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Methionine suppressors in *Aspergillus nidulans*: their genetics and behaviour in heterokaryons and diploids

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1. INTRODUCTION

Suppressors of methionine-requiring mutants in *Aspergillus nidulans* have been the subject of several investigations, although little is yet known of the pathway by which methionine is synthesized in this organism. Siddiqi (1962) has shown that the mutant *methG1* reverts at high frequency to give at least three different phenotypes. Further investigation by Lilly (1965) revealed that reversion in each of these three classes is due to suppressor mutations, and that two of the three phenotypes are each determined by at least two different genes. Alderson & Clark (1966) have used this system for determining interlocus specificity of chemical mutagens.

Several instances have been reported in fungi of differences in dominance of suppressors between diploids and heterokaryons. Pontecorvo (1963) quoted two examples of suppressors of methionine and para-aminobenzoic acid mutants of *A. nidulans*; the suppressors were fully recessive in the diploid but were semi-dominant in the heterokaryon. A similar example involving a methionine suppressor in the basidiomycete *Coprinus lagopus* has been described by Casselton & Lewis (1967).

The significance of such differences was first discussed by Pontecorvo (1963). According to one model, the differences are due to the gene products acting within the nucleus; in the heterokaryon, with the wild type and mutant suppressor alleles in different nuclei, there would be some methionine synthesis, whereas in the diploid with both alleles in the same nucleus, the mutant suppressor allele would be fully recessive. On the alternative model, the suppressor gene products act within the cytoplasm, and the separation of alleles in different nuclei might be sufficient to permit some methionine synthesis.

In the example in *Coprinus*, Casselton & Lewis (1967) were able to rule out the hypothesis of nuclear localization of suppressor action, because the suppressor was fully recessive in the dikaryon, which has two different haploid nuclei per cell. This led to the conclusion that the semi-dominance was related to the distribution of the two types of haploid nuclei within the mycelium. They were further able to show that the nuclear ratios in the heterokaryons did not significantly differ, which left uneven distribution of nuclei within the mycelium as the most likely cause of semi-dominance.

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This paper reports the investigation of the suppressors of *methB3* in *A. nidulans*. An attempt has been made to decide whether semi-dominance in the heterokaryon of one of the suppressor genes is due to adjustment of nuclear ratios. Some of the suppressors have been tested for their effect on other non-allelic methionine mutants.

2. MATERIALS AND METHODS

(i) *Strains*

The following strains of *Aspergillus nidulans* belonging to the collection in the Department of Genetics, University of Glasgow, were kindly supplied by Professor R. H. Pritchard: *bi-1methG1; bi-1methH2; yphenA2methH2; ypyro-4methB3; pabaA1Yw-3; adG14proA1pabaA1Ybi-1w-3; proA1pabaA1yw-3; adE20bi-1w-2.*

The following recombinant strains were isolated: *adG14Ybi-1w-3methH2; proA1pabaA1Ybi-1w-3methB3; proA1pabaA1yw-3methB3.*

ad, *bi*, *meth*, *paba*, *phen* and *pyro* are respectively requirements for adenine, biotin, methionine, para-aminobenzoic acid, phenylalanine and pyridoxine. *Y*, green conidia; *y* yellow conidia; *w-2* and *w-3*, white conidia.

Gene symbols and nomenclature follow the suggestions of Clutterbuck (1968), except in the case of the *meth* mutants, where different locus symbols are given to *methG1*, *methH2* and *methB3* in accord with the results of Gajewski & Litwińska (1968).

(ii) *Media*

Minimal medium (MM) was the same as that described by Pontecorvo *et al.* (1953). Amino acid supplements were added to a final concentration (w/v) of 50 μg/ml of the L isomers; vitamins were added to 1 μg/ml and adenine to 80 μg/ml.

Complete medium (CM) was: Bacto yeast, 1·5 g; Bacto peptone, 1·5 g; Bacto casitone 1·5 g; hydrolysed nucleic acid 5 ml; vitamin solution 1·0 ml; made up to 1 l. with tap water, and pH adjusted to 6·0. Techniques used were generally those described by Pontecorvo *et al.* (1953). Incubation was at 37 °C. Methionine mutants were induced by u.v. or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NNG) (Clutterbuck & Sinha, 1966) coupled with the velveteen replicating technique of Mackintosh & Pritchard (1963).

Heterokaryons were synthesized by inoculating conidia from the two component strains into a tube of 2 ml sterile water above 5 ml CM. The mycelium was teased on to selective MM after 18 h incubation and subcultured until balanced heterokaryotic growth was established. Diploids were selected following the technique of Roper (1952).

Growth measurements. Haploid and diploid strains were inoculated using conidia, heterokaryons using mycelial blocks *c.* 2 mm^2. Radial growth was measured over 120 h.

3. RESULTS

(i) *Isolation and characterization of methionine mutants*

Six new methionine mutants of the strain *ypyro-4* were isolated following u.v. treatment (*methB101*) or NNG treatment (*methH102, -103, H104, B105* and *meth-106*). These mutants were isolated mainly to see if they responded to cystathionine, since Gajewski & Litwińska (1967) reported that in a sample of 48 methionine mutants, none responded to cystathionine.

The six mutants, together with *methG1, methH2* and *methB3* were tested for growth on cysteine, cystathionine, homocysteine thiolactone or methionine. All grew well on methionine; *methG1, methB3, methB101, meth-103* and *methB105* responded to homocysteine thiolactone; but none grew on cystathionine or cysteine (*methH102, meth-103* and *meth-106* were leaky on MM). It seems likely therefore that mutants in *Aspergillus* responding to cystathionine either do not occur, or are rare, in contrast to *Neurospora crassa* (Horowitz, 1947).

