The genetics of conidiophore pigmentation in *Aspergillus nidulans*

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The grey-brown pigmentation of *Aspergillus nidulans* conidiophores depends on the functions of two 'ivory' loci. *ivoB* codes for a developmental specific phenol oxidase, and mutants accumulate its substrate N-acetyl-6-hydroxytryptophan. *ivoA* mutants are unable to make this substrate. *ygA* mutants are also poorly pigmented, and extracts require copper salts to activate both the phenol oxidase and conidial laccase. *ivoA* and *ivoB* mutants partially suppress the spore colour phenotype of *ygA* mutants. Comparisons of morphology, phenol oxidase and substrate accumulation in morphological mutants at the *brlA* locus suggest that the *brlA* protein regulates *ivoA*, *ivoB* and morphogenetic loci independently. The *medA* locus, which also affects morphology and pigmentation, may code for a modifier of *brlA* function. *abaA* mutants which are blocked at a later stage of development than *brlA* or *medA* mutants have low phenol oxidase levels, implying that by this stage of development the activity of the *ivoB* locus is declining.

Introduction

The conidiophores of the filamentous ascomycete *Aspergillus nidulans* are pigmented grey-brown. This is not conspicuous in wild-type strains, but is visible in aconidial mutants (Clutterbuck, 1969) such as 'abacus' (*abaA*) or 'bristle' (*brlA*), in which mutant derivatives of the conidiophores, metulae and phialides are exposed. 'Ivory' (*ivo*) mutants, which have lost this pigment, have been isolated in *abaA* or *brlA* mutant backgrounds (Clutterbuck, 1969) and details of their properties and interactions with other developmental mutants are presented here. A specific phenol oxidase (AHTase), lacking in *ivoB* mutants, is described in the accompanying paper (Birse & Clutterbuck, 1990a) and its substrate has been identified as N-acetyl-6-hydroxytryptophan (AHT; McCorkindale et al., 1983).

Summaries of this work have previously been included in reviews of *A. nidulans* conidiation (Clutterbuck, 1977, 1978, 1990a).

Methods

Media and general methods for *Aspergillus* genetics are described by Pontecorvo et al., (1953) and Clutterbuck (1974). All strains were derivatives of those in the Glasgow collection (Clutterbuck, 1986), and all carried the *veA1* mutation. *ivo* mutants were induced in *brlA abaA6*, *brlA abaA20* or *brlA brlA42* strains, using ultraviolet light or *N*-methyl-*N*-nitro-*N*-nitrosoguanidine as described by Clutterbuck (1969). Other genetic markers are listed in Clutterbuck (1990b). Top layer cultures on complete medium agar (Clutterbuck, 1972) were used for the growth of mycelium for extraction of AHT and AHTase: 9 cm plates containing 20 ml of agar medium were overlaid with 4 ml of liquid complete medium containing an inoculum of 10^6 conidia or ascospores per dish. Plates were incubated on a level incubator shelf at 37 °C.

AHT was extracted as described by McCorkindale et al., (1983), and assayed using the acid-diazoitated sulphanilic acid test for 6-hydroxyindoles (Jepson, 1960; Jepson et al., 1962). A 0-1 ml volume of a 5% (w/v) aqueous solution of NaNO2 was added to 0-1 g sulphanilic acid dissolved in 1 ml concentrated HCl, and left for 5 min on ice. Excess nitrous acid was destroyed by adding a crystal of ammonium sulphamate. Then 0-1 ml of the reagent was added to 2-8 ml 1 M HCl and 0-1 ml of the aqueous extract to be assayed and left for 20 min in the dark. \( A_{530} \) was read in a Pye--Unicam spectrophotometer, subtracting \((0-55 \times A_{420} + 0-45 \times A_{420})\) background. Results are expressed as total absorbance per dish. Colonies on plates were tested for 6-hydroxyindoles by overlaying with filter paper soaked in the reagent.

AHT and other indoles were detected by paper chromatography of methanolic extracts of dried mycelium (Jepson, 1960), using butanol/acetic acid/water (60:15:25 by vol.) solvent and stained with Ehrlich's reagent or diazotized sulphanilic acid.

