Phenotypes of Double Conidiation Mutants of Aspergillus nidulans

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A series of strains, doubly mutant at conidiation loci, have been made. The phenotypes of these strains reflected the epistasy of earlier blocking mutants over later ones and confirmed the order of gene sequence predicted from the phenotypes of single mutants. Oligosporogenous mutants gave complex interactions, especially between brl and med mutants.

These results indicated that (i) gene action overlapped in time, (ii) several parts of the conidial apparatus were interchangeable and (iii) nuclei leaving the vesicle were not irreversibly programmed. Structures produced by mutants were reminiscent of the conidial apparatus of other Aspergillus species and of related genera.

INTRODUCTION

Several characterized conidiation mutants isolated by A. J. Clutterbuck and others (Clutterbuck, 1969, 1976, 1977) were used to construct double mutant strains. Although the phenotype of the double mutants could be mainly predicted from the phenotypes of the single mutants, interesting epistatic interactions were expected which could add to knowledge of the gene functions and more precisely define the order of gene action. In metabolic pathways, mutations in genes acting early are epistatic to those in genes acting later in the pathway, hence double mutants may on superficial examination resemble the earlier blocked mutant. The same effect was expected in pathways of morphological development and has already been shown for spore formation in Bacillus subtilis (Coote & Mandelstam, 1973). Double mutants have also been used, for example, in the elucidation of T4 phage maturation and assembly (Levine, 1969).

METHODS

General methods were those of Pontecorvo et al. (1953).

Organisms. Conidiation mutants were kindly supplied by A. J. Clutterbuck, Glasgow, and are described in Table 1.

Media. Minimal and complete solid media and their supplements were those described by Pontecorvo et al. (1953) as modified by Cove (1966).

Characterization of double mutants. In crosses where one mutation was completely epistatic over another, a progeny ratio M1:M2:wild-type of 2:1:1 was obtained (where M1 is the morphology of the epistatic mutant and M2 is the morphology of the hypostatic mutant). Since the double mutants were necessarily indistinguishable from one of the single mutants, a selection of six M1-type progeny was out-crossed to the wild-type to identify double mutant progeny. In other crosses, double mutant progeny were clearly different from either parent giving an overall ratio M1:M2:M1M2:wild-type of 1:1:1:1. In these, out-crossing of progeny was performed as a check.

The double mutants were characterized for colony morphology and pigmentation after growth at 37 °C (2 d) and in some cases also at 25 °C (5 d). Conidial head morphology was examined and photographed after
Table 1. Properties of conidiation mutants

Only type mutations cited in the text are described.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Morphological phenotype</th>
<th>Conidiation* at 37 °C</th>
<th>Colour of conidiophore†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>stuA1</strong></td>
<td>(stunted). Short conidiophores. Poor subsequent development</td>
<td>Oli Brown</td>
<td></td>
</tr>
<tr>
<td><strong>brlA1</strong></td>
<td>(bristle). Conidiophore growth continues without developing into a vesicle, etc.</td>
<td>Asp White</td>
<td></td>
</tr>
<tr>
<td><strong>apsA6</strong></td>
<td>Anucleate primary sterigmata or metulae which develop no further. A few receive nuclei and are normal</td>
<td>Oli Brown</td>
<td></td>
</tr>
<tr>
<td><strong>apsB8</strong></td>
<td>Ivory. Unpigmented conidiophores, metulae, phialides</td>
<td>Normal White</td>
<td></td>
</tr>
<tr>
<td><strong>ivoA51</strong></td>
<td>(ivory). Unpigmented conidiophores, metulae, phialides</td>
<td>Normal White</td>
<td></td>
</tr>
<tr>
<td><strong>ivoB63</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>medA15</strong></td>
<td>(medusa). Proliferation of metulae before phialide formation</td>
<td>Oli White</td>
<td></td>
</tr>
<tr>
<td><strong>abaA1</strong></td>
<td>(abacus). Abacus-like chains of undifferentiated cells formed by apical growth</td>
<td>Asp Grey</td>
<td></td>
</tr>
<tr>
<td><strong>wetA6</strong></td>
<td>(wet-white). Apparently normal conidium formation but conidia do not pigment and autolyse</td>
<td>Oli Brown</td>
<td></td>
</tr>
<tr>
<td><strong>yA1</strong></td>
<td>Yellow conidia, lacks p-diphenol oxidase</td>
<td>Normal Brown</td>
<td></td>
</tr>
</tbody>
</table>

* Oli, Oligosporogenous, i.e. fewer conidia than wild-type; Asp, asporogenous, i.e. no conidia.
† This pigmentation may be masked in conidiating strains by coloured conidia.
‡ Illustrated in Fig. 2(j).

