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Mutations affecting cysteine synthesis in *Aspergillus nidulans*: characterization and chromosome mapping

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Summary

Selection and mapping of mutations affecting cysteine synthesis in *Aspergillus nidulans* was carried out. A new locus, *cysE*, is described, the mutants of which are deficient in *in vivo* conversion of O-acetylserine to cysteine, a step mediated by cysteine synthase. Three loci (*cysB*, *C* and *E*) were thus found to control this step *in vivo*, apparently without affecting the enzyme activity *in vitro*. By scoring for propargylglycine sensitivity of cys mutants, chromosomal map positions were obtained for all five cysteine loci (*A*, *B*, *C*, *D* and *E*).

1. Introduction

There are two pathways of *de novo* cysteine synthesis in *Aspergillus nidulans* (Pieniazek *et al.* 1974). Simultaneous impairment of both results in cysteine auxotrophy. Mutations affecting synthesis of cysteine from serine (Fig. 1, steps 1 and 2) were found to suppress mutations in loci *metA*, *metB* and *metG* (Paszewski & Grabski, 1975). This is due to de-repression of the alternative pathway of cysteine and methionine synthesis involving homocysteine synthase, cystathionine β-synthase and cystathionine γ-lyase (Fig. 1 steps 5, 6 and 7). The fact that this pathway is physiologically important only during malfunctioning of the one involving O-acetylserine as an intermediate indicates that the latter is the main route of cysteine synthesis in *Aspergillus*.

The prototrophic character of single mutants affecting either one of these pathways has hampered their allocation to linkage groups. Only the *mecA* and *mecB* genes were localized in chromosome I due to the fact that these mutants do not grow on methionine in the presence of selenate (Pieniazek *et al.* 1973). A recent observation that mutants blocked in the main pathway of cysteine synthesis are highly sensitive to propargylglycine (Piotrowska & Paszewski, 1986) was utilized in the present work in chromosome mapping of further genes involved in cysteine synthesis. We also report the identification of a new gene (*cysE*) apparently controlling O-acetylserine sulphhydrylation. This brings to three the number of genes involved in the control of cysteine synthase (EC 4.2.99.8) step *in vivo*. None of them can as yet be considered as the structural gene of the enzyme.

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2. Materials and methods

(i) *Strains*

The *cys* mutants studied in this work are listed in Table 1. Several strains carrying markers of chromosome II were kindly supplied by Professor H. N. Arst. Other strains used were from our collection. They carried markers of general use (Clutterbuck, 1984). The master strain FGSC 465 used to allocate genes to their linkage groups has the following chromosome markers: I, *sulA1*; *II AcrA1*; III, *galA1*; IV, *pyroA4*, V, *facA303*; VI, *lacA1* ; VII, *cholA1*; VIII, *chaA1*.

(ii) *Genetic analysis*

Genes were allocated to their linkage groups by mitotic haploidization (Forbes, 1959) facilitated by the use of benlate (Hastie, 1970). The positions of *cys* genes relative to other markers were established by crossing with strains carrying markers of relevant linkage groups.

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

The minimal medium described by Paszewski and Grabski (1974) with appropriate supplements was used. Cultures were grown at 33 °C for 16–18 h.

