One conclusion of this work is that the primary developmental genes so far examined appear to be regulatory, rather than encoding proteins involved in the specific biochemistry of morphogenesis. These responder genes therefore remain to be identified.

Mutational analysis (MARTINELLI and CLUTTERBUCK 1971) suggested that 45–150 gene loci are specifically involved in conidiophore morphogenesis, while TIMBERLAKE (1980) estimated by means of subtractive RNA-DNA hybridization studies that 1200 diverse poly(A)⁺ RNAs are produced during conidiogenesis. So far mutants specifically affecting conidiation have been mapped to only about a dozen loci (CLUTTERBUCK and TIMBERLAKE 1992). Spore color mutants map at a similar number of loci and mutants of a few additional genes affect both conidiation and growth or sexual reproduction. Further loci have been identified by cloning, in particular a series of spore-expressed gene clusters designated Spo genes (TIMBERLAKE and BARNARD 1981). A deletion strain for one such cluster (SpoC1) was not detectably different from the wild type (ARAMAYO *et al.* 1989). It is therefore probable that many genes involved in conidiation have quite subtle effects on the phenotype.

The mature *Aspergillus* conidiophore consists of a stalk with an apical swelling, or vesicle; on this grows a layer of support cells, or metulae, then a layer of phialides from which conidia are budded (Figure 1A). Metulae and phialides are also known collectively as sterigmata. Mutants at two loci have particularly striking effects on the conidiophore phenotype (CLUTTERBUCK

1969); "bristle" (*brlA*) null mutants fail to develop conidiophore vesicles or any subsequent structures, leaving the conidiophore as an undifferentiated bristle. "Abacus" (*abaA*) mutants are blocked at the phialide stage and proliferate layers of cells which are phialide-like in appearance, but unable to produce conidia (CLUTTERBUCK 1969; SEWALL *et al.* 1990). Leaky bristle mutants present a variety of phenotypes consisting of structures with the appearance of multibranching conidiophores or proliferated metulae. Mutations at a further locus designated "medusa" (*medA*), have phenotypes like very leaky *brlA* mutants, in which conidiation is not completely blocked, but delayed by proliferation of metulae (CLUTTERBUCK 1969); see Figure 1B.

We suggest that genes such as *medA* may be members of a polygenic series, the sum of whose actions brings about development. Mutation in any one of them will be insufficient to abolish the wild-type phenotype. With this idea in mind, we have identified secondary mutants in *medA* or leaky *brlA* backgrounds. The phenotypes of these modifiers when transferred to a wild-type background prove to be slight or undetectable.

MATERIALS AND METHODS

All *Aspergillus* strains were derived from the Glasgow stocks, and standard growth media and culture techniques were used (PONTECORVO *et al.* 1953; CLUTTERBUCK 1974). Strains are described in Table 1; note that all carry the *veA1* mutation which permits conidiation in the dark (MOONEY and YAGER 1990). Mutants were isolated as spontaneous outgrowths and were subcultured three times to obtain genetically pure strains. Since the *sth* mutants were phenotypically silent in the absence of *medA* or other morphological mutants, only *medA*⁺ progeny were examined in crosses designed to follow *sth* segregation.

RESULTS

Mutant isolation: The phenotype of *medA* mutants is similar to that of leaky *brlA* mutants such as *brlA42* (see

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FIGURE 1.—Wild-type and mutant phenotypes of conidiophore morphology. (A) Wild type; (B) medusa (*medA15*, 37°); (C) temperature-sensitive leaky Bristle (*brlA42*, 37°-restrictive temperature); (D) Ultramedusa (*medA17 sthA1*); (E) Abacus (*abaA1*); (F) Abacoid (*brlA42 sthA1*, 30°). Abbreviations: s, stalk; v, vesicle; m, metula; p, phialide; c, conidium. Both *brlA42 sthA*⁺ (30°) and *medA*⁺ *sthA1* resemble morphology seen in A. Scale: the width of the conidiophore vesicle in each figure is approximately 10 μ m.