RESULTS

Phenotypes of double mutants containing the asporogenous mutation brlA1

The brlA1 (bristle) mutation is completely unleaky. Double mutant strains constructed with this mutation and mutations presumed to block later stages in the developmental pathway, i.e. **apsA6**, **apsB8**, **medA15**, **abaA1**, **wetA6**, were like **brlA1** strains in every respect. **brlA1** was thus epistatic to all these mutations and the **brl** gene presumably acted before them.

Phenotypes of double mutants containing the oligosporogenous mutations stuA1, brlA42, apsA6, apsB8, medA15 and wetA6 and asporogenous abaA1

Each of the oligosporogenous mutants allows some later development culminating in most cases in the production of some viable conidia. In most of these crosses, the double mutant progeny were clearly recognized.

**stuA1** is the type mutation in the **stu** gene (**stunted**) and apparently produces conidia directly on the vesicles. After repeated attempts only two other mutations were successfully introduced into **stuA1** strains; these were **medA15**, the medusa type-mutation, and **brlA42**, a temperature-sensitive mutation in the **brl** gene (Fig. 1, Table 2). The morphology of the progeny from these two crosses showed the ratio 2 **stunted**: 1 **medusa or bristle**: 1 wild-type. Microscopically, it was possible to identify **stuA1**, **brlA42** double mutants by their stunted bristles but **stuA1**, **medA15** strains were only identified by out-crossing and thus resembled **stuA1** strains. **stuA1** modified the growth of bristles produced by the **brlA42** mutation by altering the normally forked bristle of **brlA42** to a simple bristle, like that produced by...
Conidiation mutants

Conidium

Phialide

Metula

Vesicle

Conidiophore

Foot cell

Branching chains of metulae

Young

medA27

Old

medA26

Conidial mass

Chains of metulae

Bristle

Fig. 1. Diagrams of single conidiation mutants of *Aspergillus nidulans*. All are drawn to the same scale, except *brlA1* which should be seven times the normal conidiophore length and old *medA26* which is reduced.
**brlA1.** *stuA1* was epistatic to *medA15* and, by inference, to all mutations in genes acting later in the pathway, but *stuA1, medA15* strains were unpigmented like single *medusa* mutants.

*apsA6* and *apsB8* (anuclear primary sterigmata or metulae), the type mutations at the two *aps* loci, are affected in nuclear division and/or migration. Only some of the metulae on the conidial vesicle receive nuclei and can develop further to form phialides and conidia (Clutterbuck, 1977). In crosses with *abaA1* (*abacus*) or *wetA6* (*wet-white*) (Fig. 2j), the double mutant progeny colonies were not clearly distinguishable from those of *apsA* or *apsB* strains until examined microscopically. The progeny ratios were 1 *aps:1 double mutant:1 abacus or wet:1 wild-type. In crosses with *medA15*, the double mutants were identified by their pale colour and *aps* appearance, contrasted with the brown *aps* colonies and white *medusa* colonies; hence an overall ratio of 1:1:1:1 was obtained. Microscopically, conidial heads from *wetA6, apsA/apsB* colonies looked mostly like *aps* except where enough conidia were produced to see a slimy aggregation of conidia from several adjacent chains. However, in *medA15, apsA/apsB* and *abaA1, apsA/apsB* strains, both phenotypes were clearly visible, the poor *aps*-like conidial heads bearing a few chains of metulae as in *medusa* strains or bearing *abacus*-like chains. Hence the oligosporogenous mutations *apsB8* and *apsA6* allowed partial expression of genes whose activity was expressed later in the pathway, i.e. *abaA1, medA15* and *wetA6.*

*apsA6* and *apsB8* mutants were crossed giving a progeny ratio of 3 *aps:1* wild-type. Closer examination revealed a ratio of 2 *apsA:1 apsB:1* wild-type morphology. The double mutant resembled the rather more extreme *apsA6* mutant (Fig. 1).