MethG1, methH2 and *methB3* were fully complementary with each other in heterokaryons, as expected. *MethB101, methH102, meth-103, methH104* and *meth-B105* were tested in heterokaryons with *methG1, -H2* and *-B3; methB101* and *methB105* were allelic with *methB3; methH102* and *methH104* were allelic with *methH2; meth-103* was not allelic with *methB3* but the results with *methG1* and *methH2* were not clear cut; and *meth-106* has not been tested.

(ii) *Isolation of revertants from methB3*

Methionine revertants were isolated by plating conidia from the strain *ypyro-4methB3* at a density of about 5×10^5 per plate. The rate of mutation was about 1·6 per 10^5 spores. Colonies with two different morphologies were distinguishable—one normal, the other sporulating poorly and possessing a brown pigment. Ten isolates were studied, *sup-101* to *sup-110*; their morphologies were as follows: *sup-101, -102, -103, -104, -105, -107*—normal (type 1); *sup-106, -108, -109, -110*—pigmented (type 2).

(iii) *Crosses of revertants with wild type*

To show that prototrophy was caused by suppressor mutations, rather than backmutations at the *methB* gene, the ten isolates were crossed with wild type. The results are presented in Table 1. These data show that in all ten isolates, prototrophy is due to mutation in suppressor genes unlinked to *methB*. In each of the crosses involving *sup-106, sup-108, sup-109* and *sup-110,* approximately 50 % of the progeny showed the same pigmentation as the parental suppressed strains, indicating that pigmentation is produced in both *methB3sup* and *meth*$^+$*sup* genotypes. *Sup-108* is closely linked to *proA1* and *pabaA1*, since out of the 122 progeny scored, 118 were of parental genotype, three showed recombination between *proA1* and *pabaA1*, and one between *sup-108* and *proA1.* No linkage was detected between any of the other suppressor genes and *pyro-4, w-3, proA1* or *pabaA1.*

Table 1. *Crosses of prototrophs with wild-type strains*

Cross	No. progeny tested	Percentage meth+	χ^{2*}
1. *y pyro-4 methB3 sup-101* × *adE20 bi-1 w-2*	158	75	0·01
2. *y pyro-4 methB3 sup-102* × *proAl pabaAl y w-3*	103	73	0·26
3. *y pyro-4 methB3 sup-103* × *proAl pabaAl y w-3*	81	63	6·26**
4. *y pyro-4 methB3 sup-104* × *proAl pabaAl y w-3*	97	71	0·77
5. *y pyro-4 methB3 sup-105* × *proAl pabaAl y w-3*	98	70·5	1·10
6. *y pyro-4 methB3 sup-106* × *proAl pabaAl y w-3*	94	72·3	0·35
7. *y pyro-4 methB3 sup-107* × *proAl pabaAl y w-3*	108	76	0·05
8. *y pyro-4 methB3 sup-108* × *proAl pabaAl y w-3*	122	71·2	0·89
9. *y pyro-4 methB3 sup-109* × *proAl pabaAl y w-3*	109	68	2·94
10. *y pyro-4 methB3 sup-110* × *proAl pabaAl y w-3*	154	76	0·08

* χ^2, 1 degree freedom, calculated on hypothesis that prototrophy is due to an unlinked suppressor mutation.

** Probability between 0·05 and 0·01. All others greater than 0·05.

(iv) *Dominance recessivity tests on suppressors*

The ten suppressed isolates were each tested in heterokaryons with the original *methB3* strain. Full growth on MM (i.e. the same as that of the heterokaryon homogenic for the suppressor) would indicate that the suppressor is dominant; absence of growth would indicate that the suppressor is recessive. Heterokaryons homogenic for *sup-101*, *-102*, *-103*, *-104*, *-105*, *-106* and *-107* were also tested together with heterokaryons *methB3/methB3*, and *methB3/methB3*$^+$. The results of these tests are shown in Table 2. As expected, heterokaryon 7, homozygous for *methB3*, does not grow on MM. Heterokaryon 8 shows full growth on MM, indicating that *methB3* is fully recessive. The tests indicate that *sup-101*, *sup-106*, *sup-108*, *sup-109* and *sup-110* are fully recessive. Since heterokaryons heterogenic for *sup-102*, *sup-103*, *sup-104*, *sup-105* and *sup-107* (heterokaryons 4) grow at a rate between that of *methB3 sup/methB3 sup* and *methB3 sup*$^+$*/methB3 sup*$^+$, these five suppressors are semi-dominant. In the case of *sup-103* the effect was shown in three different heterokaryons, with different combinations of forcing and colour makers (heterokaryons 4, 4*a*, 4*b*). Table 2 also shows that heterokaryons 1 and 5, homozygous for *sup-101* and *sup-106* respectively, show slightly less growth on MM than on MM + methionine, whereas heterokaryons 3, homozygous for *sup-102*, *-103*, *-104*, *-105* or *-107* show equal amounts of growth on both media. This was anticipated since the haploid strains *methB3 sup-101* and *sup-106* strain grow less well on MM than on MM + methionine, while *methB3sup-102* and *sup-103*, *-104*, *-105* and *-107* grow at the same rate.

