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Genetic and environmental modification of gene expression in the *brlA12* variegated position effect mutant of *Aspergillus nidulans*

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SUMMARY

The *brlA12* variegated position effect mutant is particularly suited for tests of environmental and genetic influences on variegation, but out of a large number of substances added to the medium, only salts at high concentrations and methylamine significantly increased expression of this gene. Medium shifting experiments showed that *brlA12* activity could be switched on late, but once active, was rarely switched off again during conidiation. Separate *brlA12* clones in heterokaryons were activated independently. Some *brlA12*-specific suppressor mutants, including those at loci giving almost complete suppression, have been studied. One class of suppressors also confers inability to utilize galactose as carbon source and comparison with other, pre-existing mutants showed that the *brlA12* phenotype was either suppressed or enhanced by mutants with complex phenotypes involving galactose utilization, molybdate resistance, acid phosphatase production and sulphur metabolism. Tests for the involvement of DNA methylation in *brlA12* expression gave negative results.

1. INTRODUCTION

The *brlA12* mutation is believed to be a unique example among fungal mutants in showing a variegated position effect. It is distinguished from 39 other mutants at the *brlA* locus (Clutterbuck, 1969*a*) by its variegated phenotype, i.e. the presence on, standard media, of a very small proportion of more or less normally developed conidial heads on colonies which otherwise bear only undifferentiated bristles. While cytological confirmation of the nature of the mutation is not readily obtainable, the hypothesis that the *brlA12* mutant is comparable to variegated position effect mutants in *Drosophila* and other organisms (Lewis, 1950; Baker, 1968; Spofford, 1976) was given strong support by the genetic evidence that *brlA12* strains carry a translocation which has a breakpoint very close to the *brlA* locus (Clutterbuck, 1970).

The *brlA12* mutant offers special advantages for the isolation and study of modifier mutants and also for investigation of environmental effects on variegation. The isolation of suppressors has already been briefly reported (Clutterbuck, 1970). Further studies of the properties of suppressors and enhancers, and of environmental

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effects on these and on the original *brlA12* mutant, have been carried out in order to obtain clues to the ways in which chromosome structure can be modified so as to increase or diminish transcription from a gene presumed to be inactivated by the heterochromatin spreading effect.

2. MATERIALS AND METHODS

Standard *Aspergillus* minimal (MM) and complete (CM) media, methods and strains are described by Pontecorvo *et al.* (1953) and Clutterbuck (1974). Buffered (pH 6·5) minimal medium (BMM) was made as a $\times 20$ stock solution and differed from MM in containing (final concentration) KH_2PO_4 7·0 g/l (1·52 g/l in MM) plus 4·5 g/l K_2HPO_4. Low glucose medium contained 5 mM glucose. 'Salt media' were modified by the addition of solid NaH_2PO_4 (0·7 M) after melting. Mycelium to be shifted from one medium to another was grown as a 4 ml top layer containing half strength agar and an inoculum of 10^5 conidia, separated from the bulk of the agar medium (20 ml) by a sterile 0·5 mm mesh polyester net.

The *biA1* strain was used as the wild-type control with respect to conidiation. Quantitative estimates of conidiation (Table 1) were made on colonies derived from single conidia spread at approximately ten per dish. After incubation, conidial heads per microscope field were counted under low power. Haemocytometer counts of conidia were then made of suspensions obtained by shaking 1 cm diameter discs, cut from colonies using the rim of a test tube, in 1 ml of Tween 80 solution (0·1 ml/l). The amount of conidiation varied slightly from one experiment to another, depending on the batch of medium, thickness of agar, etc. It was particularly increased if the plates were allowed to dry out more than usual.

Suppressors and enhancers were isolated after ultraviolet treatment: survival 0·1–4·3% (Clutterbuck, 1970), or nitrous acid: survival 4·5% (Siddiqi, 1962*a*) of *biA1; brlA12* or *pabaA1 yA2; brlA12* strains. Biotin or *p*-aminobenzoic acid were therefore added to MM where required.

The following mutants, in addition to those listed in Table 1, were recombined with *brlA12* and tested for effects on conidiation under the various conditions used in classifying the mutants (see Clutterbuck, 1974, 1982 for descriptions of mutants and references): *abaA1*, *acrA1*, *adC1*, *adE20*, *adF17*, *adG14*, *ahrA1*, *anA1*, *apsA1*, *apsB8*, *araA1*, *argA1*, *argB2*, *biA1*, *chaA1*, *cnxB11*, *cnxH4*, *creA*^{d}*1*, *creC27*, *creC302*, *drkA1*, *drk-2*, *drk-3*, *facB303*, *facC307*, *fpaD11*, *fwA1*, *inoB2*, *luA1*, *lysB5*, *mauA2*, *mauB4*, *meaA8*, *meaB6*, *mecC13*, *methH2*, *niaD15*, *nicB8*, *niiA4*, *nirA1*, *ornB7*, *pabaA1*, *pantoC3*, *phenA2*, *proA1*, *puA1*, *punA11*, *pyroA4*, *riboB2*, *riboE6*, *sB3*, *sC12*, *slA1*, *sod*^{III} *A1*, *spsA1*, *thiA4*, *wA3*, *wA7*, *yA2*, *ygA6*.