Figure 1C). We have observed that mature colonies of *medA*[−], *abaA*[−] and *brlA42* strains on agar frequently show outgrowths that protrude above the surface of the colony. These outgrowths arise from single conidial heads and may grow to 1 mm in diameter. They also have reduced conidiophore pigmentation (CLUTTERBUCK 1990).

Spontaneous mutants were first isolated from the *brlA42* strain AJC9.4 (Table 1). Outcrosses of all 19 mutants to the morphologically wild-type strain G12 segregated colonies of medusa-like morphology, and linkage of the determinant of this morphology to *fpaB* (Figure 2) confirmed that mutation had probably occurred at the *medA* locus. Similar secondary *medA* mutants have been also identified as the cause of frequent outgrowths on *abaA* mutant strains (A. J. CLUTTERBUCK, unpublished). The morphologies of the new *medA* mutants varied little and were indistinguishable from those isolated by CLUTTERBUCK (1969). With one exception, all were oligoconidial at 37° and almost aconidial at 30°; the exception being *medA11* which was temperature-sensitive, showing the mutant phenotype at 37° but the wild-type phenotype at 30°.

Secondary mutant outgrowths were then picked from *medA13*, *medA14* and *medA17* strains DHG54–56. These outgrowths were divisible into two classes on basis of pallor and stability. On subculture, outgrowths of the commoner and less pale class proved unstable, only normal colonies being recovered, but the paler outgrowths behaved as stable secondary mutants. Light microscopy of these mutants at 700 $\times$ showed an almost aconidial leaky Bristle-type morphology, consisting of multiple layers of elongated and branching metulae (Figure 1D).

This morphology was designated "Ultramedusa."

In these experiments, no distinction in mutation frequency was noticed between the three *medA* parent strains. In order to assess mutation frequencies, conidia from *medA15* and *brlA42* strains G0255 and G0248 were plated at densities from 2×10^5 to 3×10^6 per plate on medium + 0.1% Triton X-100 to give microcolonies. No variants were initially seen on *brlA42* plates, but on *medA15* plates the frequency of Ultramedusa colonies was estimated at 6×10^{-6} . As before, outgrowths of single conidial heads became evident on both *brlA42* and *medA15* plates as the colonies aged.

Genetic analysis: Two Ultramedusa mutants, DHG60 and DHG72, were crossed with a morphologically wild-type strain, G0112; the results are presented in Table 2, crosses 1 and 2. In both crosses approximately half of the progeny were morphologically wild type and no other abnormality was detected. This result suggested that the secondary mutation has no detectable phenotype, either morphological or nutritional, in the absence of the *medA*[−] allele. In order to test this hypothesis, morphologically wild-type progeny of both Ultramedusa strains were backcrossed with the *medA13* strain DHG54. Cross 3 (Table 2) is an example in which the segregation of Ultramedusa confirms the presence of a secondary mutation in the morphologically wild-type parent. Of two phenotypically wild-type colonies from cross 1 which gave hybrid cleistothecia with the *medA13* strain, one gave Ultramedusa progeny while the other did not. Similarly, two out of four wild-type progeny of cross 2 regenerated Ultramedusas in combination with *medA13*.

The gene whose mutants act as enhancers of *medA* was designated "sthenyo" (*sth*); in Greek mythology Sthenyo