As expected, the diploid *methB3/methB3*$^+$ grew as well on MM as on MM + methionine, whereas the diploid *methB3/methB3* grew hardly at all. Diploids homozygous for *sup-102*, *sup-103*, *sup-104* and *sup-105* grew on MM, but not as well as on MM + methionine. This result was unexpected because heterokaryons homogenic for these suppressors grew as well on MM as on MM + methionine, and also the haploid suppressed strains showed no difference in growth on MM and MM + methionine. No hypothesis has been proposed for this difference.

When diploids heterozygous for *sup-102*, *-103*, *-104*, *-105*, *-107* and *-108* were tested on MM by a number of mass conidial transfers, a variable proportion of inocula of each diploid grew. Those inocula which grew often showed obvious sectoring. These observations suggested that although the heterozygous diploids were unable to grow on MM, they segregated to produce a genotype which permitted growth. Therefore, diploids were tested by at least five conidial inoculations, and also by spreading dilute conidial suspensions on MM + methionine and MM to

Table 2. *Dominance recessivity tests on suppressors in the heterokaryon*

	Genotype of heterokaryon	Growth on: Methionine	Growth on: MM	Interpretation
1.	*methB3 sup-101/methB3 sup-101*	+ + + +*	+ + +	.
2.	*methB3 sup-101/methB3 sup-101*$^+$	+ + + +	−	*sup-101* recessive
2a.	*methB3 sup-101*$^+$*/methB3 sup-101*	+ + + +	−	*sup-101* recessive
3.	*methB3 sup-102/methB3 sup-102* (and heterokaryons homogenic for *sup-103*, *-104*, *-105* and *-107*)	+ + + +	+ + + +	.
4.	*methB3 sup-102/methB3 sup102*$^+$ (and heterokaryons heterogenic for *sup-103*, *-104*, *-105* and *-107*)	+ + + +	+ +	*sup-102*, *-103*, *-104*, *-105* and *-107* semi-dominant
4a.	*methB3 sup-103*$^+$*/methB3 sup-103*	+ + + +	+ +	*sup-103* semi-dominant
4b.	*adG14 y pyro-4 methB3 sup*$^+$*/ proA1 pabaA1 Y bi-1 w-3 methB3 sup-103*	+ + + +	+ +	*sup-103* semi-dominant
5.	*methB3 sup-106/methB3 sup-106*	+ + + +	+ + +	.
6.	*methB3 sup-106/methB3 sup-106*$^+$ (and heterokaryons heterogenic for *sup-108*, *-109* and *-110*)	+ + + +	−	*sup-106*, *-108*, *-109* and *-110* recessive
7.	*methB3 sup*$^+$*/methB3 sup*$^+$	+ + + +	−	.
8.	*methB3/methB3*$^+$	+ + + +	+ + + +	*methB3* fully recessive

Except in heterokaryon 4b, first component carries additional markers *y pyro-4*, second carries *proAl pabaAl Y bi-1 w-3* (or *proAl pabaAl y w-3*).

* Radial growth in 120 h: + + + +, 20–28 mm; + + +, 15–20 mm; + +, 5–13 mm; − < 3 mm.

give about 20–100 viable spores per plate. This second method gave clear results. In the case of diploids homozygous for a suppressor, about equal numbers of colonies developed on both media, whereas in the case of heterozygous diploids, colonies rarely developed on MM. From these tests it was concluded that *sup-101*, *-102*, *-103*, *-104*, *-105*, *-106*, *-107* and *-108* are recessive in the diploid. Although *sup-109* and *-110* have not been tested in singly heterozygous diploids, they have been shown to be recessive in diploids heterozygous for two different suppressors, as shown later in Table 3. In summary, *sup-101*, *sup-106*, *sup-108*, *sup-109* and *sup-110* are recessive in the heterokaryon and diploid, whereas *sup-102*, *sup-103*, *sup-104*, *sup-105* and *sup-107*, although recessive in the diploid, are semi-dominant in the heterokaryon.

The exact level of semi-dominance of *sup-103* has been measured in heterokaryons, and the results are shown in Fig. 1; measurements on equivalent diploids

are given in Fig. 2. In Fig. 2, it should be noted that the results for the diploid *sup-103/sup*$^+$ on MM are expressed as highest and lowest readings, as opposed to all other readings in Figs. 1 and 2, which are expressed as averages. The lowest reading is assumed to indicate growth of the original heterozygous genotype, *sup-3/sup*$^+$, while the highest reading is growth of the homozygous diploid *sup-3/sup-3* which is selected on MM. The reason for these assumptions is given in the next paragraph.

There are two mechanisms by which heterozygous diploids could segregate to give a genotype which would give growth on MM. They are: (1) mitotic crossing

