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A new gene controlling sulphite reductase in *Aspergillus nidulans*

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Summary

A new gene designated *sG* was identified in *Aspergillus nidulans* by mutation affecting the enzyme sulphite reductase and leading to a strong derepression of arylsulphatase and enzymes constituting the alternative pathway of cysteine synthesis. The results indicate that proper physiological functioning of this pathway is strongly dependent on full activity of the sulphate assimilation pathway.

1. Introduction

Aspergillus nidulans, like many other fungi, possesses two pathways for *de novo* cysteine synthesis in which sulphide is an inorganic sulphur precursor (Fig. 1). A double enzymic block is necessary to obtain a cysteine-requiring auxotroph (Pieniazek *et al.* 1974; Paszewski & Grabski, 1975). For example, a double mutant *cysB1, mecB10* grows only on a cysteine-supplemented medium. The *cysB1* mutation apparently impairs the last step of cysteine synthesis, the sulphydrylation of *O*-acetylserine, catalysed by the enzyme cysteine synthase. It was found that another anzyme, homocysteine synthase (Fig 1, step 2) exhibits cysteine synthase activity *in vitro* (Paszewski *et al.* 1984), but apparently not *in vivo*, as otherwise the *cysB, mecB* strains would be cysteine prototrophs.

We have looked for suppressor mutations in a *cysB1, mecB10* strain leading to cysteine prototrophy. We expected that one type of suppressor may result from a mutation altering the structure of homocysteine synthase protein so that the enzyme has cysteine synthase activity *in vivo*, as is probably the case in *Saccharomyces cerevisiae* (Yamagata, Takeshima & Naiki, 1974). Another type of suppressor which can be envisaged is a mutation causing strong derepression of the cystathionine γ-lyase, such that its low activity due to a slightly leaky *mecB* mutation is compensated for by an increase in enzyme synthesis.

In this communication we describe a suppressor mutation of the latter type, in gene designated *sG*, which causes a significant reduction of sulphite reductase activity (Fig. 1, step 6) and a high elevation of levels of the enzymes involved in the alternative pathway of cysteine synthesis – homocysteine synthase, cystathionine β-synthase and cystathionine γ-lyase. It was found that the efficiency of this pathway is strongly dependent on full activity of the sulphate assimilation pathway.

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Material and Methods

(i) *Strains*

The following strains of Aspergillus nidulans from our collection were used: *cysB1, mecB10, phenA2, yA1*; *proA2, pabaA2, biA1*; *methH2, pyroA4, yA1*; *anA1, phenA2, biA1*; *pyroA4, yA1*. The last two strains were used as reference wild-type strains in enzyme assays and growth tests. Other strains were derived by standard crossing procedures described by Pontecorvo *et al.* (1953) and selection of segregants of desired genotype: *phen* (phenylalanine), *pro* (proline), *an* (aneurine), *paba* (para-aminobenzoic acid), *bi* (biotin), *met* (methionine), *Pyro* (pyridoxin), *cys* (cysteine). The symbol *mec* denotes methionine catabolism.

(ii) *Mutagenesis*

Cysteine-independent revertants of the *cysB1, mecB10* strain were selected following UV irradiation of conidia at 1–3% survival rate.

(iii) *Media, culture conditions and enzyme assays*

Liquid minimal medium described previously (Paszewski & Grabski, 1974), with appropriate supplements was used. Cultures were grown at 30 °C unless

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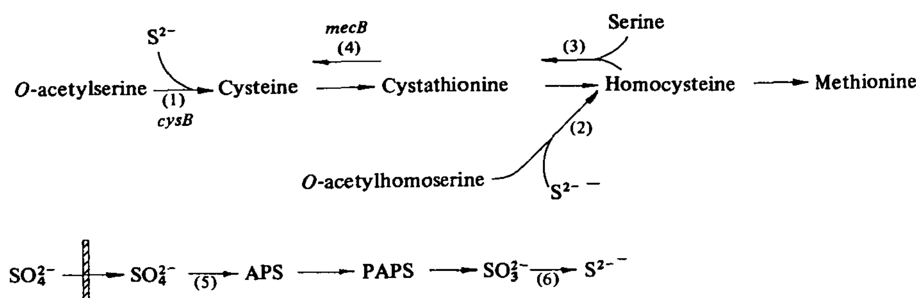

Fig. 1. Two pathways of cysteine synthesis in *Aspergillus nidulans*. Enzymes: (1) cysteine synthase (EC 4.2.99.8); (2) homocysteine synthase (EC 4.2.99.10); (3) cystathionine β-synthase (EC 4.2.1.22); (4) cystathionine γ-lyase (EC 4.4.1.1); (5) ATP-sulphurylase (EC 2.7.7.4); (6) sulphite reductase (EC 1.8.1.2).

otherwise stated. Preparation of cell-free extracts and enzyme assays were as described by Paszewski *et al.* (1984).

3. Results and Discussion

Two revertants of *cysB1. mecB10* strain able to grow without cysteine were obtained among 5×10^4 survivors of UV irradiation. One, which resulted from reversion of the *cysB* mutation, grew like the wild-type strain and was discarded. The second revertant, which grew more slowly than the wild type but much faster than the parental strain (Fig. 2), resulted from a new mutation, designated *sG8*, that leads to elevated levels of homocysteine synthase, cystathionine synthase and cystathionine γ-lyase (Table 1). These activities are the highest observed so far in *Aspergillus* strains (Paszewski & Grabski, 1975; Paszewski *et al.* 1984). The *sG8* strain exhibits a very high level of arylsulphatase and a low level of sulphite reductase (Table 2), but the latter enzyme reaches wild-type level when mycelia are grown at 24 °C. The *sG8* strains phenotypically strongly resemble the *sul-reg* strains, some of which carry leaky mutations in the sulphate assimilation pathway (Paszewski *et al.* 1984). The *sul-reg* mutants show elevated levels of the same enzymes as *sG8*, though to a lesser degree. It is therefore likely than the observed derepression of several enzymes of sulphur metabolism in *sG8* is a secondary effect of mutation affecting the sulphate assimilation pathway.

