A new gene controlling sulphite reductase in 
Aspergillus nidulans

JANINA NADOLSKA-LUTYK* AND ANDRZEJ PASZEWSKI†
Institute of Biochemistry and Biophysics, Polish Academy of Sciences, 36 Rakowiecka, 02-532 Warsaw, Poland
(Received 2 June 1987 and in revised form 11 August 1987)

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 cysBl, mecB10 grows only on a cysteine-supplemented medium. The cysBl mutation apparently impairs the last step of cysteine synthesis, the sulphhydration of O-acetylserine, catalysed by the enzyme cysteine synthase. It was found that another enzyme, 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 cysBl, 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.

Material and Methods
(i) Strains
The following strains of Aspergillus nidulans from our collection were used: cysB1, mecB10, phenA2, yAl; proA2, pabaA2, biA1; metH2, pyroA4, yA1; anA1, phenA2, biA1; pyroA4, yA1. The last two strains were used as reference wild-type strains for 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 (anurine), 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
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 x 10⁴ 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.

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

| Relevant genotype | Specific activity (nmole/min/mg protein) |
|-------------------|----------------------------------------|
|                   | 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.
### Sulphite-reductase controlling gene in *A. nidulans*

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

| Strain  | ATP-sulphurylase (nmole/min/mg protein) | Arylsulphatase (nmole/min/mg protein) | Sulphite reductase (nmole/min/mg protein) |
|---------|----------------------------------------|--------------------------------------|----------------------------------------|
| 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 *cysB sG* 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:

```
metH  sG  phenA
|164 ± 0.6|220 ± 5.6|336 ± 0.6|
```

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-regB*, 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.

This work was supported by the Polish Academy of Sciences within the project CPBR 3-13.

**References**

Clutterbuck, A. J. (1984). Loci and linkage map of the filamentous fungus *Aspergillus nidulans* (Eidam) Winter (n = 8). *Genetic Maps* 3, 265–273.

Paszewski, A. & Grabski, J. (1984). Regulation of S-amino acids biosynthesis in *Aspergillus nidulans*: role of cysteine and homocysteine as regulatory effectors. *Molecular and General Genetics* 132, 307–320.

Paszewski, A. & Grabski, J. (1975). Enzymic lesions in methionine mutants of *Aspergillus nidulans*: role and regulation of an alternative pathway for cysteine and methionine synthesis. *Journal of Bacteriology* 124, 893–904.

Paszewski, A., Prazmo, W., Nadowska, J. & Regulski, M. (1984). Mutations affecting the sulphur assimilation pathway in *Aspergillus nidulans*: their effect on sulphur amino acid metabolism. *Journal of General Microbiology* 130, 1113–1121.

Pieniazek, N. J., Bal, J., Balbin, E. & Stepien, P. P. (1974). An *Aspergillus nidulans* mutant lacking serine trans-acetylase: evidence for two pathways of cysteine synthesis. *Molecular and General Genetics* 132, 363–366.

Pontecorvo, G., Roper, J.A., Hemmonds, L.M., McDonald, K.D. & Butlin, A.W. (1953). *Advances in Genetics* 5, 141–238.

Yamagata, S., Takeshima, K. & Naiki, N. (1974). Evidence for the identity of O-acetylseryl sulphydrolase with O-acetylserylhomoserine sulphydrolase in yeast. *Journal of Biochemistry* 75, 1221–1229.