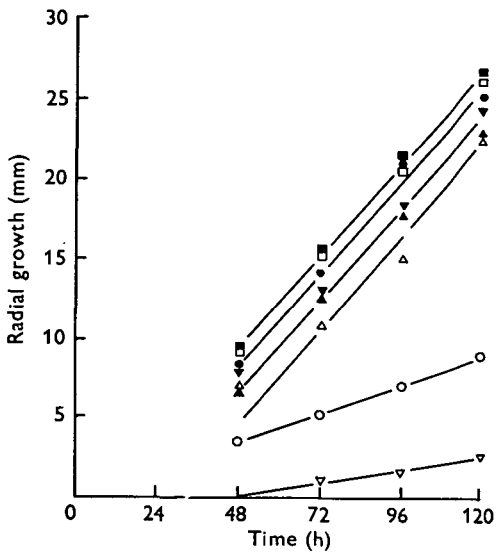

Fig. 1

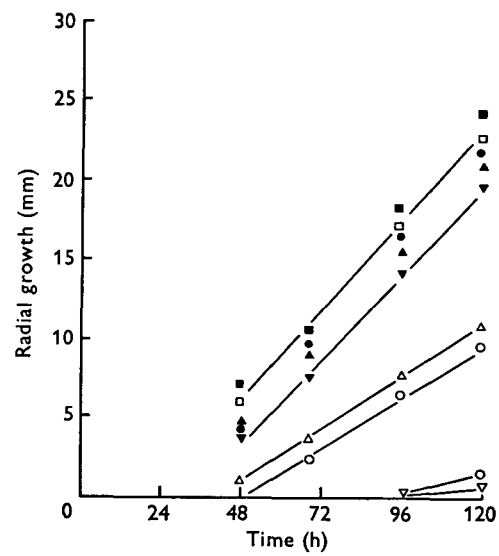

Fig. 2

Fig. 1. Growth of heterokaryons on MM + methionine, and on MM. ●, ○, *sup-103/sup*$^+$; ▲, △, *sup-103/sup-103*; ▼, ▽, *methB3/methB3*; ■, □, *methB3/meth*$^+$. Closed points, MM + methionine. Open points MM. All readings are averages for five colonies.

Fig. 2. Growth of diploids on MM + methionine, and on MM. ●, ○, *sup-103/sup*$^+$; ▲, △, *sup-103/sup-103*; ▼, ▽, *methB3/methB3*; ■, □, *methB3/meth*$^+$. Closed points, MM + methionine. Open points, MM. All readings averages for four colonies, except for *sup-103/sup*$^+$ on MM, where highest and lowest of four values are given.

over, leading to homozygosity for the suppressor, (2) breakdown of the diploid to give haploid segregants carrying the *sup* rather than the *sup*$^+$ allele. To investigate whether either mechanism was operating here, five inocula from diploids *sup-102/sup*$^+$ and *sup-104/sup*$^+$ growing on MM from mass conidial inocula were found to be still diploid on conidial diameter measurements. Conidia from these two diploids on MM, and on MM + methionine, were plated on CM + para-fluorophenylalanine, in order to induce haploidization (Morpurgo, 1961). The resulting haploid segregants were tested to see whether they required methionine or not. The results indicated that, at the time the conidia were sampled for plating on para-fluorophenylalanine, both diploids on MM + methionine were heterozygous for the suppressor (for diploid *sup-102/sup*$^+$ the allele ratio was 4 *sup-102* to 4 *sup*$^+$, for diploid

sup-104/*sup*$^+$ the ratio was 20 *sup-104* to 3 *sup*$^+$). On MM, both diploids were homozygous for the *sup* allele (for the diploid originally *sup-102*/*sup*$^+$ the allele ratio was 18 *sup-102* to 0 *sup*$^+$, for diploid *sup-104*/*sup*$^+$ the ratio was 5 *sup-104* to 0 *sup*$^+$). These results suggest that the heterozygous diploids undergo mitotic recombination which leads to homozygosity for the suppressor.

(v) *Complementation tests between suppressors*

From crosses of the type *y pyro-4 methB3 sup* × *proA1 pabaA1 Y bi-1 w-3 methB3*, suppressors 101–107 were isolated with markers *proA1 pabaA1 Y bi-1 w-3 methB3* (in the case of *sup-102*, *sup-103*, *sup-104*, *sup-105* and *sup-107*) or *proA1 pabaA1 yw-3 methB3* (in the case of *sup-101* and *sup-106*). The suppressors were tested against each other for complementation in heterokaryons, and the results are presented in Table 3. In the case of heterokaryons between two fully recessive suppressors, absence of growth on MM indicates that the suppressors are complementary; growth indicates that they are non-complementary (that is, the reverse situation from that found with auxotrophic markers). With heterokaryons where one or both suppressors is semi-dominant, partial growth (5–13 mm in 120 h) indicates complementation, whereas full growth (20–28 mm) indicates non-complementation.

Heterokaryons between non-complementing suppressors and those homogenic for the same suppressor (Fig. 1) showed 20–28 mm radial growth in 120 h on MM, whereas heterokaryons between different semi-dominant suppressors showed 5–13 mm growth. This applied to all heterokaryons except those where both components were of the pigmented type. The heterokaryons *sup-106*/*sup-106* and *sup-106*/*sup-109* grew to between 15 and 20 mm radius in 120 h on MM, whereas *sup-106*/*sup-108* and *sup-106*/*sup-110* showed less than 2 mm radial growth in the same period.

From the data the suppressors can be divided into five complementation groups comprising (1) *sup-101*, (2) *sup-102* and *-105*, (3) *sup-103*, *-104* and *-107*, (4) *sup-106* and *-109*, (5) *sup-108* and *-110*. (Although complementation tests have not been carried out between *sup-108* and *-110*, they are certainly alleles of different genes since *sup-108* is closely linked to *proA1*, unlike *sup-110*.)

Twenty-two of the combinations of suppressors tested in the heterokaryon were further tested in the diploid, as indicated in Table 3. The tests were performed in the same way as those on heterozygous diploids, i.e. by plating dilute spore suspensions to obtain colonies from single conidia. With one exception, the results were in agreement with the heterokaryon tests. In particular, those combinations of suppressors which were non-complementary in the heterokaryon (*sup-102*/*sup-105*, *sup-104*/*sup-103*, *sup-107*/*sup-103*, and *sup-103*/*sup-107*, *sup-104*/*sup-107*) were also non-complementary. In the one exceptional case, the diploid between *sup-103* and *sup-105* grew on MM; the diploid obviously needs analysing to confirm that it is still heterozygous for both suppressors.