TABLE 1

Aspergillus nidulans strains

Strain	Genotype
G01	<i>biA1; abaA14</i>
G0112	<i>yA2; pyroA4; methB3</i>
G0248	<i>biA1; brlA42</i>
G0255	<i>medA15 biA1</i>
G12	<i>fpaB37 adF17 pabaA1 yA2</i>
G116	<i>riboA1 adG14 proA1 pabaA1 yA2</i>
G141	<i>proA1 adF9 pabaA1 biA1</i>
G197	<i>suA1adE20 lysF88 pabaA1 adE20 biA1</i>
G52	<i>adG14 yA2; lysB5</i>
G53	<i>yA2; nicA2 pA3 facA303 riboD5; methB3</i>
G54	<i>yA2; nicA2 facA303 riboD5</i>
G523	<i>proA1 pabaA1 yA2; cysA1</i>
G95 (MSF)	<i>suA1adE20 yA2 adE20; acrA1; galA1; pyroA4; facA303; sB3; nicB8; riboB2</i>
AJC11.7	<i>medA15 riboA1 yA2; argB2</i>
AJC9.4	<i>pabaA1; argB2; brlA42 ivoB63</i>
DHG54	<i>medA13 adF17 pabaA1</i>
DHG55	<i>medA14 adF17 pabaA1</i>
DHG56	<i>medA17 biA1</i>
DHG60	<i>medA13 adF17 pabaA1; sthB2</i>
DHG68	<i>medA13 biA1; sthB68</i>
DHG69	<i>medA13 sthA69 biA1</i>
DHG72	<i>medA17 sthA1 biA1</i>
DHG76	<i>medA17 sthA1 yA2; argB2; methB3</i>
DHG77	<i>medA13 biA1; sthB2</i>
DHG87	<i>medA17 adF17 sthA1</i>
DHG88	<i>medA13 adF17; sthB2</i>
DHG90	<i>medA17 sthA1 biA1; pyroA4</i>
X1a82	<i>sthA1; methB3</i>
X2a80	<i>yA2; sthB2; methB3</i>
X4a6	<i>riboA1 adG14 sthA1 biA1</i>
X7b23	<i>medA13 yA2; sthB2 nicA2 facA303 riboD5</i>
X9a12	<i>medA13 yA2; sthB68 nicA2 riboD5</i>
X9b17	<i>medA13 yA2; sthB68 nicA2</i>
X10b9	<i>medA13 yA2; sthB68 lysB5</i>
X10a12	<i>medA13 yA2; sthB68 lysB5</i>

Strains prefixed with G, AJC and DHG are from the Glasgow or the authors' stocks, respectively. DHG strains were mainly derived from preliminary crosses. Strains prefixed with X followed by a cross number are progeny of crosses described in this work. All strains also carry *veA1*. New mutants are here given the locus letters which they received as a result of the investigations described.

was one of the sisters of the gorgon, Medusa.

Haploidization and preliminary mapping: As a first step in locating the *sth* loci, diploids were constructed between Ultramedusa strains and the G95 master strain MSF (McCULLY and FORBES 1965). Since *sth* mutants appeared to be silent in the absence of *medA*, only *medA*⁻ haploids were selected for classification. Haploids from the diploid containing DHG87 (*sth-1*) showed segregation of all markers from linkage groups II–VIII, but all 55 *medA*⁻ haploids proved to have Ultramedusa morphology, implying that *sth-1* is on the same chromosome as *medA* (chromosome I).

Classification of haploids derived from diploid G95 (MSF) + DHG88 (*sth-2*) is shown in Table 3. These results show *sth-2* linked to both *facA* (chromosome V) and *nicB* (chromosome VII). This implies that *sth-2* could be on either of these chromosomes and that there may be a translocation between them.

It was concluded that *sth-1* and *sth-2* are clearly at

Chromosome I

Chromosome V

FIGURE 2.—Genetic map locations of the *sthA* and *sthB* loci. Quoted linkages, shown in parentheses, are from KÄFER (1958, 1977), CYBIS *et al.* (1988) and CLUTTERBUCK (1969) and are summarized in (CLUTTERBUCK 1993).

separate loci which are therefore designated *sthA* and *sthB*, respectively.

In preliminary meiotic crosses *sthA1* was found to be linked at 6% recombination to *pabaA* on the right arm of chromosome I. Ad⁺ progeny were selected from a cross between DHG90 (relevant markers *medA17*, *sthA1*) and G141 (*adF9 pabaA1*); out of 57 progeny, 2 were Paba⁻, and both of these were also Sth⁺, indicating that *sthA* is to the right of *pabaA*.