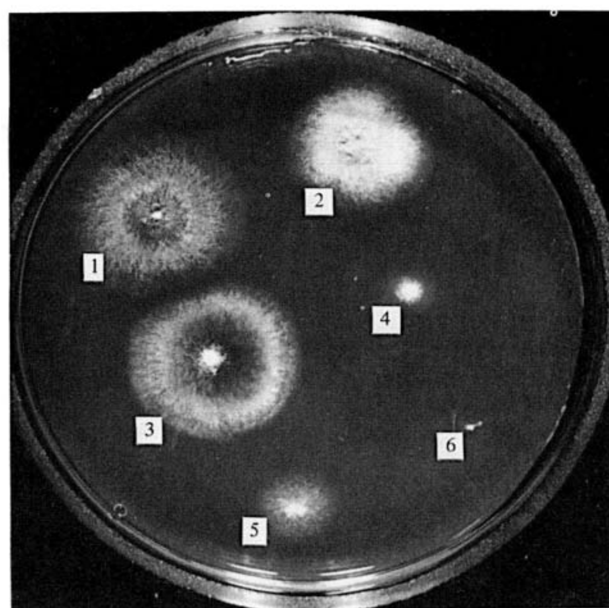

Fig 2. Growth of the following strains on minimal medium: (1) *sG8(phenA2, biA1, pabaA2)*; (2) *CysB1 (pyroA4, yA1)*; (3) wild type (*anA1, phenA2, biA1*); (4) *sG8, cysB1 (phenA2, yA1)*; (5) *cysB1, mecB10, sG8(phenA2, yA1)*; (6) *cysB1, mecB10 (phenA2, yA1)*. Colonies were grown at 37 °C for 48 h (1–4) or 96 h (5–6). The latter were inoculated into the plate 48 h before the former. Appropriate supplements were added to supplement the nutritional requirements (in parentheses) of the strains.

Table 1. *Activities of enzymes of the alternative pathway of cysteine synthesis in strains of various genotypes*

	Specific activity (nmole/min/mg protein)		
Relevant genotype	Homocysteine synthase	Cystathionine β-synthase	Cystathionine γ-lyase
Wild type	65·6 ± 9·9	1·67 ± 0·24	0·48 ± 0·10
sG8	379·0 ± 17·6	9·00 ± 1·20	1·77 ± 0·50
sG8, cysB1, mecB10	855·6 ± 108·9	9·70 ± 1·30	0·41 ± 0·04

The results represent the means of three or more experiments ± S.E. The levels of cystathionine γ-lyase in *mecB10* strains are 0·0–0·1 nmole/min/mg protein, but are imprecise because they are close to blank values.

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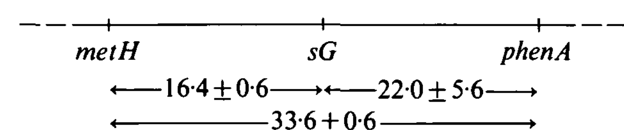
Table 2. *Activities of ATP-sulphurylase, arylsulphatase and sulphite reductase in* sG8 *and wild-type strains*

	Specific activity (nmole/min/mg protein)		
Strain	ATP-sulphurylase	Arylsulphatase	Sulphite reductase
sG8	132·6±27·9	247·0±29·3	0·24±0·18 (1·53±0·37)
Wild type	71·0±12·0	3·1±0·8	1·02±0·30 (1·33±0·27)

Values for cultures grown at 24 °C are given in parentheses. The results represent the means of three or more experiments±S.E.

It should be noted that the level of cystathionine γ-lyase in the *cysB1, mecB10, sG8* strain approaches that of the wild type, which is probably why the former is prototrophic for cysteine. It is very likely that this strain produces more enzyme than the wild type but of lower specific activity due to the *mecB10* mutation. It is of interest that a double mutant *cysBsG* grows much more slowly on a minimal medium than *cysB* and *sG* single mutants (Fig. 2). As growth of *cysB* depends entirely on the activity of homocysteine synthase, the first enzyme of the alternative pathway of cysteine synthesis (Fig. 1), poor growth of the double mutant could mean that the functioning of this pathway unlike that of the main one, is highly sensitive to a shortage of sulphide. *cysB*, *sG* strains are stimulated by sulphide and sulphite.

A high activity of arylsulphatase allows the identification of *G8* segregants by staining of colonies with indoxylsulphate (Paszewski *et al.* 1984). This technique helped in chromosome mapping of this mutation, which was found linked to *phenA* and *metH* loci located in chromosome III. From results of two- and three-point crosses involving these mutations the following map order was obtained:



The localization of the *sG* gene shows that it is distinct from previously known genes, *sA* to *sF*, also involved in the sulphate assimilation pathway (Clutterbuck, 1984). No linkage between *sG* gene and *sul-reg* loci was found. Mutation in one of these loci, *sul-regB2*, leads to a low level of sulphite reductase (Paszewski *et al.* 1984). In the progeny of a cross between the *sul-regB2* and *sG8* strains no sulphide-requiring auxotroph was found, but about 50 per cent of slower growing colonies (*sul-regB* and double mutants) were stimulated by methionine and cysteine and, after longer incubation, also by sulphide.

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