In crosses between *medA15* and *abaA1* or *wetA6*, superficial phenotypic ratios of 2 *medusa:1 abacus or wet:1* wild-type were obtained, reflecting the partial epistasy of *medA15* over the other mutations. Double mutants, however, developed no conidial colour since either *abaA1* or *wetA6* alone prevented the expression of conidial pigment and *medA15* prevented the expression of brown–grey conidiophore pigment. Conidial heads of *medA15, abaA1* strains were interesting since they bore long chains of metulae followed by intermediate cells then *abacus* chains, clearly showing the expression of both mutations (Fig. 2d). The *med* gene clearly expressed itself before the *aba* gene. *medA15, wetA6* double mutants also showed *medusa* conidial heads, but with normal chains of conidia replaced by an autolysing sticky mass of white conidia. The *wetA6* mutation was presumably expressed later than *medA15.*

Double mutants synthesized from the asporogenous mutation *abaA1* and *wetA6* were indistinguishable from *abaA1* strains hence *abaA1* was epistatic over *wetA6*, and the *aba* gene acted prior to the *wet* gene.

**Interactions between several brl and med alleles**

Even in maturity, all known *medusa* mutants have pale or unpigmented conidiophores, vesicles, metulae and phialides. Since double mutants of *medA15* with either *abaA1* or *aps* or *stuA1* mutations also lacked this pigment, it was interesting to look at the interaction between four *med* alleles and four *brl* alleles including leaky *brl* alleles which normally make pigment. In addition, the morphology of leaky *brl* and *med* mutants is so similar that *medusa* was originally thought to represent another type of *bristle* mutation (A. J. Clutterbuck, personal communication).

Each of four *brl* alleles, *brlA1, 9, 7, 42,* was crossed with each of four *med* alleles, *medA15, 26, 27, 30.* The *bristle* mutants represent a series of modifications ranging from unleaky *brlA1* to very leaky *brlA42* (Clutterbuck, 1969). The four *medusa* mutants are all similar. In some cases, double mutants were identified by their unique morphology and in all cases by out-crossing the putative double mutant to the wild-type. These double mutants have been classified for colony morphology, pigment and conidial head structure. The results are summarized in Table 2.
Table 2. Phenotypes of brl and med mutants and of double mutant strains

The presence or absence of structures was scored, not the quantity (but see †).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Growth temperature (°C)</th>
<th>Colour of conidiophores etc.</th>
<th>Secondary conidiophores with vesicles</th>
<th>Secondary bristles (with septa, s)</th>
<th>Long metulae or short bristles*</th>
<th>Normal-sized metulae†</th>
<th>Conidia</th>
<th>Summary of phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>37</td>
<td>Brown</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Wild-type</td>
</tr>
<tr>
<td>Any medA allele</td>
<td>37</td>
<td>White</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Wild-type</td>
</tr>
<tr>
<td>brlA1</td>
<td>37</td>
<td>White</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Unleaky bristle</td>
</tr>
<tr>
<td>brlA9</td>
<td>25</td>
<td>Brown</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Unleaky bristle</td>
</tr>
<tr>
<td>brlA7</td>
<td>37</td>
<td>Brown</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Leaky bristle</td>
</tr>
<tr>
<td>brlA42</td>
<td>25</td>
<td>Brown</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Leaky bristle</td>
</tr>
<tr>
<td>brlA1, with medA alleles</td>
<td>37</td>
<td>White</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Unleaky bristle</td>
</tr>
<tr>
<td>brlA9, with medA alleles</td>
<td>25</td>
<td>Pale brown</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Unleaky bristle</td>
</tr>
<tr>
<td>brlA7, medA15 or medA30</td>
<td>37</td>
<td>Almost white</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Leaky bristle with weak medusa expression</td>
</tr>
<tr>
<td>brlA7, medA26 or medA27</td>
<td>37</td>
<td>Almost white</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Leaky bristle with strong medusa expression</td>
</tr>
<tr>
<td>brlA7, medA26 or medA30 or medA27§</td>
<td>25</td>
<td>Almost white</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Bristle and medusa</td>
</tr>
<tr>
<td>brlA42 with medA alleles</td>
<td>37</td>
<td>White</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Weak bristle and very strong medusa</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>White</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

* It is difficult to distinguish between the two possibilities unless the initial cell gives rise to another one either by budding (metula) or by septation (bristle). Length varies from 1-5 to 5 times that of normal metula.
† Wild-type has one row of metulae (designated +); some leaky bristle strains produce more than one row of normal-sized metulae without becoming medusoid (+ +); medusa expression is recognized by many branching chains of metulae (+ + +).
‡ brlA9 is structurally identical with brlA1 at 37 °C, therefore unleaky, but it produces pigmentation characteristic of most leaky bristle mutants.
§ brlA7, medA15 was not tested at 25 °C.
Double mutants containing *brlA1* and any of the four *medusa* alleles were all identical with *brlA1* strains in producing stiff, white, elongated bristles instead of conidiophores, etc. *brlA1* was completely epistatic to all the *med* alleles tested.