Crosses between *sthB2* strains and strains carrying markers on chromosomes V and VII, showed no linkage of *sthB* to *nicB*, *wetA* or *malA* (chromosome VII) or to *lysB*, *facA* or *riboD* (chromosome V). On the other hand *sthB2* was found to give only 22% recombination with *nicA* on chromosome V (see cross 6, Table 7).

Allelism tests for further mutants: Heterokaryons between *sthA1* and *sthB2* strains (DHG76 and DHG88), both in *medA* backgrounds, had the Ultramedusa aconidial phenotype, which indicated that complementation between *sthA* and *sthB* was not detectable in heterokaryons. Heterokaryons would therefore be unsuitable for complementation tests between further *sth* mutants. The oligoconidial phenotype of *medA* mutants would also make it difficult to isolate diploids in this background, and furthermore, *medA* mutants are self-sterile so that direct linkage tests between *medA sth* strains were ruled out. For these reasons subsequent sthenyo mutants were first tested for linkage to markers already shown to be linked to *sthA* and *sthB*.

The results of such tests, summarized in Table 4, suggest that *sth-67*, *sth-69* and possibly *sth-70* are all in the same map region as *sthA1*, and that *sth-68* shows the same linkage relationships as *sthB2*. On the other hand, *sth-65* and *sth-66* are linked to neither chromosome I nor chromosome V markers, so these mutants probably represent at least one additional locus. A mass plating of a cross of constitution *medA*⁺ *sthA1* (X4a6) × *medA13 sth-69* (DHG69) gave no progeny of unmodified medusa morphology, implying that *sth-69* is closely linked to *sthA1* and that these two mutants are probably allelic. This result also implies that *sthA1* interacts with *medA13*

TABLE 2
Segregation of Medusa and Ultramedusa morphologies in crosses 1-3

Cross	Parent strains and morphology		Progeny				
	P1	P2	Wild type	Medusa	Ultra-medusa	$\chi^2(1)^a$	$\chi^2(2)^b$
1	DHG72 Ultramedusa	G0112 Wild type	40	20	12	0.8	2.0
2	DHG60 Ultramedusa	G0112 Wild type	36	9	27	0	4.5*
3	X2a80 Wild type	DHG54 Medusa	44	17	20	0.6	0.2

For full genotypes of parent strains see Table 1. P1 in cross 3 is a wild-type morphology segregant from cross 2.

^a $\chi^2(1)$ tests the fit of the wild type: (medusa + ultramedusa) ratio to 1:1.

^b $\chi^2(2)$ tests the fit of the medusa: ultramedusa ratio to 1:1. * = significant at the 0.05 probability level; the remaining χ^2 values are non-significant.

to give the same phenotype as that seen in the *medA17* background where it was isolated. Similarly, no plain medusa progeny were obtained from a cross between strains of constitution *medA*⁺; *sthB2* (X2a80) and *medA13*; *sth-68* (X9a12), implying *sth-68* should, at least provisionally, be regarded as an *sthB* allele.

Linkage mapping: Table 5 gives details of cross 4 which shows the linkage of *sthA* to a series of chromosome I markers. The most obvious location of *sthA* is between *pabaA* and *yA* as indicated in Figure 2. This position is confirmed by the three point analysis shown in Table 6.

Mapping *sthB* proved less straightforward. Since hap-

loidization suggested that *sthB2* strains might carry a V-VII translocation, *sthB68* was analyzed in parallel with *sthB2*. In the event, no clear difference in the behavior of *sthB2* and *sthB68* was seen. Results of a series of crosses are summarized in Table 7. *sthB* is clearly linked to *cysA*, *nicA* and *lysB*, and is more distant from *facA* and *riboD*. Three point analysis of crosses 7 and 11-14 in which more than one of these markers was segregating failed to give a clear order: in no case were members of one recombinant class rare enough to indicate that this was the double recombinant class. The *cysA* marker should be able to test the location of *sthB* relative to *nicA* and *lysB*; however, it is common for crosses involving