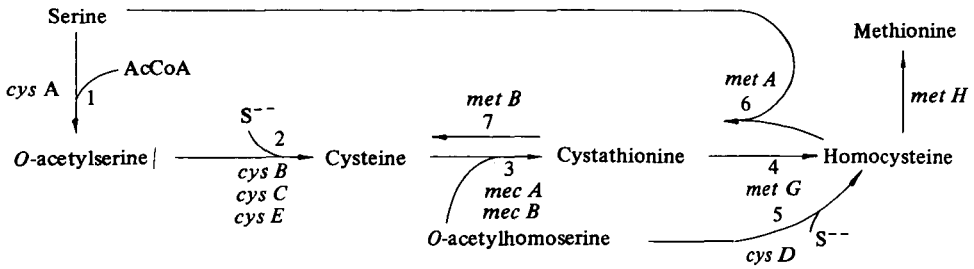

Fig. 1. An outline of sulphur amino acid biosynthetic pathways in *Aspergillus nidulans*. Enzymes: 1, serine transacetylase (EC 2 . 3 . 3 . 1 . 30); 2, cysteine synthase (EC 4 . 2 . 99 . 8): 3, cystathionine γ-synthase (EC 4 . 2 . 99 . 9) 4, cystathionine β-lyase (EC 4 . 4 . 1 . 8); 5, homocysteine synthase (EC 4 . 2 . 99 . 10); 6, cystathionine β-synthase (EC 4 . 2 . 1 . 22); 7, cystathionine γ-lyase (EC 4 . 4 . 1 . 1).

Table 1. *Mutations impairing cysteine biosynthesis in* Aspergillus nidulans

Locus and mutant number	Remarks	References
cysB102[cysB1]	Isolated as suppressor of *metB3* mutation	Paszewski & Grabski (1975)
cysB10[cysE1]	Isolated in *mecB10* strain, later found to be an allele of *cysB*	Bal *et al.* (1975) and This paper
cysB212	Isolated as suppressor of *metA17* mutation	This paper
cysB220	Isolated as suppressor of *met A17* mutation	This paper
cysC103[cysC1]	Isolated as suppressor of *metB3* mutation	Ayling, (1969), Paszewski & Grabski, (1975)
cysA1	Isolated in *mecB10* strain, lacks serine transacetylase	Pieniazek *et al.* (1974)
cysD11[cysD1]	Isolated in *cysB102* strain, lacks homocysteine synthase	Paszewski & Grabski, (1975)
cysE98	Isolated in *mecB10* strain	This paper
cysE121	Isolated in *mecB10* strain	This paper

The symbols used previously are given in parentheses. We are presently assigning new mutant numbers in order to conform to the rule proposed by Clutterbuck (1973) that no two mutants with the same primary gene symbol be given the same mutant number. In symbols *cysB102* and *cysC103* the original suppressor numbers designated by Ayling (1969) are retained.

Mycelial extracts and cysteine synthase activity assays were as described previously (Pieniazek *et al.* 1973)

(iv) *DEAE-cellulose chromatography*

The separation of cysteine synthase from homocysteine synthase on DEAE-cellulose was performed as described by Paszewski *et al.* (1984) except that the enzymes were eluted with a stepwise salt gradient.

3. Results and discussion

The synthesis of cysteine from serine involves two enzymatic steps (Fig. 1 steps 1 and 2). Serine transacetylase catalyzing the first step was found to be impaired in *cysA* strains (Pieniazek *et al.* 1973). The second step, sulphhydrylation of acetylserine, is apparently blocked in *cysB*, *cysC* and *cysE* mutants. When the *mecB* mutation (Fig. 1 step 7) is combined with any of the above mutations the resulting double mutants are auxotrophs which grow only on cysteine. This suggests that *cysB*, *C* and *E* mutants should lack cysteine synthase activity. However, this activity was present in extracts from all the mutants tested (Table 2). The strains used in these experiments carry the *cysD11* mutation which reduces considerably the level of homocysteine synthase. The latter enzyme exhibits also an *in vitro* cysteine synthase activity which hinders determination of the physiological cysteine synthase. With the possible exception of the *cysE98* strain, all double mutants exhibit a synthase level comparable to that of the *cysD* single mutant strain. When cell-free extracts from these strains are chromatographed on DEAE-cellulose, the cysteine

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synthase activity, which is not retained on the column under the conditions used, is found in all the extracts. A representative elution profile is shown in Fig. 2. The biochemical nature of the *cysB*, *C* and *E* mutations is not clear. None of them seems likely to be in the structural gene of cysteine synthase. However, no firm conclusion as to the nature of these genes is possible without further characterization of the enzyme from the mutant strains. Bal *et al.* (1975) presented evidence that the synthase is located in the mitochondria and shows mislocation in the *cysB10* mutant. It is, therefore, possible that these three genes are involved in processing the enzyme protein and its mitochondrial localization. They may also affect interaction with the true *in vivo* sulphhydryl donor for the enzyme, which might not be sulphide.