In auxanographic tests for effects of graded concentrations of nutrilites, or other substances used to classify mutants, these were added in excess to wells in minimal agar which incorporated conidia of the strain concerned. The following antimetabolites, etc., were tested similarly against *biA1; brlA12* or other *brlA12* strains: *N*-acetyl-L-alanine, agmatine, D-alanine, DL-alanine hydroxamate, L-alanine methyl ester, allopurinol, 3-2-aminoethyl-L-cysteine, aminopterin, 4-aminopyrazolo(3,4-di)pyrimidine, 3-amino-L-tyrosine, amphotericin B, arcaine sulphate, aspartic acid β-hydroxamate, 8-azaadenine, 5-azacytidine, 8-azaguanine, 6-

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azauridine, L-azetidine-2-carboxylic acid, bacitracin, benomyl, α-*N*-benzoyl-L-alanine, bipyridyl, 5-bromodeoxyuridine, butyric acid, cadaverine, colchicine, creatin, creatinin; cysteamine, L-cysteine, dehydroproline, 1-4-diaminobutanone, dibutyrl cyclic AMP, 3,4,dihydro-DL-proline, dimethylsulphoxide, ethanol, DL-ethionine, ethylamine, ethylene diamine tetraacetic acid, *N*-ethyl maleimide, ethyl urea, fluorodeoxyuridine, *p*-fluorophenyl-alanine, β-fluoropyruvic acid, 5-fluorouracil, glutamic acid hydroxamate, griseofulvin, histamine, D-histidine, hydroxylamine, 4-hydroxy-L-proline, hydroxyurea, iproniazid phosphate, D-isoleucine, isoniazid, isopropylphenyl carbamate, D-lysine, L-methionine sulphoxide, methylamine, methyl-DL-tryptophan, methyl-urea, monodansylcadaverine, naladixic acid, nystatin, D-ornithine, D-phenylalanine, polymixin B, propionic acid, selenate, selenomethionine, D-serine, spermidine, spermine, L-thiazolidine-4-carboxylic acid (thioproline), thiodisuccinic acid, thiourea, D-threonine, trimethylene diamine, tryptamine, D-tryptophan, D-tyrosine, ultraviolet light, D-valine.

3. RESULTS

(i) *Stability of the* brlA12 *phenotype*

As already reported (Clutterbuck, 1970) more than 99% of the conidiophores of the *brlA12* mutant on MM or CM medium have the mutant phenotype; i.e., they are undifferentiated bristles (see Table 1). The very few conidiating heads that are formed appear randomly scattered and vary morphologically from normal, wild type conidial heads to more deformed structures bearing fewer conidia. This is evidence that the bristle locus activity is switched on in clones of nuclei which are not larger than that found in one conidial head, but may be smaller, in which case they will give rise to the partially developed conidial heads.

Observations were made of mycelium shifted between minimal medium and salt (0·7 M-NaH_2PO_4) medium which gives increased conidiation (Clutterbuck, 1970, and Section (v) below). Both media contained low glucose to give sparse colonies suitable for observation. Conidial heads formed on the salt medium continued to develop normally when shifted to salt-free medium although new undifferentiated bristles also grew alongside them. On the other hand bristles formed on salt-free medium developed conidial heads at their tips on shifting to salt.

The stability of the conidiating state was further checked using a *brlA12 abaA1* recombinant which forms abacus (beaded stick-like) structures in place of chains of conidia (Clutterbuck, 1969*a*); these have the advantage that they do not break up when mounted in liquid. On salt medium heads could readily be seen with 30 beads (each representing one uninucleate cell) which indicated stability of the switched-on state of the bristle locus for at least this number of mitotic divisions. Out of many heads observed on colonies shifted from salt to salt-free medium, only one abacus structure was observed which had evidently reverted to the bristle (unbeaded) morphology after the shift.

As a control for this observation, it has been shown (Clutterbuck, unpublished) that where *abaA1* is combined with the temperature sensitive *brlA42* allele, the switch to the bristle morphology, obtained in this case by a temperature shift-up, was readily observed in all *abacus* structures.

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(ii) *Nuclear interaction in heterokaryons*

In order to test the possible influence on each other of nuclei in different states of *brlA12* activation, heterokaryons were constructed between pairs of *brlA12* strains. The heterokaryons were in all cases balanced by *argA1* and *inoB2* auxotrophic markers and were grown on low glucose MM + salt (on non-salt medium conidiation was too sparse to be studied). The two component strains were distinguished by the spore colour mutant *yA2* (yellow) in one strain and *abaA1* (abacus) in the other.