TABLE 3
Classification of haploids from the diploid MSF + DHG88

Haploid	Chromosome markers													
	II <i>acrA</i>		III <i>galA</i>		IV <i>pyroA</i>		V <i>facA</i>		VI <i>sB</i>		VII <i>nicB</i>		VIII <i>riboB</i>	
	s	r	+	-	+	-	+	-	+	-	+	-	+	-
<i>medA</i> ⁺ <i>sth</i> ⁺	4	8	3	9	6	6	0	12	7	5	0	12	7	5
<i>medA</i> ⁺ <i>sth</i> ⁻	7	5	8	4	6	6	12	0	5	7	12	0	6	6

See Table 1 for parental genotypes. For auxotrophic markers, "+" = wild type, "-" = mutant. For *acrA*, "r" = resistant, "s" = sensitive. Only *medA*⁺ haploids were classified and all were *yA*⁺ *adF*⁻ (all three of these markers being on chromosome I).

TABLE 4
Linkage of additional *sth* mutants to chromosome I and V markers

Mutant	Crosses to G141 linkage to <i>pabaA</i> (chromosome I)			Crosses to G53 linkage to <i>nicA</i> (chromosome V)		
	Progeny	% rec.	χ^2 test	Progeny	% rec.	χ^2 test
<i>sth-65</i>	24	62 ^a	1.5	37	43	0.7
<i>sth-66</i>	70	50	0	117	51	0.1
<i>sth-67</i>	17	24	4.3*	39	44	0.6
<i>sth-68</i>	71	44	1.1	130	32	16.3***
<i>sth-69</i>	80	8	57.8***	39	59	1.3
<i>sth-70</i>	19	36	1.3	19	47	0.1

For genotypes of parents, see Table 1. χ^2 probabilities: * $P < 0.05$, *** $P < 0.001$. In the remaining cases deviations from 50% recombination (rec.) are not significant.

^a Cross to G197: linkage to *adE* (0.1% from *yA*).

TABLE 5
Classification of progeny of cross 4: DHG72 × G116

	<i>riboA</i>		<i>adG</i>		<i>proA</i>		<i>pabaA</i>		<i>yA</i>		<i>biA</i>		Total
	+	-	+	-	+	-	+	-	+	-	+	-	
<i>medA</i> ⁻ <i>sthA</i> ⁺	16	15	21	10	9	22	4	27	4	27	21	10	31
<i>medA</i> ⁻ <i>sthA</i> ⁻	44	24	52	16	59	9	59	9	64	4	5	63	68
Total	60	38	73	26	68	31	63	38	68	31	26	73	99
Recombinants (%)	40.4		37.4		18.2		13.1		8.1		15.2		

See Table 1 for parent genotypes.

TABLE 6
Three-point analysis of cross 4

Crossover interval			No. of segregants	Crossovers
<i>pabaA</i>	A <i>sthA</i>	B <i>yA</i>		
+	-	+	57	
-	+	-	23	
+	+	-	4	A
-	-	+	7	A
+	-	-	2	B
-	+	+	4	B
+	+	+	0	A, B
-	-	-	2	A, B
			99	

See Table 5 for full progeny of cross 4.

medA parents to show reduced fertility, and no further hybrid cleistothecia could be obtained from crosses involving *medA*, *sthB* and *cysA*.

Phenotypes of further mutant combinations: Hybrid cleistothecia of a cross between *sthA1* and *sthB2* strains (X4a6 and X2a80, both *medA*⁺) were plated on complete medium at 37°. No morphological variation was detected among the progeny, implying that the *sthA1 sthB2* double mutants either conidiate normally or are lethal. The latter explanation was ruled out by the normal segregation of *yA* and *biA* markers which are linked to *sthA*. The *sth*⁻ genotype of the parent strains was confirmed by control crosses to a *medA*⁻ strain (AJC11.7).