AHTase is an extracellular enzyme, extractable by shaking mycelium with buffer. This procedure, and the colorimetric assay using hydroquinone monomethyl ether as substrate, are described by Birse & Clutterbuck (1990a). In the *medA/medA* comparison 10 mM phosphate buffer (pH 7-0) was substituted for ammonium tartrate. Tests of AHTase thermostability and electrophoretic mobility are also described by Birse and Clutterbuck (1990a).
Results

Ivo mutants

A total of 200 ivo (‘ivory’ – unpigmented conidiophore) mutants have been isolated in A. nidulans as described in Clutterbuck (1969), starting with an abaA or brlA42 aconidial background in which the conidiophore is directly visible. The ivo mutants had no evident effect on either growth or morphology.

Complementation tests performed by stabbing strains close together on complete medium divided the mutants into two main classes: ivoA and ivoB. ivoA mutants were approximately seven times commoner than ivoB. A third class of partially defective mutants failed to complement in diploids with ygA6. This mutant is characterized by yellow-green spore pigment, and is believed to be deficient in copper distribution (Clutterbuck, 1972).

The pattern of pigmentation at the junctions of complementing colonies suggested that a diffusible material accumulated in ivoB mutants would cross-feed, and produce pigmentation in ivoA strains. We have purified this material and identified it as N-acetyl-6-hydroxytryptophan (AHTase) which converts the substrate to a melanin in an aconidial background in which the conidiophore is directly visible. These data have been summarized by Clutterbuck (1990).

Genetics of ivoA, ivoB and ygA mutants. Fig. 1 represents regions of the A. nidulans linkage map to which these three mutations have been located. These data have been summarized by Clutterbuck (1990b).

The adD to ygA linkage was determined using two new ygA alleles (ygA7 and ygA8) induced in an adD3 strain. The resulting strains were crossed with a trpA strain, selecting for ad+ (Clutterbuck, 1981). Five out of more than 5000 colonies were yellow-green, and four of these were trpA, implying that trpA and ygA are on opposite sides of adD.

The ivoB to ureD linkage was obtained from a cross between the two single mutants in which no recombinants were obtained among 400 fully analysed colonies plus more than 1000 colonies plated selectively for ureD4.

The ivoB locus. These mutants lack AHTase and accumulate its substrate, AHT (Table 1). Evidence that ivoB is the structural gene for AHTase was provided by a temperature-sensitive mutant ivoB192 which had an ‘ivory’ phenotype at 37 °C, but was more or less wild-type at 25 °C. This mutant was shown to be allelic to other ivoB mutants by complementation and recombination tests, and enzyme extracts from the mutant were more thermostable than wild-type enzyme (Birse & Clutterbuck, 1990a).

IvoB mutants accumulate AHT (see Fig. 4), and to a lesser extent small quantities of a compound tentatively identified as 6-hydroxytryptophan (McCorkindale et al. 1983). Attempts were made to alter AHT accumulation by ivoB strains by supplementing the medium: acetate, leucine, anthranilic acid, indole, mevalonolactone, phenylalanine and tyrosine all failed to increase AHT accumulation, and L-tryptophan and DL-N-acetyltryptophan significantly decreased AHT yield.

The ivoA locus. Mutants at this locus possess AHTase, but are assumed to be defective in biosynthesis of AHT, since they fail to accumulate it when in combination with an ivoB mutation. Chromatograms of extracts of ivoA ivoB double mutants revealed no 6-hydroxyindoles identifiable with diazotized sulphanilic acid, nor did they show any conspicuous indole staining with Ehrlich’s reagent. A spot corresponding to N-acetyltryptophan was sometimes seen, but was not conspicuous. A tryptophan spot was detectable in all extracts and sometimes appeared more intense in those from ivoA strains.

IvoA mutants have approximately 60% more AHTase activity than ivoA+ strains (compare brlA7 and brlA7

<table>
<thead>
<tr>
<th>Mutant</th>
<th>Pigment</th>
<th>AHTase</th>
<th>AHT accumulation</th>
<th>AHT accumulation in ivoB background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>ivoA</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ivoB</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>ygA</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>+</td>
</tr>
</tbody>
</table>
Genetics of Aspergillus conidiophore pigment