Control heterokaryons where both components were *brl*$^+$ formed conidial heads of which approximately 10% were morphologically mixed, indicating that both types of nuclei contributed to their formation (Pontecorvo *et al.* 1953). On the other hand, in biparentally *brlA12* heterokaryons, heads bearing conidial structures of both types were extremely rare, as would be expected if the component nuclear clones did not interact and mixed heads occurred only if both components happened to be activated simultaneously.

In order to increase the proportion of mixed heads, the experiment was repeated with heterokaryons whose parents also different in carrying *apsA1* and *apsB8* mutations respectively. These mutations prevent the formation of normal conidial heads by interfering with nuclear migration into the sterigmata (Clutterbuck, 1977); however, the mutations can complement each other in heterokaryons so that in mixed heads development is normal. In homozygous *brl*$^+$ heterokaryons of this type virtually all phenotypically *aps*$^+$ conidial heads were indeed seen to be morphologically mixed, bearing both yellow conidia and abacus structures. On the other hand, heterokaryons where both parents were *brlA12* gave conidial heads of which more than 70% were morphologically unmixed, i.e., they bore either yellow conidia or abacus structures – with approximately equal frequencies of the two types. This indicated that in most of these mixed conidial heads, which must contain both types of nuclei in order to allow complementation of *apsA1* and *apsB8*, only one of the clones of nuclei had an active *brlA* gene.

(iii) *Suppressors of* brlA12

Since, as described above, the phenotype of *brlA12* on standard media is predominantly mutant, suppressors are detectable more readily than enhancers. However some enhancers were isolated from salt medium and classified according to their residual conidiation level, but they were not studied further (but see Section (iv) below).

(*a*) *Classes of suppressors*

Clutterbuck (1970) described three classes of suppressors distinguished by the degree of suppression obtained. Further representatives of these three phenotypic classes have been isolated and examined more closely. Table 1 shows a comparison of the conidiation of the wild type (*biA1*), *biA1; brlA12* and strains carrying mutations representative of the three classes of suppressors.

A representative of the most effective class of suppressors has been mapped on

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linkage group II (Clutterbuck, 1982) and is designated *drkB5* on account of the dark colour of its conidia (Clutterbuck, 1970). A further 56 suppressors of this phenotypic class have now been isolated and 16 of them have been tested for complementation in heterokaryons with *drkB5*: all gave negative results and are therefore regarded as allelic. On CM these *brlA12; drkB* strains conidiate almost as well as wild type, but on MM they may show some unsuppressed bristles.

Table 1. *Conidiation of wild-type and of* brlA12 *and its suppressors*

(Haemocytometer counts (the mean of three estimates, expressed as conidia/mm^2 of colony surface) of conidial suspensions prepared from discs cut from single colonies on various media incubated for 4 days at 37 °C or 12 days at 25 °C.)

Strain	Temperature (°C)	Media: BMM	CM	MM+0·7 M (NaH_2PO_4)	CM+0·7 M (NaH_2PO_4)
biA1	37	670000	780000	70000	670000
	25	630000	840000	410000	610000
biA1; brlA12	37	340	590	15000	52000
	25	680	1600	32000	65000
biA1; brlA12; drkB5	37	180000	360000	—	—
biA1; brlA12; galG2	37	60000	700000	—	—
biA1; brlA12; vbsC7	37	210000	100000	—	—

The type-mutant of the second most effective class of suppressors also has a distinctive secondary characteristic: the inefficient use of galactose as carbon source, hence the designation *galG*. Unlike the *drkB* strains, *galG* suppressors rarely showed any unsuppressed bristles but their conidiation was often reduced because of a slightly slower growth rate and thinner colony density than normal, especially on MM. *galG2* maps in linkage group VIII, but some distance (approximately 80 map units) from the *brlA* locus (Clutterbuck, 1982). Twenty-nine further mutants sharing the same phenotype have been tested for complementation in heterokaryons with *galG2*. One mutant did show some complementation for galactose utilization which may mean that there is more than one locus giving rise to galactose negative suppressors. However, negative results from heterokaryon tests of the remaining mutants may not be a reliable indicator of allelism since it has been shown (see Section (iii)(b)) that *galG2* behaves autonomously with respect to *brlA12* suppression in heterokaryons. In addition to these mutants, ten others giving the same degree of suppression as *galG2*, but not noticeably impaired in galactose utilization, have been isolated. No tests of allelism have been made on these mutants.

The least effective class of suppressors reported by Clutterbuck (1970) is represented in Table 1 by a mutant, now designated *vbsC7* (*v*ariegated *b*ristle *s*uppressor) which was located to linkage group VII but not mapped further. This mutant is included in the weakest class of suppressors because, although it actually conidiates quite well, especially on MM (Table 1), the conidiating heads are generally hidden beneath a mat of unmodified bristles. Suppressors with a low degree of suppression are common and almost certainly represent a genetically

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heterogeneous class. The growth of 131 such suppressor strains on galactose varied from slightly worse than the wild type to slightly better.