Table 3. *Complementation tests between suppressors 101 to 110 on MM*

proA1 pabaA1 Y6 i-1 methB3	*y pyro-4 methB3*									
	sup-101	*sup-102*	*sup-105*	*sup-103*	*sup-104*	*sup-107*	*sup-106*	*sup-109*	*sup-108*	*sup-110*
sup-101	+ + +*	+ +*	+ +*	+ +*	+ +	+ +*	.	.	.	.
sup-102	.	+ + + +*	.	+ +*	+ +*	+ +	+ +	+ +	.	+ +*
sup-105	.	+ + + +*	+ + + +*	+ +*	+ +	+ +	.	+ +	.	+ +*
sup-103	.	.	.	+ + + +*	+ + + +*	+ + + +*	+ +	.	.	.
sup-104	.	.	.	+ + + +	+ + + +*	+ + + +	.	.	.	.
sup-107	.	.	.	.	+ + + +*	+ + + +*	+ +*	.	.	.
sup-106	.	.	.	.	.	.	+ + +	+ + +	−*	−*
sup-109	.	.	.	.	.	.	.	.	.	.
sup-108	.	.	.	.	.	.	.	.	.	.
sup-110	.	.	.	.	.	.	.	.	.	.

* Twenty-two combinations of suppressors were also tested in diploids. See text.

Radial growth in 120 h on MM: + + + +, 20–28 mm; + + +, 15–20 mm; + +, 5–13 mm; −, < 3 mm.

(vi) *Crosses between suppressors*

Since one of the objects of this investigation was to see whether there were combinations of non-allelic suppressors which failed to complement, crosses were analysed between suppressors from the same complementation groups. Crosses between suppressors in different groups were also analysed, to check the reliability of the complementation tests previously described. The results are summarized in Table 4. Crosses 1–9 confirm the results of the complementation tests in Table 3.

Table 4. *Crosses between different suppressors*

Cross	No. progeny tested	Percentage *meth*$^+$	χ^2 (* where appropriate)
1. *sup-102* × *sup-101*	77	76·5	0·11
2. *sup-105* × *sup-101*	128	85·0	6·0**
3. *sup-103* × *sup-101*	101	74·3	0·03
4. *sup-104* × *sup-101*	113	70·0	1·56
5. *sup-107* × *sup-101*	124	76·5	0·17
6. *sup-103* × *sup-105*	99	75·8	0·03
7. *sup-103* × *sup-102*	99	64·6	5·66**
8. *sup-104* × *sup-105*	131	66·5	5·15**
9. *sup-107* × *sup-105*	132	75·7	0·04
10. *sup-102* × *sup-105*	250	100	.
11. *sup-103* × *sup-107*	120	100	.

* χ^2 Calculated on hypothesis that two different, unlinked suppressors are involved.
** Probability between 0·05 and 0·01. All other probabilities greater than 0·05.

No recombination was detected between *sup-102* and *sup-105*, or *sup-103* and *sup-107*, indicating that these suppressors are almost certainly allelic. (These data by themselves do not rigorously exclude the possibility that the pairs of non-complementary suppressors are mutants in different, closely linked genes.) Unfortunately several attempts to obtain fertile perithecia from crosses between *sup-103* and *sup-104*, and *sup-104* and *sup-107* failed. It is therefore still possible that *sup-104* is non-allelic with *sup-103* and *sup-107*.

(vii) *Specificity of suppressor action*

To investigate whether the suppressors would act on methionine mutants other than the ones from which they were isolated, crosses of the following type were analysed:

$$\frac{methB3}{+} \quad \frac{sup}{+} \quad \frac{+}{methG1}.$$

If the suppressor is unlinked to the *meth* mutant against which it is being tested, then eight genotypes would be produced in equal frequencies as shown in Table 5.

Therefore suppression of one *meth* mutant gives 37·5% ($\frac{3}{8}$) prototrophy (genotypes 1, 4 and 6), whereas suppression of both *meth* mutants gives 62·5% ($\frac{5}{8}$) prototrophy (genotypes 1, 4, 5, 6 and 7). The effect of linkage between the suppressor and the second *meth* gene would be to give a value between 37·5% and

50 % in the former case, and 50 % and 62·5 % in the latter. The exact values between these limits would be directly proportional to the recombination values. The results of crosses between *methB3 sup-101* to *sup-107* with *methG1*, and *methB3 sup-101* to *sup-107* with *methH102* are given in Table 6.