With the exception of *cysD*, all other *cys* mutants are highly sensitive to propargylglycine (Piotrowska & Paszewski, 1986). Their growth is completely inhibited by 1 μM concentration of the antibiotic, which strongly inhibits cystathionine γ-lyase. This property facilitated the chromosome mapping of the *cys A*, *B*, *C*, *D* and *E* genes (Fig. 3). It was fortunate that the *cysD* gene happened to be linked with *cysE*, since the *cysD* mutations are phenotypically wild type but the *cysE*, *cysD* double mutants are auxotophs. These two genes are located on chromosome II but their precise position remains unknown. No linkage with the following markers of chromosome II was found: *cysB*, *AcrA*, *adH*, *riboE*, *palcA*, *adD*, *puA*, *AcrB* and cnxE

Table 2. *Activity of cysteine synthase in strains blocked in the alternative pathways of cysteine synthesis*

Strain	Cysteine synthase nmole/min/mg protein
cysD11	2·6±0·6
cysB102, cysD11	2·3±0·7
cysB10, cysD11	2·8±0·4
cysB212, cysD11	3·3±0·3
cysC103, cysD11	2·0±0·6
cysE98, cysD11	1·3±0·2
cysE121, cysD11	2·1±0·8
Wild type	3·9±0·1

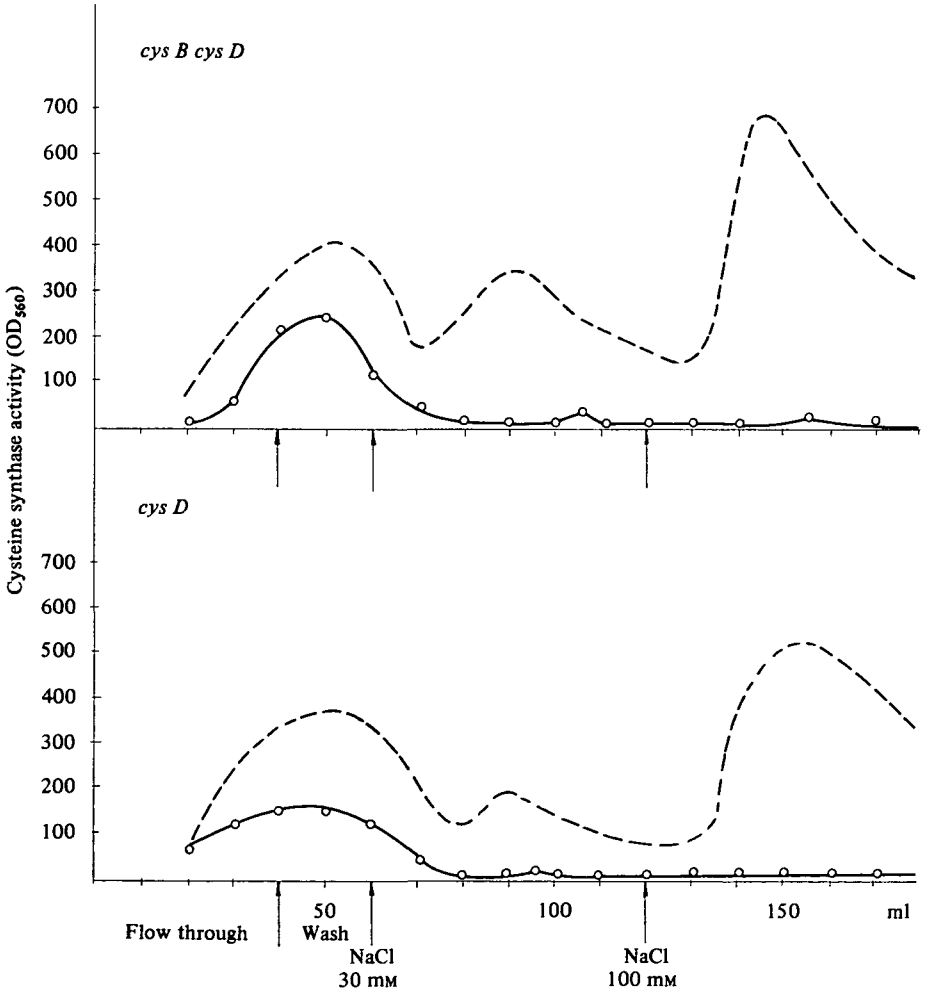

Fig. 2. DEAE-cellulose elution profile of cysteine synthase from the *cysB102, cysD11* and *cysD11* strains of *A. nidulans*. 100000 g supernatants (80 mg protein) were applied onto the column (1 × 15 cm). ----, protein, –O---O–, cysteine synthase activity.

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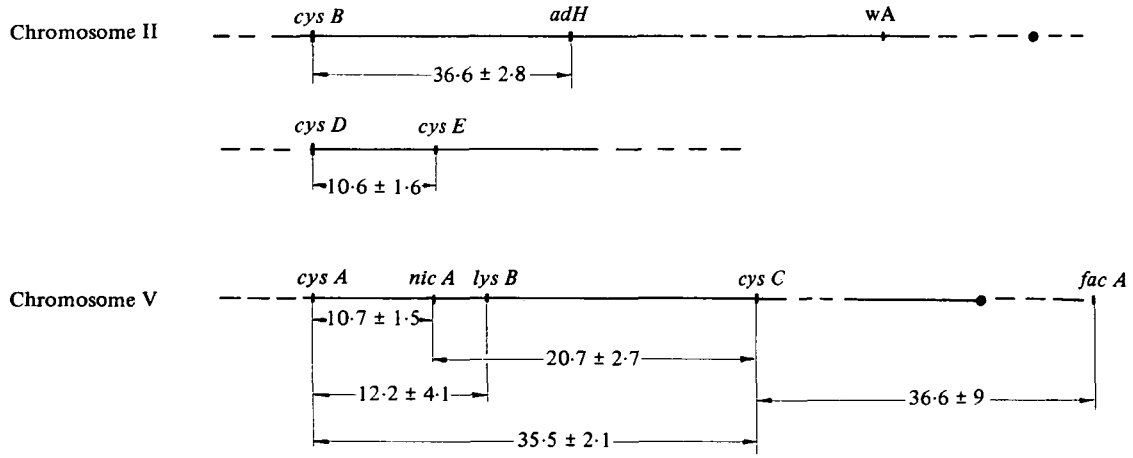