(b) *Dominance tests in heterokaryons*

For a representative of each class of suppressor, heterokaryons of the following constitutions were constructed:

(1) *brlA12 vbs* y^+ + *brlA12* vbs^+ *yA2*.
(2) *brlA12 vbs* y^+ + brl^+ vbs^+ *yA2*.
(3) brl^+ *vbs* y^+ + *brlA12* vbs^+ *yA2*

(*vbs* stands for a suppressor, vbs^+ for its wild type allele, and the yellow conidial colour marker *yA2* is used to distinguish conidia from the two components of the heterokaryon). The heterokaryons were grown on minimal medium and balanced by suitable auxotrophic markers.

In tests with *drkB5* all three heterokaryons carried many unsuppressed bristles, indicating that the suppressor is largely recessive, although some green (y^+) conidia in heterokaryons (1) and (2) showed that it was not entirely overridden by its wild type allele. Furthermore, a small but significant proportion of yellow conidia in heterokaryons (1) and (3) (more than in the *brlA12* vbs^+ *yA2* strain on its own) suggested some dominant action of the suppressor.

vbsC7 gave very similar results to *drkB5*, but showed slightly more evidence of dominance, despite the fact that it is a weaker suppressor.

galG2, a member of the second class of suppressors, appeared to be largely autonomous: heterokaryons (1) and (3) produced mainly green conidia and bristles, while heterokaryon (2) produced only yellow and green conidia, i.e. the two components of the heterokaryon behaved much as they would on their own.

As reported before (Clutterbuck, 1970), control heterokaryons between *brlA12* and a brl^+ strain gave no evidence that conidiation of *brlA12* is altered in heterokaryons with normal strains.

(c) *Specificity of suppression to* brlA12

It was reported by Clutterbuck (1970) that *drkB5* does not suppress *brlA9* which is a leaky, salt-sensitive, but non-variegated mutant at the *brlA* locus (Clutterbuck, 1969*a*). *vbsC7* and *galG1* have now also been combined with *brlA9*: neither showed any signs of suppression. All three classes of suppressor can therefore be regarded as specific for the variegated mutant. Despite frequent mutation experiments, no variegated mutants at other loci have been obtained so the locus specificity of the suppressors is untested.

(d) *Mutability and recombination*

It was noticed that old colonies of *galG2* strains developed tufts of fluffy mycelium scattered across the colony, suggesting an increased mutation rate in these strains. Tests on the spontaneous mutability of fresh conidia of *brlA12 galG2*, brl^+ *galG2*, *brlA12* and control strains failed to show any consistent differences in mutation frequency either to morphological abnormality or to selenate resistance (a mutation test system: Jansen, 1972), however, when conidia from 4-week old slopes were tested, the *brlA12 galG2* did give a high frequency of 'fluffy'

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morphological mutants. These mutants are characterized by their ability to overgrow other colonies (Dorn, 1970) and it is therefore possible that they could be selected for in the interstices of old colonies of strains such as *galG2* which grow somewhat sparsely and therefore may not completely exhaust the medium.

Crosses homozygous for *drkB5* or *galG2* were tested for effects of these suppressors on recombination between *pabaA1* and *pabaA18* alleles and the adjacent markers *adF17* and *yA2* (Siddiqi, 1962*b*). No significant differences from control crosses were found in either intergenic or intragenic recombination.

(*e*) *Mycelial pigmentation*

Mutants of the *drkB* group have deeper than normal spore colours and also develop a brown mycelial pigmentation which is intensified in the presence of some amino acids, especially arginine. A few of the low efficiency suppressors also become heavily pigmented under a variety of circumstances.

Suppressors of the *galG* class develop a red pigment, again intensified by arginine, but more strikingly by putrescine. Putrescine also partially inhibited the growth of the *brlA12 galG2* strains, at concentrations as low as 0·06 mM, the concentration required to supplement putrescine auxotrophs (Sneath, 1955; Spathas, Pateman & Clutterbuck, 1982). Analysis of the components of these strains showed that while the *galG2* mutant was responsible for growth inhibition by putrescine, strains carrying *galG2* or *brlA12* alone developed red coloration on putrescine medium. Further investigation showed that other *brlA* mutants responded similarly, but only if the medium contained nitrate or nitrite as nitrogen source. Pigmentation was blocked by the presence in the strains of *niiA* or *nirA* which prevent nitrite assimilation (Cove, 1979) and a *brlA12 cnxB11* strain, which lacks nitrate reductase activity, only developed the pigment on medium containing nitrite. Hydroxylamine, a possible intermediate in nitrite reduction, did not support pigmentation.