Table 5. *Genotypes and phenotypes produced by a cross between* methB3 sup *and* methH2

Genotype	Growth on MM with suppression of: *methB3*	Growth on MM with suppression of: *methB3* and *methG1*
1. *methB3 sup* +	+	+
2. + + *methG1*	−	−
3. *methB3* + *methG1*	−	−
4. + *sup* +	+	+
5. *methB3 sup methG1*	−	+
6. + + +	+	+
7. + *sup methG1*	−	+
8. *methB3* + +	−	−

The results clearly indicate that *methG1* is suppressed by *sup-102*, *sup-103*, *sup-104*, *sup-105* and *sup-106* (crosses 2, 3, 4, 5 and 6), whereas *methH2* is not suppressed by *sup-102*, *sup-104*, *sup-106* or *sup-107* (crosses 8, 10, 12 and 13). The remaining crosses in Table 6 are more difficult to interpret. The data from crosses 1 and 7 do not indicate whether *sup-101* can suppress *methG1* or *methH2*. Although linkage between the suppressor and one of the methionine mutants could result in 50 % prototrophy as already described, it obviously could not explain both results since *methG1* and *methH2* are on different chromosomes. Crosses 9 and 11 testing the action of *sup-103* and *sup-105* on *methH2*, where again the data do not show good fits to either 37·5 or 62·5 % prototrophy, cannot be explained by linkage because crosses 8, 10 and 13 involving alleles of these two suppressors, give normal results. In fact the data from crosses 1, 7, 9 and 11 give good fits to a hypothesis of 50 % prototrophy. It is possible that *sup-101* can suppress *methB3*, *methG1* or *methH2*, but not the double mutants: this would result in 50 % prototrophy. In the cases of *sup-103* and *sup-105* it would be necessary for *methB3*, *methG1*, *methH2* and the double mutant *methB3 methG1* to be suppressed, but not the double mutant *methB3 methH2*. Further investigation is needed on these points.

(viii) *Conidial ratios in heterokaryons involving semi-dominant suppressors*

In the heterokaryon, nuclear ratios, and hence allele ratios, can vary within the limits imposed by the forcing markers. Consequently, in a heterokaryon heterogenic for a suppressor, it might be possible for an adjustment of the nuclear ratio to occur on MM in favour of the suppressor allele. This would result in a suppressor gene, which was recessive in the diploid, showing a degree of dominance in the heterokaryon. It is possible to test this hypothesis of nuclear adjustment since it

seems likely that nuclear ratios are in close agreement with conidial ratios (Clutterbuck & Roper, 1966).

Conidial ratios in heterokaryons heterogenic for *sup-103* growing on MM + methionine, and MM, are given in Table 7. In the two heterokaryons *y pyro-4 methB3 sup-103/proA1 y w-3 methB3* and *y pyro-4 methB3 proA1 pabaA1 y w-3 methB3 sup-103* on MM + methionine, the results suggest that the component nuclei are present in equal numbers, whereas on MM, there is a highly significant excess of the nuclei carrying the active suppressor allele. A similar situation was found for the

Table 6. *Analysis of crosses between suppressed* methB3 *strains, and* methG1 *and* methH2

Cross	No. progeny tested	Percentage *meth*+	χ^{2}**
1. *methG1* × *methB3 sup-101*	216	54	$\frac{5}{8}$ = 7·1**; $\frac{1}{2}$ = 1·18
2. *methG1* × *methB3 sup-102*	212	65·5	$\frac{5}{8}$ = 1·64
3. *methG1* × *methB3 sup-103*	365	59·5	$\frac{5}{8}$ = 1·4
	214	56	$\frac{5}{8}$ = 2·41
4. *methG1* × *methB3 sup-104*	215	56	$\frac{5}{8}$ = 3·3
5. *methG1* × *methB3 sup-105*	214	60	$\frac{5}{8}$ = 0·45
6. *methG1* × *methB3 sup-106*	214	59	$\frac{5}{8}$ = 0·98
7. *methH2* × *methB3 sup-101*	137	49·5	$\frac{5}{8}$ = 8·6**; $\frac{1}{2}$ = 0·08
8. *methH2* × *methB3 sup-102*	162	42·5	$\frac{3}{8}$ = 1·15
9. *methH2* × *methB3 sup-103*	124	47·5	$\frac{3}{8}$ = 5·37**; $\frac{1}{2}$ = 0·52
10. *methH2* × *methB3 sup-104*	215	40	$\frac{3}{8}$ = 0·85
11. *methH2* × *methB3 sup-105*	107	47·8	$\frac{3}{8}$ = 6·1**; $\frac{1}{2}$ = 0·36
12. *methH2* × *methB3 sup-106*	216	40	$\frac{3}{8}$ = 0·08
13. *methH2* × *methB3 sup-107*	207	38	$\frac{3}{8}$ = 0·06

* χ^{2} Calculated on hypothesis that expected fraction of prototrophs is $\frac{3}{8}$, $\frac{5}{8}$, or $\frac{1}{2}$, as indicated.
** Probability less than 0·05. All other probabilities greater than 0·05.

Table 7. *Conidial ratios in heterokaryons heterogenic for semi-dominant suppressors*

Heterokaryon	Medium	No. colonies scored	y (%)	w (%)	χ^{2}***
sup-103/+ *	Methione	999	48·5	51·5	58·89,
	MM	903	66·1	33·9	$P < 0·01$
+/*sup-103*	Methionine	362	50	50	26·37,
	MM	146	25·2	74·8	$P < 0·01$
sup-107/+	Methionine	159	35·8	64·2	16·96,
	MM	479	54·5	45·5	$P < 0·01$
+/*sup-107*	Methionine	332	31·3	68·7	6·83,
	MM	246	21·5	78·5	$P < 0·01$

Heterokaryons were established on MM + methionine, and inoculated on to MM + methionine, and MM. Dense conidial suspensions were prepared after 120 h growth, and dilutions spread on CM.

* First component of heterokaryon carries additional markers *y pyro-4 methB3*, second carries *proA1 pabaA1 y w-3 methB3*.

** Contingency χ^{2} value, 1 D.F.

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two heterokaryons heterogenic for *sup-107* although on MM + methionine, both these heterokaryons showed an excess of the nuclei carrying the white spore colour marker. These results are therefore compatible with the hypothesis of adjustment of nuclear ratio as a mechanism for semi-dominance in heterokaryons.