The situation was much more complicated in the case of leaky *brl* mutations. All the latter when present as single mutants produced a brown pigment in their conidial apparatus thus colouring the whole colony brown. When combined with any *med* allele, the colonies and conidial heads had little or no pigmentation. This reduction in pigment was always visible after growth at 25 °C, but in some cases the colonies were intermediate in colour at 37 °C. Since lack of pigment is a characteristic of *med* mutations, *med* alleles were epistatic over leaky *brl* alleles in this respect.

The conidial apparatus seen on colonies of leaky *bristle* type was very variable, hence it is not possible to illustrate typical *brlA9*, 7, 42 structures (but see Table 2). Each *brl* strain and each double mutant was examined after growth at 37 and 25 °C. The strains were scored for the presence or absence of normal conidiophores, vesicles, metulae, phialides and conidia and for various abnormal structures such as bristles (Fig. 1), secondary conidiophores (Fig. 2e), secondary bristles (Fig. 2g), medusa-like proliferation of short cells (Figs 2f, i, k) and various types of longer cell (Fig. 2h) or short bristle. In several cases, more than one structure arose from one vesicle. Seldom were the cells growing from the vesicle recognizable as metulae and phialides.

All single and double mutant strains carrying the *brlA9* mutation were identical in having long brown bristles with no further development at 37 °C, but double mutants were paler brown. The expression of *med* alleles was visible in double mutants with *brlA7* or *brlA42* grown at 37 °C since either a medusa-like chain of cells or secondary bristles divided by cross-walls at frequent intervals were visible (Fig. 2e). The overall morphology strongly resembled that of the respective *brl* allele, but colonies were pale or unpigmented reflecting *med* action.

The expression of *med* mutations was more obvious at 25 than 37 °C and increased with the leakiness of the *brl* alleles. The *brlA9* mutation was leakier at 25 °C than at 37 °C and produced vesicles, secondary conidiophores, secondary bristles and several rows of normal-looking metulae (Fig. 2i). Double *brlA9*, *med* mutants possessed these characters at 25 °C and, in addition, either medusa-like chains of cells or divided bristles, characteristic of *med* expression (Fig. 2k).

The *brlA7* mutation was leakier than *brlA9* at both temperatures since *brlA7* strains bore vesicles with secondary conidiophores (Fig. 2e) and secondary bristles divided by cross-walls. Cells resembling short bristles or long metulae were also produced (Fig. 2h). At 25 °C, some normal metulae and phialides were formed (Fig. 2i). *med* mutations barely influenced the development of *brlA7* strains at 37 °C except for the possession of a few medusa-like chains of cells in the double mutants. At 25 °C, *med* mutations exercised greater influence by producing obvious medusa-like chains of cells from vesicles, in addition to some structures characteristic of *brlA*.

The *brlA42* mutation was very leaky at 37 °C and gave rise to wild-type morphology at 25 °C. While having typical leaky *bristle* structures, it also displayed slight *medusa* character at 37 °C. The addition of *med* mutations to these strains was very dramatically expressed after growth at 25 °C when conidial heads were almost completely medusoid (Fig. 2f). At 37 °C, conidial heads expressed both mutations.

Since the appearance of colonies depended on the type of conidial apparatus present, colony morphology closely reflected the pattern of mutant expression discussed above. At 37 °C, *brlA9*, *med* strains were indistinguishable from *brlA9* strains, but at 25 °C appeared to have slightly modified leaky *bristle* morphology. *brlA7*, *med* strains most closely resembled *brlA7* strains at 37 °C but were intermediate between *brlA7* and *med* strains at 25 °C. *brlA42*, *med* strains were obviously intermediate in morphology at 37 °C and rather medusa-like at 25 °C.
Conidiation mutants

All the med alleles used gave rise to similar morphological aberrations. At 37 °C, they could be classified quite early for their medusa character of producing several rows of metulae before normal phialide formation (Fig. 2c). At 25 °C, they all resembled a form intermediate between medusa at 37 °C and very leaky bristle (Fig. 2a). Conidiation was extremely poor at 25 °C compared with 37 °C. Some strains produced secondary conidiophores with medusoid metulae at 25 °C (Fig. 2b). There were no consistent differences in the effects of these alleles on the expression of brl mutations.