The fact that putrescine-induced pigmentation of *galG2* was reduced by isoniazid and iproniazid, which are inhibitors of amine oxidase, suggested that the pigment could be a derivative of Δ'pyrroline, an oxidation product of putrescine and possible precursor of prodigiosin, the red pigment of *Serratia marcescens* (Bachrach, 1981; Gerber, 1975). Furthermore, pigmentation was also blocked by *o*-aminobenzaldehyde which can combine with Δ'pyrroline. Normal levels of putrescine oxidase and aminotransferase, two putrescine catabolizing enzymes found in the wild type (Spathas, Clutterbuck & Pateman 1983*b*), were found in *brlA12* and *galG2* strains (data not shown).

A preliminary analysis of the pigment produced by *brlA12 galG2* grown in the presence of putrescine showed that it was soluble in methanol and acetone, but not in water. Chromatography on Sephadex LH-20 revealed a complex mixture of at least five pigments, none of which was seen in mycelium grown without putrescine.

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(iv) *Other mutants modifying* brlA12

(*a*) *Auxotrophs and other mutants*

In the course of mapping and other studies, the opportunity has been taken to examine the effect of a wide variety of auxotrophic, resistance and other markers on *brlA12*: these are listed in the Methods Section. In addition, the effects of deficiency or excess of the test substances required to classify these markers have been noted. Very few of the mutants or test media had any clear effects on the conidiation of *brlA12*, but slight effects might have been obscured by unknown, low-efficiency modifiers which were found to segregate in many of the crosses. The Glasgow *A. nidulans* strains are all derived from a single wild isolate (Pontecorvo *et al.* 1953) so they should be relatively isogenic, but there is a strong possibility that during subculture, the *brlA12* strains might have been subject to unconscious selection for partial suppressors.

(*b*) *Mutants affecting galactose utilization, molybdate resistance, acid phosphatase and sulphur metabolism*

Since one type of mutant (*galG2*), isolated as a suppressor of *brlA12*, is unable to grow well on galactose, other *gal* mutants were also tested for effects on *brlA12*. In addition, since correlations have been found between galactose utilization, molybdate resistance and acid phosphatase phenotype (Arst & Cove, 1970), mutants affecting these features were also investigated. The results, summarized in Table 2, justify interest in these areas of metabolism, although the picture is complex.

The *gal* mutants with known defects in the Leloir pathway: *galA1*, *galD5* and *galE9* (Roberts, 1963, 1970) have no effect on *brlA12*, nor have they been found to affect molybdate resistance (Arst & Cove, 1970). On the other hand *galC4*, *galC7*, *gamA55*, *gamB65* and *gamC66*, in which no specific defect has been identified, but which confer molybdate resistance as well as galactose non-utilization, all acted as enhancers of *brlA12*. It was therefore of interest to test *galG2* for molybdate resistance: it proved to be hypersensitive. The molybdate resistant mutant *molB35*, which grows as a thin, rapidly spreading colony on galactose agar, was a slight suppressor, but *molA33*, whose molybdate resistance may have a different basis, involving the nitrate reductase cofactor (Arst, MacDonald & Cove, 1970), had no effect on *brlA12*. C. R. Bailey (quoted in Arst, 1981) has shown that *molB* is the same locus as *creB*, mutants of which have multiple defects in carbon catabolism. The *creC27* and *creC302* mutants, which have similar phenotypes to *creB* mutants, had no effect on *brlA12*, but *creA*^{d}*1*, which according to Arst (1981) probably acts by a different mechanism, did suppress conidiation to some degree.

The acid phosphatase deficient mutants *pacC5* and *suB2palB7* (Dorn, 1965) which are also resistant to molybdate (Arst & Cove, 1970) were identified as *brlA12* enhancers. The *pacA1* mutant, which apparently has normal internal phosphatases (Dorn, 1965) did not affect *brlA12*, nor did the alkaline phosphatase (*pal*) mutants, although they are hypersensitive to molybdate. For comparison with molybdate toxicity, sodium arsenate, sodium pyrophosphate and lead nitrate were tested for conidiation effects and differential toxicity. The only conclusion of interest is that the growth of *gamA55* is resistant to arsenate.

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The double mutants combining *drkB5* or *galG2* with some of the other mutants discussed in this section generally behaved (Table 2) as if these mutants acted independently, i.e., the resulting phenotypes were approximately as predicted for the sum of the two components.