4. DISCUSSION

The absence of mutants responding to cystathionine in *Aspergillus* could indicate either that cystathionine is not an intermediate in methionine synthesis in this organism, or that it is unable to enter the cell when supplied in the medium. In *Neurospora*, Horowitz (1947) isolated cystathionine-requiring mutants, and more recently Kerr & Flavin (1968) have shown that in fresh extracts of *Neurospora* there is a rapid synthesis of cystathionine, which is known to be synthesized from *O*-acetylhomoserine and cysteine (Nagai & Flavin, 1967). Mutants in the *me-3* and *me-7* genes of *Neurospora* which respond to cystathionine lack this enzymic activity (Kerr & Flavin, 1968). It is possible in *Aspergillus* that cystathionine is by-passed in methionine synthesis, and that homocysteine is synthesized directly from *O*-acetylhomoserine and sulphide, by a reaction similar to that demonstrated to occur at a low rate in *Neurospora* (Kerr & Nagai, 1967 and Wiebers & Garner, 1967). These latter authors suggest that this reaction plays a significant role in methionine production in *Neurospora*.

The ten suppressors of *methB3*, blocked before homocysteine, have been divided into five complementation groups on the basis of heterokaryon, and, in some cases, diploid, complementation tests: (1) *sup-101*; (2) *sup-102*, *sup-105*; (3) *sup-103*, *sup-104*, *sup-107*; (4) *sup-106*; (5) *sup-108*, *sup-110*. Complementation groups 1, 2 and 3 correspond to suppressors of type 1 morphology, and groups 4 and 5 to type 2 morphology, in the terminology of Gajewski & Litwińska (1968). *Sup-108* and *sup-110* are certainly non-allelic, since *sup-108* is closely linked to *proA1* on chromosome I, whereas *sup-110* shows no linkage with *proA1*, so that a total of at least six different suppressor genes can be distinguished. Gajewski & Litwińska (1968) have classified eight type 1 suppressors into two complementation groups, and seventeen type 2 suppressors into five groups, so that in the present work an additional type 1 complementation group has been identified.

The large number of suppressor genes suggests that the *methB3* suppressor system is very complex, but since no enzyme studies have yet been carried out on methionine mutants in *Aspergillus nidulans*, it is difficult to discuss possible mechanisms of suppression. Four suppressor genes are able to act on *methG1*, which like *methB3* can utilize homocysteine, but not on *methG2*, which responds only to methionine. The cross suppression data of Gajewski & Litwińska show that in fact three different *methB* suppressor genes can act on 24 mutants at four different *meth* loci blocked before, but not on the two loci after, homocysteine. This degree of non-specificity rules out any hypothesis of suppression based upon correction at the translational level; suppressors of nonsense or missense mutations (Benzer & Champe, 1962; Yanofsky & St Lawrence, 1960) are highly allele-specific and would

not act on all the mutants before homocysteine, also they would be dominant in the heterozygous diploid. It is much more likely that these suppressors act by opening an alternative pathway so that the original lesions in the methionine pathway are by-passed. Suppressors of acetate mutants in *Neurospora crassa* are thought to operate in this way (Lein & Lein, 1952; Strauss & Pierog, 1954) and a similar mechanism has been postulated for cysteine suppressors of *cysA* and *cysF* in *Salmonella typhimurium* (Howarth, 1958).

Of the three different type 1 suppressor genes, the two with wild-type growth on MM were semi-dominant in heterogenic heterokaryons, while the third, which had a reduced growth rate, was recessive. All three suppressors were recessive in the diploid. Gajewski & Litwińska (1968) showed that eight mutants belonging to two type 1 suppressor genes were also semi-dominant in heterokaryons, but do not report on their behaviour in diploids, so it is not possible to say whether these suppressors behave differently in heterokaryons and diploids. The data on conidial ratios in heterokaryons *sup-103*/$^+$ and *sup-107*/$^+$ suggest that an adjustment on MM in favour of the *sup-103* and *sup-107* nuclei may be responsible for the semi-dominance effect. It is possible that irregular distribution of nuclei within heterogenic heterokaryons accentuates the semi-dominance, since these heterokaryons had a noticeably more irregular distribution of conidial colour on MM than on MM + methionine. This irregularity did not indicate that these heterokaryons were breaking down on MM because they grew normally when transferred back to MM + methionine. In spite of this suggestive evidence, it is not possible to exclude the possibility that the suppressor product is nuclear (as has been done in the case of a methionine suppressor in *Coprinus lagopus* (Casselton & Lewis, 1967)). The diploid cannot be compared with a dikaryon since *Aspergillus* does not possess a persistent dikaryotic phase.

Unlike the situation in *C. lagopus* (Lewis, 1961), there is no evidence in the present work of non-complementation between different suppressor genes. In *Coprinus*, three methionine suppressor genes, two of which were loosely linked, unexpectedly failed to complement in pairwise combinations in the dikaryon. Subsequent work (Casselton & Lewis, unpublished) has shown that all these combinations of suppressors also fail to complement in diploids. In the present work, out of four cases of non-complementation between suppressors in heterokaryons (*sup-102*/*sup-105*, *sup-103*/*sup-107*, *sup-103*/*sup-104* and *sup-106*/*sup-109*), the combinations of mutants in each of the first two heterokaryons have been shown to be allelic, and unfortunately, the second two heterokaryons have failed to yield fertile perithecia.