Linkage group III

\[
\begin{align*}
\text{str} & \quad \text{ad} & \quad \text{mo} & \quad \text{ivo} & \quad \text{al} & \quad \text{pal} & \quad \text{meth} \\
\text{40} & \quad 1.2 & \quad 27.3 & \quad 14.1 & \quad 10.5 & \quad 13.7 & \quad \text{standard error} \\
\text{zC} & \quad \pm 1.5 & \quad \pm 0.3 & \quad \pm 2.0 & \quad \pm 1.5 & \quad \pm 5.1 & \quad \pm 3.5 \\
\end{align*}
\]

\[ \text{ivoA} \quad \text{alX} \quad \text{palG} \quad \text{methH} \]

\[ \text{ivoA} \quad \text{alX} \quad \text{palG} \quad \text{methH} \]

Linkage group II

\[ \text{trpA} \quad \text{adC} \quad \text{adD} \quad \text{ygA} \]

Linkage group VIII

\[ \text{palB} \quad \text{ivoB} \quad \text{chaA} \]

\[ \text{pppA} \quad \text{ureD} \]

Fig. 1. Linkage map showing positions of ivoA, ivoB and ygA loci. The data are derived from single crosses except as indicated by numbers in square brackets. Linkage distances are percentages of recombinants ± standard error. Figures in parentheses are linkage estimates from Hankinson (1974) (in which ureD = uZ).

Table 2. AHTase activities of ygA strains assayed in the presence of copper salts

Two other ivoB mutants gave similar results to those of the brlA42; ivoB63 strain. Results are expressed as 100 \( \times \Delta A_{470} \) min\(^{-1} \) per culture dish. Figures are the means of duplicate assays.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Copper salt</th>
<th>Copper concn (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>brlA42</td>
<td>CuCl</td>
<td>51.2</td>
</tr>
<tr>
<td>brlA42; ivoB63</td>
<td>CuCl</td>
<td>3.2</td>
</tr>
<tr>
<td>brlA42; ygA6</td>
<td>CuCl</td>
<td>3.2</td>
</tr>
<tr>
<td>brlA42; ygA6</td>
<td>CuSO₄</td>
<td>3.2</td>
</tr>
</tbody>
</table>

ivoA1 in Table 3). It seems probable that in ivoA+ strains tanning destroys some of the AHTase enzyme by incorporating it into a melanin complex, but that this does not occur in the absence of substrate. A similar phenomenon has been seen with the spore pigment enzyme laccase I (Clutterbuck, 1972; Kurtz & Champe, 1982). The \( K_m \) and electrophoretic properties of the enzyme were unaffected by ivoA (data not shown).

The ygA locus. AHTase, like other phenol oxidases (Malström & Rydén, 1968), contains copper and, less typically, zinc (Birse & Clutterbuck, 1990a). Mutants at the ygA locus are partially deficient in both conidial laccase (Clutterbuck, 1972) and AHTase. It is possible to restore laccase activity to extracts of a ygA mutant (Clutterbuck, 1972), or a yB mutant (Kurtz & Champe, 1981) by addition of copper salts. Table 2 similarly demonstrates the restoration of AHTase activity to extracts of a ygA mutant by incorporation of copper salts in the assay mixture. Both cuprous chloride and cupric sulphate significantly activated the ygA extract, but not extracts of wild-type or ivoB controls. The cuprous salt, however, was inhibitory at high concentrations. The restored AHTase activity was maintained after dialysis against buffer (data not shown).

Analysis of the progeny of appropriate crosses demonstrates that ivo mutants (both A and B) partially suppressed the yellow-green conidial phenotype of ygA.
Table 3. AHTase and AHT accumulation in morphological mutants

Colonies were grown for 3 d at 37°C from point inocula on CM agar. All assays are the means of two or more replicate colonies. AHTase and AHT levels are expressed as percentages relative to brlA42. The AHTase level in the brlA42 iuoB63 strain, and in brlA1-3, is taken as background activity from other phenol oxidases and spontaneous oxidation. The differences between brlA42 medA15 and brlA42 were assessed on two independent recombinants of each genotype: the differences in both AHTase activity and AHT accumulation are significant at the 0.05 probability level. Similarly, the brlA7/brlA7 iuoA1 difference in AHTase was tested on 14 recombinants of each genotype, and was significant at the 0.001 probability level. Missing entries (-) were untested. Morphologies are coded according to the lettering of Fig. 2.