Table 2. *Galactose utilization and molybdate resistance of mutants modifying* brlA12

Mutants	Effect on conidiation of *brlA12*	Growth on galactose	Growth on molybdate	Other features
drkB5	+ + + +	0	0	Dark conidia
galG2	+ + +	− −	−	Slightly thinnish growth on all media
vbsC7	+	0	0	
molB35	+	+	+	
molA33	0	0	+	
gamA55, *-B65*, *galC4*, *-C7*	−	− −	+	Pale conidia
galA1, *-D5*, *-E9*	0	− − −	0	
pacC5, *suB2palB7*	−	0	+	Acid phosphatase deficient, pale conidia
pacA1	0	0	0	Acid phosphatase deficient
palB7, *-C4*, *-F15*	0	0	−	Alkaline phosphatase deficient
palcA2, *-B3*	0	0	0	Acid and alkaline phosphatase deficient
Double mutants				
drkB5 galC7	+ + +	− −	+	Darkish conidia
galG2 galC7	0	− − −	n.t.	
galG2 gamA55	+	− − −	0	Orange mycelial pigment
galG2 gamC66	0	− − −	+	
galG2 molB35	+ + +	−	−	Orange mycelial pigment

Key: 0, no effect on conidiation or growth; + to + + + +, better growth or conidiation than unmodified *brlA12*; − to − − −, worse growth or conidiation than unmodified *brlA12*; n.t., not tested.

Another mutant conferring molybdate resistance is the sulphate uptake mutant *sB3* (Arst, 1968). This mutant also acted as a mild suppressor of *brlA12*, but the effect was obvious only at room temperature, and was not influenced by the level of sulphite or thiosulphate used to replace sulphate as sulphur source. Other mutants affecting sulphur metabolism: *sA1*, *sC12* and *methH2*, had no effect, nor did *mecC13* which is partially deficient in methionine adenosyltransferase (Pieniążek, Kowalska & Stępień, 1973). However, the failure of *mecC* mutants to convert methionine into an effector for repression of sulphate and selenate uptake (Pieniążek, Kowalska & Stępień, 1973) is tested on medium containing 0·1 mM selenate + 0·1 mM methionione, and on this medium the five *galG*-type suppressors tested had a 20 % reduction in growth rate and five out of ten of the least effective suppressors had a 50 % reduction in growth rate. Other suppressors were unaffected and none differed from the wild type in sensitivity to selenate alone.

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(v) *Environmental influences on* brlA12 *and its modifiers*

It was reported by Clutterbuck (1970) that high concentrations of salts or other solutes, especially at low pH, give rise to increased conidiation (i.e., phenotypic suppression) of *brlA12*. These observations have been extended by testing a variety of environmental influences on *brlA12* as well as *gal G2 brlA12*, *drkB5 ; brlA12*, and *vbsC7 ; brlA12* strains as representatives of the suppressors.

(*a*) *Temperature*

Conidiation of *brlA12* was somewhat better at 25 than at 37 °C, (Table 1) but only after rather prolonged incubation. The temperature of incubation did not dramatically affect the influence of modifiers on *brlA12*.

(*b*) *pH*

Low pH of the medium, down to pH 3·5, which is the lowest tolerated by *A. nidulans*, slightly improved *brlA12* conidiation, but was more effective in the presence of high salt concentrations. pH values above 7 have a correspondingly deleterious effect on conidiation. As with salt, low pH can convert even poor suppressors into good ones, while at high pH, only the galactose non-utilizing suppressors (e.g., *galG2*) retained any effectiveness.

(*c*) *Solutes at high concentrations*

In initial experiments molar concentrations of glucose, NaCl and NaH_2PO_4 were found to be effective in improving conidiation of *brlA12* (Clutterbuck, 1970). Later, the following salts (listed in order of increasing effectiveness) were compared: $CaCl_2$, Na_2HPO_4, sodium tartrate, $NaNO_3$, ammonium tartrate, $(NH_4)_2SO_4$, NaCl, KCl, Na_2SO_4, KH_2PO_4, NaH_2PO_4. It appeared from these tests that the main differences were due to the final pH of the medium and the tolerance of the fungus for particular salts at high concentrations (up to 2 M) rather than specific effects of particular ions on conidiation.

The optimal concentration of NaH_2PO_4 was found to be 0·7 M and this concentration was regularly employed in 'salt media' when conidiation of *brlA12* was required. This concentration gives rather sparse growth of all strains on MM at 37 °C, hence the reduced conidiation of the wild-type shown in Table 1. On these salt media approximately 15 % of the conidiophores of the *brlA12* strain developed conidia, but since most of the heads were smaller than normal, the number of conidia formed was less than 10 % of the wild-type.

At high salt concentrations most *brlA12* suppressors, even the least effective ones, give conidiation comparable with the wild type. Enhancers, on the other hand were isolated on the basis of their reduced conidiation at high salt concentrations and they retained that property on all media tested. The *sltA* mutant which confers inability to grow on media containing 1 M salt (Spathas, 1978) had no effect on the conidiation of *brlA12* irrespective of salt concentrations below the toxic level, nor do any of the mutants isolated as modifiers of *brlA12* affect ability to grow at high solute concentrations.