In summary, double mutants most closely resembled their brl mutation in expression at 37 °C, but at 25 °C medusa characters were seen increasingly clearly with progressive leakiness of the brl allele. There was, therefore, no clear-cut epistasy of one group of mutants over the other.

DISCUSSION

The epistasy of the asporogenous mutant brlA1 over apsA6, apsB8, abaA1, wetA6 and four med alleles and that of asporogenous abaA1 over wetA6 is consistent with the predicted order of gene expression in this developmental pathway, and confirms the usefulness of this approach. Within the crosses performed in this study, complete epistasy of brl, aba and wet mutations over conidial colour mutations cha, fwn, w, y and yg was also observed. The order of expression is brl, aps and med, aba, wet.

The time of expression of genes which give oligosporogenous mutations is more difficult to interpret. stuA1 strains have shortened conidiophores, but their form is determined by the brl allele. The stuA gene must therefore act over the same period as the brlA gene; however, brlA42 is not phenotypically leaky in a stuA1 background but produces simple bristles like brlA1. This could imply an overlap in gene function. stuA1 is epistatic over medA15 and by inference over apsA, apsB, abaA and wetA, but not over colour mutations. Mutations in genes apsA and apsB also give rise to an oligosporogenous phenotype. Since med, aba and wet mutations appear to alter events occurring later than the formation of the initial metulae, their expression in cells produced from the few nucleated metulae of aps strains is only to be expected. The intermediate phenotypes illustrate the action of aps before that of med, aba or wet.

The most complex series of phenotypes was observed in strains constructed from one of four medA alleles which are oligosporogenous and one of three leaky brlA alleles. Generally, the double mutants most strongly resembled the bristle parent at 37 °C and had medusoid morphology at 25 °C. The exceptions to this were brlA9, medA strains in which brlA9 was epistatic over medA alleles for structural phenotype. These results are consistent with the leaky bristle mutants being more extreme at 37 °C and the medusa mutants at 25 °C. The complex phenotypes are found because neither mutant completely blocks development and growth, and because the action of the two genes must overlap in time since both are concerned with metula and phialide production. The overall effect of medA alleles on brlA alleles is that of increasing septation either in secondary bristles or by encouraging metula proliferation. The overall shape of the brlA conidial apparatus is, however, maintained. Clearly, brlA action begins before that of medA and provides a basic structure for med alleles to modify. The possession of multiseriate metulae by leaky bristle strains and that of secondary conidiophores by medusa strains indicates a large degree of overlap in gene function, as well as time of action. Indeed, Clutterbuck (1969) originally expected medusa mutations to map at the bristle locus. The more variable results obtained with combinations of brlA7 and medA is probably a reflection of the delicate balance in gene expression between two oligosporogenous mutants. Repeated observation ruled out the possibility of experimental error in this matter.

Interactions of conidiation mutants also produced an interesting effect on pigmentation. The introduction of any medA allele into a strain carrying any apsA, apsB, abaA, stuA or leaky brlA mutation inhibited pigmentation of these mutants. All normally form a grey-
Fig. 2. Characteristic structures produced by some single and double conidiation mutants of
A. nidulans. (a) medA26 at 25 °C showing medusoid proliferation of metulae. (b) medA26 at 25 °C showing secondary conidiophores. (c) medA30 at 37 °C. (d) abaA1, medA15 at 37 °C showing medusa and abacus features. (e) brlA7, medA27 at 37 °C showing secondary conidiophores and medusoid character. (f) brlA42, medA27 at 25 °C showing strong medusoid expression. (g) brlA9, medA15 at 25 °C showing metulae-bristle transitions. (h) brlA7, medA30 at 37 °C showing long metulae or short bristles. (i) brlA7 at 25 °C showing bristle proliferation of metulae. (j) wetA6 at 37 °C showing autolyzing conidia. (k) brlA9, medA30 at 25 °C showing medusoid expression.