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Morphology</th>
<th>Conidiophore pigment</th>
<th>AHTase</th>
<th>AHT in iuoB63 derivative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild-type</td>
<td>e</td>
<td>+</td>
<td>16.5</td>
<td>34.3</td>
</tr>
<tr>
<td>brlA1</td>
<td>a</td>
<td>-</td>
<td>7.2</td>
<td>-</td>
</tr>
<tr>
<td>brlA2</td>
<td>a</td>
<td>-</td>
<td>7.7</td>
<td>0.0</td>
</tr>
<tr>
<td>brlA3</td>
<td>a</td>
<td>-</td>
<td>7.0</td>
<td>-</td>
</tr>
<tr>
<td>brlA4</td>
<td>a</td>
<td>-</td>
<td>56.6</td>
<td>2.9</td>
</tr>
<tr>
<td>brlA9</td>
<td>a</td>
<td>-</td>
<td>84.9</td>
<td>0.0</td>
</tr>
<tr>
<td>brlA6</td>
<td>a-b</td>
<td>+</td>
<td>99.0</td>
<td>18.3</td>
</tr>
<tr>
<td>brlA10</td>
<td>b</td>
<td>-</td>
<td>71.9</td>
<td>2.4</td>
</tr>
<tr>
<td>brlA9</td>
<td>b</td>
<td>+</td>
<td>134.4</td>
<td>43.4</td>
</tr>
<tr>
<td>brlA7</td>
<td>b-c</td>
<td>+</td>
<td>192.2</td>
<td>22.8</td>
</tr>
<tr>
<td>brlA35</td>
<td>b-c</td>
<td>+</td>
<td>157.9</td>
<td>56.1</td>
</tr>
<tr>
<td>brlA42</td>
<td>c</td>
<td>+</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>abaA1</td>
<td>d</td>
<td>+</td>
<td>25.9</td>
<td>-</td>
</tr>
<tr>
<td>abaA6</td>
<td>d-e</td>
<td>+</td>
<td>17.5</td>
<td>58.8</td>
</tr>
<tr>
<td>abaA20</td>
<td>d</td>
<td>+</td>
<td>21.7</td>
<td>81.9</td>
</tr>
<tr>
<td>brlA42 iuoB63</td>
<td>c</td>
<td>-</td>
<td>91.1</td>
<td>(100)</td>
</tr>
<tr>
<td>brlA42 medA15</td>
<td>c</td>
<td>(+)</td>
<td>192.3</td>
<td>57.2</td>
</tr>
<tr>
<td>brlA7 iuoA1</td>
<td>b-c</td>
<td>-</td>
<td>305.6</td>
<td>0.0</td>
</tr>
</tbody>
</table>

On the other hand, mutants in yA, the structural gene for conidial laccase, did not affect the conidiophore pigmentation of ygA mutants. It is possible that by abolishing conidiophore melanization, ivo mutants prevent the locking-up of copper resources by chelation. Tanning of the copper-containing AHT oxidase would also amount to a loss of copper, if this could otherwise have been recycled for use in conidial laccase. Since pigmentation of spores follows conidiophore pigmentation, it is to be expected that conidiophore pigment mutants would affect spore colour, but not vice versa.

The conidial colour of ygA strains is dependent on the pH of the growth medium, being yellow (mutant) at high pH and green (wild-type) at low pH. The conidiophore pigmentation was less obviously responsive to pH, but while the wild-type had approximately twice the AHTase activity when grown at pH 8.2 rather than pH 6.5, ygA had similar activities on both media.

Morphogenetic loci

The brlA locus. Twenty-one null-phenotype mutants at the brlA locus develop undifferentiated bristles in place of conidiophores (Fig. 2a). These are unpigmented, and neither AHT nor AHTase is produced (see Table 3, brlA1-3 and Clutterbuck, 1969; Birse & Clutterbuck, 1990a). In contrast, all brlA mutants (brlA6, 7, 9, 10, 12, 31, 33, 35, 42) giving 'leaky' phenotypes, as judged by partial morphological progression, tested positively for AHTase (Table 3). These included two mutants (brlA10 – see Table 3; and brlA31 – not tested quantitatively) whose conidiophores are not visibly pigmented. Furthermore, two mutants (brlA14 and brlA19) which show neither pigmentation nor morphological development were also AHTase positive.

AHTase extracted from leaky or temperature-sensitive brlA mutants (brlA9, 14, 35 and 42) was not distinguishable from the wild-type enzyme in thermolability or electrophoretic mobility.