Double mutants of constitution *sth*⁻ *brlA42* were constructed by further crosses (AJC9.4 × X1a82 and X2a80). Strains carrying *brlA42* conidiate normally at 30°, but produce an aconidial, leaky Bristle phenotype at 37° [CLUTTERBUCK (1969) and Figure 1C]. It was found that whereas at 37° *brlA42* was unaffected by *sthA1*, at 30° the double mutants had a new, complex phenotype, designated "Abacoid" (Figure 1F). Here multiple layers of branched metulae developed into the "beads on a stick" phialide-like structures displayed by *abaA* mutants (Figure 1E). *sthB2* had a similar, but less pronounced, Abacoid effect than *sthA1* on the morphology of *brlA42*.

Close examination of *brlA*⁺ progeny from the cross used to obtain the *sthA1 brlA42* double mutant showed that at 30° they could be divided into two classes showing a slight difference in intensity of conidial pigmentation.

The determinant of the paler form proved to be linked to *pabaA*, which suggests that it may be *sthA1*. Microscopic examination did not reveal any morphological defect in these strains. The *sthB2* mutation did not appear to affect spore color.

A double mutant combination was also constructed with *abaA14* which is a temperature-sensitive *abaA* mutant whose phenotype is mutant at 37° but normal at 30° (CLUTTERBUCK 1969). The *abaA14 sthA1* combination, obtained from cross G01 × X1a82, was phenotypically indistinguishable at 30° from strains carrying *sthA1* alone, but when grown at 37° bore conidial heads which were slightly less compact than those of the *abaA14* control. No sign of interaction of *sthB2* with *abaA14* was detected among the progeny of the cross G01 × X2a80.

DISCUSSION

By isolating enhancers of morphological mutants which are partially blocked in conidiation, we have identified a number of new conidiation loci whose mutants had not been picked up by mutational screening of the wild type (CLUTTERBUCK 1969; MARTINELLI and CLUTTERBUCK 1971).

The frequency of outgrowths on both *medA* and leaky *brlA* colonies suggested that these strains might be particularly mutable, either in general, or perhaps for specific loci, during conidiation. However, while the frequency of sthenyo mutations on *medA* plates was quite high, it may be at least partially accounted for by the existence of more than one *sth* locus, and it is also probable that the conspicuousness of the proliferating outgrowths contributes to the apparent high frequency of mutation.

Our original hypothesis was that failure to identify morphogenetic responder genes in the past might be because such genes are members of polygenic series acting in concert to bring about conidiogenesis. Mutation of any one of them might have little or no effect on the phenotype, but multiple deficiencies would act cumulatively. We have mapped two *sthenyo* loci, and have evidence that there is at least one more. Failure to observe any defect in the *sthA1 sthB2* double mutant is therefore disappointing, although it does not deny the possibility

TABLE 7
Recombination of *sthB* with linkage group V markers

Cross	Parents		Percent recombination with <i>sth</i>					<i>sth</i> ⁺ :- ratio	Progeny analyzed	
			<i>cysA</i>	<i>nicA</i>	<i>lysB</i>	<i>facA</i>	<i>riboD</i>			
<i>sthB2</i> crosses										
5	DHG77	G523	15					17:30	47	
6	DHG77	G53		22				64:40	104	
7	DHG77	G54		16		39	52	40:17**	57	
8	X7.b23	G52		13	11	32	48	18:28	46	
<i>sthB68</i> crosses										
9	DHG68	G54		29		27	31	12:33**	45	
10	DHG68	G52			26			8:19	27	
11	X10a12	G523	22		19			10:22*	32	
12	X10b9	G54		13	8.7	23	26	42:27	69	
13	X9a12	G52		6.9	6.9		45	26:32	58	
14	X9b17	G52		19	19			27:22	49	

For genotypes of parents see Table 1.

* Deviation of *sth*⁺:*sth*⁻ ratio from 1:1 significant at 0.05 probability level; ** significant at 0.01 probability level.

that more complex combinations might give a recognizable phenotype. This contrasts with a case in *Caenorhabditis elegans* where two unlinked mutants, *lin-8(n111)II* and *lin-9(n112)III*, each of which is silent on its own, in combination confer a Multivulva phenotype (HORVITZ and SULSTON 1980; FERGUSON and HORVITZ 1985).