Abbreviations: m, metulae; cp, conidiophore; c, conidia; p, phialide; a, abacus-like cell; b, bristle. Bar markers represent 50 μm.
S. D. MARTINELLI

Colour genes except stuA ivoB bdA niedA

CONIDIOPHORE

PHIALIDES

METULAE

VESICLE

CONIDIOPHORE

Fig. 3. Diagrammatic representation of gene expression during conidial development of Aspergillus nidulans. The sequence of action is shown along the horizontal axis and the period of gene action is shown vertically: ——, genes essential for production of viable conidia; ——, non-essential genes; ———, conjectural.

brown pigment in the conidial apparatus. This synthesis depends on the presence of wild-type alleles in the ivoA and ivoB genes (Clutterbuck, 1977), the former being concerned with phenol production and the latter with phenol oxidase synthesis. The enzyme is called cresolase from its action on cresols in vitro. Those mutants tested, e.g. leaky brlA and abaA, have normal cresolase levels (A. J. Clutterbuck, personal communication). The lack of pigment in medusa strains has not been explained but it is not due to repression of the ivoB gene, hence an absence of cresolase. Mycelial extracts of medA15 strains contain at least wild-type amounts of cresolase and possibly excess enzyme (S. Martinelli, unpublished results; confirmed by A. J. Clutterbuck, personal communication). It is curious that this inhibition occurs in mutants which are presumably blocked at an earlier structural stage than medusa, i.e. aps, brl, stu as well as mutants blocked later, i.e. aba, wer. This may extend backwards in the time period over which the medusa gene acts.

The simplified diagram of this developmental pathway published previously (Martinelli, 1974) is no longer tenable in view of the overlapping gene action seen in this work. A diagrammatic representation of conidiation in the wild-type is presented in Fig. 3, with an indication of the order in which gene action commences and finishes.

The phenomenon of ‘mixed conidial heads’ in which one vesicle gives rise to several different structures, i.e. conidiophores, bristles, metulae, is an interesting one and shown not only by brlA and medA mutants but also by aps (A. J. Clutterbuck, personal communication). In these cases, incorrectly programmed nuclei are leaving the vesicle and instead of developing further they either revert to a previous stage (conidiophore) or produce a more extreme phenotype (bristle) or form metulae. It is tempting to consider a quantitative
explanation of gene action. The nuclei in the conidial apparatus probably never become committed to the whole conidiation process, but sequentially to small developmental steps. Under normal circumstances this programming is synchronous and cancels previous programme instructions.

Some results point to the conclusion that various parts of the conidial apparatus are dispensable and interconvertible, at least in mutant strains. _stuA1_ strains can apparently produce conidia directly from a vesicle. Either metulae and phialides are dispensable or the conidiophore is physiologically a phialide. A similar phenomenon occurs when wild-type strains are grown in submerged liquid culture (Martinelli, 1976). The conidial apparatus is reduced to a few phialides produced apically on a conidiophore, or, in conditions of carbon or nitrogen starvation, to single apical or lateral phialides. In leaky _brlA, apsA, apsB_ and _medA_ mutants, cells which resemble metulae can be converted to bristles or conidiophores (Figs 2g, h) and bristles can revert to metulae.

Both interconvertibility and dispensability could be expected from a review of conidial apparatus in the genus _Aspergillus_, as described by Raper & Fennell (1965). Within the genus both uniseriate (phialides only) and biseriate (metulae and phialides) species occur, indicating that metulae are dispensable, e.g. _A. japonicus_ and _A. carbonarius_, respectively. Vesicles must also be dispensable since _A.asper aes_ can produce two rows of sterigmata from a conidiophore lacking a vesicle, as can _A. biplanus_ from mycelium at the agar surface. In _A. cre mie us_, a complex situation exists since the conidial head changes from being uniseriate to biseriate after conidial production has started, bearing septate sterigmata at the intermediate stage. Phialides are thus converted to metulae. Other features of mutants anticipated within the genus are the long, club-shaped, occasionally septate metulae of the _A. niger_ and _A. candidus_ groups, similar to the situation in _bristle_ and _medusa_ mutants. Other genera such as _Penicillium_ have similar forms.

It was hoped initially that a study of double mutant phenotypes would add to the knowledge of gene function in this pathway. Unfortunately, these results only confirm Clutterbuck's interpretation of the genetic lesions (1969, 1976, 1977), although they perhaps emphasize some of the peculiarities of the conidiation apparatus, and the flexibility of the process.

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**REFERENCES**