Quantitative AHTase comparisons on 3-d-old colonies (Table 3) showed a range of activities, the highest coming from the brlA7, 9 and 35 mutants which have moderately leaky morphologies. Birse & Clutterbuck (1990b) found similar results for 26 h cultures assayed for both AHTase and iuoB mRNA.

Bristle mutants on their own accumulated only traces of AHT, if any, but the pigment precursor accumulated in iuoB63 recombinants at levels which correlated well with the degree of visible pigmentation.
The abaA locus. All abaA mutants contain AHTase, and none accumulate AHT. While AHTase levels were low in abaA mutants, approaching the level of the conidiating wild-type, substrate levels in the ivoB derivatives were as high as for many leaky brlA mutants. The assays reported in Table 3 were made on whole colonies which would include conidiophores representing a spectrum of developmental stages. Low AHT or AHTase levels in the wild-type and abaA mutants therefore probably indicate down-regulation at the later stages of conidiation, whereas high levels in some brlA mutants indicate that these mutants are stuck at stages of maximum ivoA and ivoB activity.

The medA locus. Mutants at this locus have a partial 'bristle' phenotype in which a normal vesicle bears multiple layers of metulae surmounted by phialides and conidia (Clutterbuck, 1969). All mutants so far mapped to this locus exhibit only delayed conidiation. They also differ from brZA partial mutants in that the conidiophore pigmentation is reduced. In order to decide which component of the pigmentation system was deficient in medA mutants, a brlA42 medA15 strain was compared to a brlA42 control for AHTase levels, and a similar pair of strains, both carrying ivoB63, was used to assay AHT accumulation. Table 3 shows that medA strains had reduced AHT levels, and as in ivoA mutants (see above), this was accompanied by increased AHTase.

Developmental timing of AHT and AHTase production

The production of AHTase by developing cultures has been shown to correspond closely to the time of formation of conidiophore vesicles (Clutterbuck, 1977), i.e. shortly before the conidiophore pigment becomes visible and about 4 h before the appearance of conidial laccase (Clutterbuck, 1972). Fig. 3 shows that accumulation of AHT in a brlA42 ivoB63 strain coincides with the rise in AHTase in a brlA42 ivoB+ strain.

Discussion

The conidiophore pigmentation system described here employs the fourth distinct phenol oxidase to be studied in A. nidulans. The other three, distinguished by substrate preference and developmental specificity, are hyphal tyrosinase (Bull & Carter, 1973; Martinelli & Bainbridge, 1974), conidial laccase I (Clutterbuck, 1972; Kurtz & Champe, 1982) and cleistothecial laccase II (Hermann et al., 1983). Phenol oxidases in other fungi are similarly diverse, in both substrate utilization and function (Bell & Wheeler, 1986).

In A. nidulans, conidial laccase I contributes to ultraviolet light resistance (Wright & Pateman, 1970), and the other two systems are postulated to confer resistance to lysis (Kuo & Alexander, 1967; Polacheck & Rosenberger, 1977; Hermann et al., 1983). It is possible that conidiophore melanin might also provide some protection against ultraviolet light, but since the conidiophore is hardly pigmented before it becomes covered with conidia, this seems unlikely to be important.

The alternative role of melanins in consolidating polysaccharide walls against degradation, a function comparable to insect cuticle tanning (Andersen, 1985) or seed coat maturation (Marbach & Mayer, 1978) seems more pertinent. While we have not demonstrated any structural deficiency in ivo mutants in morphologically normal conidiophores, pigmented brlA mutants have
conspicuously more rigid bristles than unpigmented ones (see Fig. 5D and E in Birse & Clutterbuck, 1990b).

Phenol oxidases are copper enzymes (Malström & Rydén, 1968). The reactivation of AHTase activity in extracts of ygA strains by the addition of copper is circumstantial evidence for the role of copper in this enzyme. However, AHTase is unusual among phenol oxidases in containing zinc as well as copper (Birse & Clutterbuck, 1990a), and although the necessity of zinc for AHTase activity remains to be tested, its presence may indicate an evolutionary origin distinct from other phenol oxidases.

To have a tryptophan derivative as principal substrate is unusual, although indoles are standard intermediates in the formation of melanin from tyrosine. We have suggested (Birse & Clutterbuck, 1990a) that the final melanin incorporates other materials oxidized in secondary reactions with oxidized AHT, and we cannot exclude the possibility that AHTase also reacts with some of these directly.