It is possible that a polygenic series might consist of genes of related biochemical function such as the set of three chitin synthase genes in *Saccharomyces cerevisiae*, where deficiencies in one gene can be partially masked by the activities of other members of the series (SHAW *et al.* 1991; VALDIVIESO *et al.* 1991). However, in the development of a structure as complex as the *A. nidulans* conidiophore (TIMBERLAKE 1991b; CLUTTERBUCK and TIMBERLAKE 1992) interacting polygenes need not be related in biochemical function; *e.g.*, the precise morphology of metulae might be determined collectively by numerous genes encoding cell wall structural components as well as enzymes involved in their synthesis and assembly.

The role of *sth* genes in conidiation can best be considered by relating them to the intensively studied genes *brlA*, and *abaA*, and to *medA*. The *brlA* gene is essential for conidiation (CLUTTERBUCK 1969), and there is compelling evidence (ADAMS *et al.* 1988; TIMBERLAKE 1991b; CHANG and TIMBERLAKE 1992) that it is a developmental regulator. The phenotypes of *brlA* mutants can be graded on a scale of "leakiness" on the supposition that each increment in *brlA*⁺ activity induces responder genes that allow conidiophore development to proceed to the next stage (CLUTTERBUCK 1969). In the absence of such an increment, subsequent growth will lead to repetition of the morphology of the current stage.

Medusa mutants present a morphology rather similar to *brlA* leaky mutants which are able to form normal vesicles and metulae, but are delayed in progression to the phialide stage, instead producing multiple layers of metulae. CLUTTERBUCK (1969) isolated 12 medusa mutants, all of which displayed a similar oligo-

conidial phenotype but MARTINELLI (1979) was able to distinguish slight differences in severity of 4 *medA* alleles, particularly in combination with other morphological mutants. No *medA* mutants isolated in this or previous studies were completely aconidial, suggesting that the *medA* null phenotype is probably oligoconidial. *medA11* is the first temperature sensitive mutant reported, other *medA* mutants showing a more serious deficiency at lower temperatures.

sth mutants enhance the *medA* defect, delaying morphological progression indefinitely. They also interact with the *brlA42* mutant, but in this case have no effect on morphology at 37°, when morphogenesis is blocked at the vesicle-metula stage. This suggests that *sth* genes are inactive at this stage. However at 30° when the *brlA42* on its own is morphologically normal, the *brlA42 sth* double mutant appears to repeat the phialide stage, giving a phenotype that we describe as "Abacoid" because of its similarity to that given by *abaA* mutants. One implication is that although the *brlA42* mutant is apparently morphologically normal at this temperature, it is in fact cryptically defective in phialide determination.

Secondly we conclude that the *sth* genes, like *abaA*, probably act mainly at the phialide stage of conidiophore development (CLUTTERBUCK 1969; MIRABITO *et al.* 1989; SEWELL *et al.* 1990). Late action of *sth* genes is consistent with the failure of *sthA* and *sthB* mutants to complement in heterokaryons. The latest stage in conidiation at which nuclei of different genotypes could interact is in the multinucleate vesicle, *sth* gene action therefore occurs later than this, in the uninucleate sterigmata, and noncomplementation of *sth* mutants implies that their products cannot diffuse between sterigmata.

The above description of *brlA* action implies that its control of developmental progression depends on increasing activity of this gene in successive stages. While recent molecular evidence gives support for this idea (TIMBERLAKE 1991b) it is also possible that if the *BrlA* protein is the primary regulator, its different activities in

vesicles, metulae and phialides are due to successive modifications of this protein, or to a varying sequence of effector molecules. The *medA* and/or *sth* loci might be responsible for these modifying influences; they might code for additional DNA-binding proteins which interact with BrlA at particular sites, or they might be structural genes responding to BrlA induction.

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