SUMMARY

Nine methionine mutants of *Aspergillus nidulans* (six new mutants plus three isolated previously) were examined; five responded to homocysteine, none responded to cystathionine or cysteine.

Ten revertants of one of the mutants, *methB3*, blocked before homocysteine, were shown to be due to suppressor mutations. The suppressors were divided into

six genes on the basis of complementation tests and recombination data. Mutants of two of the genes were semi-dominant in the heterokaryon but recessive in the diploid. Experiments in which conidial ratios in the heterokaryons were determined suggested that semi-dominance of these suppressors is due to a shift in nuclear ratios in the heterokaryon in favour of the suppressor nuclei.

Some of the suppressors were tested for suppression of two other methionine loci; they acted on *methG1* blocked before homocysteine, but not on *methH2* blocked after homocysteine, although four out of 13 crosses tested gave ambiguous results.

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REFERENCES

Alderson, T. & Clark, A. M. (1966). Interlocus specificity for chemical mutagens in *Aspergillus nidulans*. *Nature, Lond.* **210**, 593–595.

Benzer, S. & Champe, S. P. (1962). A change from nonsense to sense in the genetic code. *Proc. natn. Acad. Sci. U.S.A.* **48**, 1114–1121.

Casselton, L. A. & Lewis, D. (1967). Dilution of gene products in the cytoplasm of heterokaryons in *Coprinus lagopus*. *Genet. Res., Camb.* **9**, 63–71.

Clutterbuck, A. J. (1968). Gene symbols and nomenclature: proposals and notes on them. *Asp. News Letter*, no. 9, pp. 26–29.

Clutterbuck, A. J. & Roper, J. A. (1966). A direct determination of nuclear distribution in heterokaryons of *Aspergillus nidulans*. *Genet. Res., Camb.* **7**, 185–194.

Clutterbuck, A. J. & Sinha, U. K. (1966). *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) as a mutagen for *Aspergillus nidulans*. *Asp. News Letter*, no. 7, pp. 12–13.

Gajewski, W. & Litwińska, J. (1967). Methionine mutants of *Aspergillus nidulans* and their suppressors. *Asp. News Letter*, no. 8, pp. 9–10.

Gajewski, W. & Litwińska, J. (1968). Methionine loci and their suppressors in *Aspergillus nidulans*. *Molec. Gen. Genetics* **102**, 210–220.

Horowitz, N. H. (1947). Methionine synthesis in *Neurospora*. The isolation of cystathionine. *J. biol. Chem.* **171**, 255–267.

Howarth, S. (1958). Suppressor mutations in some cystine-requiring mutants of *Salmonella typhimurium*. *Genetics* **43**, 404–418.

Kerr, D. S. & Flavin, M. (1968). Synthesis of cystathionine from *O*-acetlyhomoserine in *Neurospora*: a step in methionine biosynthesis. *Biochem. biophys. Res. Commun.* **31**, 124–130.

Kerr, D. & Nagai, S. (1967). Enzymic formation of acetylhomoserine and its utilization for methionine synthesis. *Fedn Proc.* **26**, 387.

Lein, J. & Lein, P. S. (1952). Studies on a suppressor of non-allelic acetate requiring mutants of *Neurospora*. *Proc. natn. Acad. Sci. U.S.A.* **38**, 44–48.

Lewis, D. (1961). Genetic analysis of methionine suppressors in *Coprinus*. *Genet. Res., Camb.* **2**, 141–155.

Lilly, L. J. (1965). An investigation of the suitability of the suppressors of *meth 1* in *Aspergillus nidulans* for the study of induced and spontaneous mutation. *Mutation Res.* **2**, 192–195.

Mackintosh, M. E. & Pritchard, R. H. (1963). The production and replica plating of microcolonies of *Aspergillus nidulans*. *Genet. Res., Camb.* **4**, 320–322.

MORPURGO, G. (1961). Somatic segregation induced by *p*-fluoro-phenylalanine (PFP). *Asp. News Letter*, no. 2, p. 10.

NAGAI, S. & FLAVIN, M. (1967). Acetylhomoserine. An intermediate in the fungal biosynthesis of methionine. *J. biol. Chem.* **242**, 3884–3895.

PONTECORVO, G. (1963). Microbial genetics: retrospect and prospect. *Proc. R. Soc.* B **158**, 1–23.

PONTECORVO, G., ROPER, J. A., HEMMONS, L. M., MACDONALD, K. D. & BUFTON, A. W. J. (1953). The genetics of *Aspergillus nidulans*. *Adv. Genet.* **5**, 141–238.

ROPER, J. A. (1952). Production of heterozygous diploids in filamentous fungi. *Experientia* **8**, 14–15.

SIDDIQI, O. H. (1962). Mutagenic action of nitrous acid on *Aspergillus nidulans*. *Genet. Res., Camb.* **3**, 303–314.

STRAUSS, B. S. & PIEROG, S. (1954). Gene interactions: the mode of action of the suppressor of acetate-requiring mutants of *Neurospora crassa*. *J. gen. Microbiol.* **10**, 221–235.

WIEBERS, J. L. & GARNER, H. R. (1967). Acyl derivatives of homoserine as substrates for homocysteine synthesis in *Neurospora crassa*, yeast and *Escherichia coli*. *J. biol. Chem.* **242**, 5644–5649.

YANOFSKY, C. & ST LAWRENCE, P. (1960). Gene action. *A. Rev. Microbiol.* **14**, 311–340.