The conidiophore pigmentation system is one aspect of Aspergillus development where we know something of the biochemistry behind the phenotype. We wish now to learn something about the developmental control of this process. It should first be noted that a simple induction pathway of AHTase by its substrate does not seem to operate, since ivoA mutants, which lack AHT, nevertheless have high levels of AHTase.

A scheme integrating the morphogenetic and pigmentation pathways should explain the following observations.

1. Mutants at two loci, brlA and medA, have quantitatively correlated effects on pigmentation and morphogenesis.

2. ivoA mutants which are blocked in AHT synthesis do not accumulate the assumed precursor, N-acetyltryptophan.

3. Addition of tryptophan or N-acetyltryptophan to the medium has the effect of decreasing rather than increasing the level of AHT accumulated by an ivoB mutant.

4. Tryptophan auxotrophs grown on limiting tryptophan conidiate poorly and have relatively unpigmented conidiophores (Yelton et al., 1983).

5. We have found that a tryptophan auxotroph will not grow on N-acetyltryptophan, but where tryptophan is limiting, addition of N-acetyltryptophan allows full conidiation.

Fig. 4 illustrates two possible schemes. Scheme A suggests that the product of medA modifies the action of brlA, and thus plays a part in regulation of ivoA, ivoB and unknown morphogenetic loci. The medA product might for instance be involved in post-translational modification of a brlA-encoded regulator. This scheme requires two further postulates: firstly, that tryptophan and N-acetyltryptophan are readily interconverted only during conidiation; and secondly, that exogenous indoles induce their own breakdown and thereby lead to a shortage of

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**Fig. 4.** Two possible schemes for the genetic control of conidiophore pigmentation and morphogenesis. Two alternative routes for the formation of the minor component 6-hydroxytryptophan are common to both schemes. Other features are discussed in the text. Degradation of tryptophan to anthranilic acid is shown by a broken arrow, signifying that it is postulated in scheme A to be induced by exogenous indoles. Interconversion of tryptophan and N-acetyltryptophan are similarly assumed in scheme A to be conditional upon conidiation.
AHT precursor in the conidiophores. G. Griffith (personal communication) has found evidence that exogenous indoles are degraded to anthranilic acid in conidiating cultures.

In scheme B, AHT is synthesized indirectly, via an unknown morphogenetic compound, and brlA acts independently of medA except in this step. This gives N-acetyltryptophan a unique role in conidiophore morphogenesis, and feedback controls of morphogen synthesis might explain the failure of exogenous indoles to enhance AHT synthesis.

At present both schemes depend on hypothetical features to explain morphology: morphogenetic loci in scheme A and a postulated morphogen in scheme B. In both schemes brlA plays a crucial regulatory role. It has been demonstrated that transcription of ivoB is regulated, directly or indirectly, by brlA (Adams et al., 1988; Birse & Clutterbuck, 1990b). There is also good evidence that brlA encodes a DNA-binding protein; it has been sequenced and the inferred peptide contains a zinc-finger DNA-binding motif (Boylan et al., 1987). Furthermore, mis-scheduled expression of brlA is sufficient to activate transcription of many genes characteristic of later conidiophore development, including ivoB (Adams et al., 1988). brlA and ivoA or -B mutants complement well in heterokaryons, implying that the brlA product is not confined to the nucleus which codes for it (cf. Cove, 1979; Scorzacco et al., 1982).

The presence of AHTase in brlA14 and 19, which show no signs of BRL + morphology, implies that levels of brlA gene activity insufficient for morphogenesis can induce ivoB at a low level. On the other hand, the fact that brlA mutants 14, 19, 10 (and 31, not shown here) possess AHTase but are unpigmented and have low AHT accumulation figures implies that the ivoA gene (or possibly some other step in the AHT biosynthetic pathway) is inactive in these mutants. While there is a correlation between morphology and pigmentation, it is not complete: brlA6 is less developed but more pigmented than brlA10 (Clutterbuck, 1969). It therefore seems probable that if brlA acts as a transcriptional regulator, the BRLA proteins formed by various mutants have differing affinities for the promoters of ivoA and ivoB, and for those of the genes governing morphogenesis. We are now cloning the ivoA gene, and hope to be able to measure its transcription in brlA mutants and investigate the mechanism by which both ivo genes are induced.

References


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