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(d) Constituents of standard media

Omission or excess of the major components or trace elements from minimal medium had no effects on the conidiation of *brlA12* or its suppressors which were not also shown by the wild type. Some low-efficiency suppressors developed heavy mycelial pigmentation in the presence of high concentrations of zinc or cobalt. Some of the less effective suppressors, e.g., *vbsC7* in Table 1, showed slight differences in efficiency (in either direction) on complete as compared to minimal medium.

Carbon and nitrogen sources could also be replaced by a variety of alternatives without effect on conidiation. Addition of certain amino acids to the medium affected the conidiation of some of the suppressors: histidine generally improved conidiation slightly, methionine and lysine depressed it. Lysine also partially inhibited the growth of *drkB5*. Arginine reversed the effects of lysine and it also improved the growth of *galG2*. As reported in Section (iii) (e)) above, arginine also increased *galG2* and *drkB5* pigmentation.

(e) Antimetabolites

A wide variety of amino acid or nucleotide analogues, antibiotics, and other potentially toxic substances were tested for effects on *brlA12*. The chemicals (listed fully in Materials and Methods) were spotted onto MM agar containing conidia of a *brlA12* strain with or without 0·7 M-NaH_2PO_4. The majority had no conspicuous effect on conidiation, or if they inhibited it, did so equally to *brlA12* and the wild type. A few compounds (aminopterin, hydroxylamine, L-alanine hydroxamate and thioproline) gave a ring of slightly increased conidiation, but only in the presence of 0·7 M-NaH_2PO_4. In the case of thioproline, this was quantified by testing it at various concentrations incorporated in the medium: the most effective concentration (5 mM) increased the conidiation of *brlA12* in this experiment from 1·3 to 6·7 % of the wild-type level.

The only compound effective without salt was methylammonium chloride: the highest non-toxic level (60 mM) gave 6·5 % of the wild type level of conidial heads where none were visible in its absence.

A few compounds also appeared to decrease conidiation of *brlA12* on salt medium without reducing conidiophore (bristle) formation or decreasing conidiation in the wild type: these were butyric acid, D-alanine, cysteamine and 1,4-diaminobutanone.

(f) Delayed conidiation

Quite dense conidiation was found on parts of colonies on salt medium where regrowth had occurred at room temperature after the colony surface had been scraped off to harvest conidia. A similar phenomenon may have been responsible for a false impression that some growth inhibitors, such as 8-azaguanine, could stimulate conidiation on auxanography plates although they did not do so if incorporated at any fixed concentration in the medium: if delayed growth occurred at room temperature on areas initially inhibited by high concentrations of the test substance, these areas conidiated much better than control plates of standard medium, although the conidia normally took at least a week to develop. This phenomenon, however, was conspicuous only on salt media.

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4. DISCUSSION

Whereas in *Drosophila* (Spofford, 1976) and the mouse (Russell & Bangham, 1961) there is often a considerable interval between establishment of a clone of nuclei in which the variegating locus will be active, and the time of expression of that activity, the two events are too close together to be separable in the *brlA12* mutant. Manipulation of the environment shows that when conidial heads become more frequent, they also become larger, presumably indicating the earlier establishment of an active clone of nuclei, but there was no evidence for clones large enough to include more than one conidiophore initial. If conidiophores are initiated from ordinary hyphal compartments, which are estimated to contain about four nuclei then only three or four division cycles may be needed to provide enough nuclei for entry into the 30 or so sterigmata on a standard conidial head (Clutterbuck, 1969*b*) and it is during this short period that the phenotypic effects of bristle locus activity are seen in the form of vesicle and sterigma development and pigmentation.

Observation of long chains of conidia, or the equivalent *abacus* structures, has established that activation of the *bristle* gene is rarely reversed even in colonies shifted to non-salt medium, and in fact a tendency towards increased activation was seen in colonies regenerating on salt medium at room temperature. This is reminiscent of the progressive increase in pigmentation with age of a variegated position effect mutant in the mouse (Cattanach, 1974).

The finding that separate *brlA12* clones in a heterokaryon variegate independently is in keeping with the fact that variegation in any organism depends on cellular, or at least local, autonomy of gene expression. Moreover, variegation in mouse position effect mutants demonstrates autonomy of active and inactive X chromosomes in the same cell.

The significance of the simpler environmental effects on *brlA12* is difficult to evaluate: high salt concentrations and low pH, if applied directly to chromatin, might be expected to reduce the binding of both histones and non-histones to DNA, but it cannot be assumed that exogenous application has a comparable effect inside the cell. While any solute at high concentration in the medium may also accumulate within the cell, other standard osmotic stabilizers, such as glucose, glycerol and other polyols may also be accumulated (Luard, 1982, Hocking & Norton, 1983). The slight increase in activation of *brlA12* at low temperatures is the opposite of the usual response of *Drosophila* variegated position effect mutants (Spofford, 1976).