Fig. 3. Location of *cys* genes in the linkage groups of *Aspergillus nidulans*. The distances were calculated from the results of the following crosses: *cysB102* × *adH23, wA1*; *cysB212* × *adH23, wA1*; *cysE98* × *cysD11*; *cysE121* × *cysD11*; *cysE121, cysD11* × *wild type*; *cysE98, cysD11* × *wild type*; *cysA1* × *nicA2*; *cysA1* × *lysB5*; *cysA1, nicA2* × *lysB5*; *nicA1* × *cysC103*; *cysA1* × *cysC103* and *cysC103* × *facA303*.

Among the 83 newly isolated suppressors of the *methA17* mutation, 55 were sensitive to 1 μM propargylglycine. Complementation analysis revealed that 39 of them were *cysA* alleles and 15 were *cysB* alleles. One mutant phenotypically resembles *cysA* (in that its growth inhibition by propargylglycine is reversed by O-acetylserine), but complementation tests and recombinational analysis suggest that it may represent a new locus, closely linked to *cysA*; however intragenic complementation cannot be excluded. It is somewhat surprising that no mutations in *cysC* and *cysE* genes were found, as mutations in these loci also suppress *metA* mutations. It is worth noting that among the four cysteine auxotrophs isolated in the *mecB10* strain two were *cysB* and two were *cysE* alleles. This shows that, as in the other systems, the genetic background and the method of selection determine the distribution of mutant alleles between the loci.

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