A number of theories for clonal activation of chromatin, either in the course of differentiation or as part of a euchromatin–heterochromatin switch, invoke the properties of methylated DNA (Holliday & Pugh, 1975; Riggs, 1975; Sagar & Kitchin, 1975). These theories are particularly attractive since they can explain the replication of an activation state as a result of the preferential methylation of half-methylated DNA created by the replication of a fully methylated duplex (Wigler, 1981). Methylated DNA is generally less transcriptionally active than unmethylated DNA (Razin & Riggs, 1980) and 5-azacytosine, an inhibitor of DNA methylation, reactivates inactive mammalian X chromosomes (Venolia *et al.* 1982),

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it is therefore disappointing to find that although it inhibited the growth of *Aspergillus*, indicating that it is taken up by this fungus, it had no effect on the conidiation of *brlA12*. Ethionine, a general methylation inhibitor, was also without any specific effect, and so was the *mecC13* mutant which is deficient in the methyl donor S-adenosyl methionine.

R. L. P. Adams (personal communication) has found that *A. nidulans* DNA is substantially degraded by the restriction enzyme HpaII, giving no sign that any detectable proportion of its sites of action are protected by methylation of cytosine residues. Adams has also assayed DNA methylase in the wild type and *drkB5* and *galG2* mutants, but found that activity is not more than 1 % of that in mouse ascites cell controls in any of these strains. Methylation of DNA therefore seems unlikely to have any major role in this fungus. The same conclusion has been reached by Tamame *et al.* (1983).

Modification of nuclear proteins has also been invoked in position effect variegation, and butyrate and propionate, which are believed to inhibit deacetylation of histones (Candido, Reeves & Davie, 1978), have also been shown to reactivate position effect mutants in *Drosophila* (Mottus *et al.* 1980). In *Aspergillus,* both compounds were again inhibitory, but the only specific effect on *brlA12* was that butyrate, when added to salt medium, increased the frequency of long aconidial bristles, which presumably signified a reduction of *brlA* locus activity.

Polyamines had no detectable effect on *brlA12*, even in recombinants containing the *spsA1* mutant which allows much more effective uptake of spermidine and spermine than in the wild type (Spathas, Clutterbuck & Pateman, 1983*a*). There is evidence of interaction of the *galG2* class of suppressors with putrescine, resulting in decreased growth and increased pigmentation, but the significance of this remains obscure.

Methylamine, the most effective promoter of conidiation of *brlA12* other than salt medium, is an inhibitor of transglutaminase, a protein crosslinking enzyme (Folk & Finlayson, 1977). Ethylamine was similarly active on *brlA12*, but ammonia, bacitracin, and the most potent transglutaminase inhibitor, monodansylcadaverine, were without effect.

Colchicine, which was reported to suppress a *Drosophila* variegating mutant (Spofford, 1976), had no effect on *brlA12* and nor did benlate or *p*-fluorophenylalanine which are more effective spindle inhibitors for fungi. Methotrexate (amethopterin) although inhibitory to growth, also failed to influence *brlA12* although it was effective in *Drosophila* (Schulz, 1956).

Suppressor mutants were strikingly more effective than any environmental influence in restoring conidiation to *brlA12*, and investigation of their mode of action must be a priority for further work. One area for speculation is suggested by the inclusion of acid phosphatase mutants among those affecting *brlA12* function as well as molybdate resistance and galactose utilization (Table 2): these might vary in the phosphorylation of nuclear and other proteins.

The chromosomes of *A. nidulans* are too small for heterochromatin to be detected cytologically, but according to the genetic evidence (Clutterbuck, 1970) the translocation associated with *brlA12* has a breakpoint close to *brlA* and shifts this locus and the whole of linkage group VIII distal to it to an apparently terminal

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position on linkage group III. Similar nonreciprocal translocations in *Neurospora* have been described as 'quasiterminal' (Perkins & Barry, 1977) on the assumption that translocated fragments can only become attached to broken chromosome ends, even when as in this case, there is evidence that no genetically essential material has been displaced. Such apparently terminal translocations are not in fact rare in fungi (Burr, Roper & Relton, 1982) and are now recognized in *Drosophila* (Novitski *et al.* 1981). Both the heterochromatic nature of chromosome telomeres and their apparent genetic inertness are in accord with the finding of repetitive DNA comprising the cloned telomeres of *Tetrahymena* and *Saccharomyces* (Szostak & Blackburn, 1982). *Aspergillus* telomeres are clearly another area which would repay direct study.

Most of the *Drosophila* variegated position effect mutants have the variegating locus translocated adjacent to centromeric rather than telomeric heterochromatin. This may account for some of the differences between *brlA12* and the classical *Drosophila* examples. Alternatively, *brlA12* may be an example of a translocation of a 'heterochromatic' gene into euchromatin (Spofford, 1976). One other possibility exists: in *Neurospora* the nucleolus organizer is terminally located. In *Aspergillus* its position is unknown, but it is possible that it may, as in *Drosophila* (Hannah-Alava, 1971) act as heterochromatin.

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