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Minireview

Sulfur amino acid metabolism and its regulation in fungi: studies with *Aspergillus nidulans**

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The filamentous fungus *Aspergillus nidulans*. like many other microorganisms, is able to synthesize the sulfur amino acids – cysteine and methionine – from inorganic sulfate which is metabolized to sulfide by the sulfate assimilation pathway (Fig. 1). Sulfide is utilized for cysteine synthesis in a reaction catalyzed by cysteine synthase (Fig. 1, step 7) [1].

Methionine is produced from cysteine and *O*-acetylhomoserine *via* cystathionine and homocysteine (Fig. 1, steps 9 and 10). Mutants impaired either in cystathionine γ-synthase (*metA, metB*) or in cystathionine β-lyase (*metG*) are auxotrophs [2, 3] indicating that these enzymes are essential for the fungus.

As shown in Fig. 1 *A. nidulans*, similarly to mammals, is able to convert homocysteine to cysteine through the reverse transsulfuration pathway (Fig. 1, steps 12, 13) which does not exist in Enterobacteriacae. Since mutations in *mecA* and *mecB* genes do not lead to auxotrophy, this pathway appears dispensible for the organism. Homocysteine is made from methionine in reactions 18 and 19 (Fig. 1). Genetic and biochemical data suggest that there may be two *S*-adenosylmethionine synthetases in *A. nidulans* – one constitutive (the "house keeping enzyme") and the second inducible by methionine which is missing in the *mecC* mutant [4].

Alternative pathways of cysteine synthesis

The existence of two pathways for *de novo* cysteine synthesis in fungi was demonstrated for the first time in *A. nidulans* [5]. In the main pathway the amino acid is made from *O*-acetylserine and sulfide (Fig. 1, step 7). The second pathway consists of three enzymes: *O*-acetylhomoserine sulfhydrylase (homocysteine synthase), cystathionine β-synthase and cystathionine γ-lyase (Fig. 1, steps 12, 13). Cysteine auxotrophy results from a simultaneous impairment of both pathways [3, 5]. Interestingly, mutations in the main pathway (*cysA, cysB, cysC*) suppress lesions in *metA, metB* and *metG* loci [3] due to derepression of all three enzymes of the alternative pathway of cysteine synthesis (Table 1). Homocysteine synthesized in the reaction catalyzed by homocysteine synthase can be used both for cysteine and methionine synthesis. The *cys(A,B,C)* mutants were found to exhibit elevated levels of the sulfate assimilation enzymes [3] suggesting that cysteine is a small molecular weight effector in a regulatory system controlling these enzymes along with those of the alternative pathway of cysteine synthesis. Starvation for cysteine of double mutants requiring this amino acid led to

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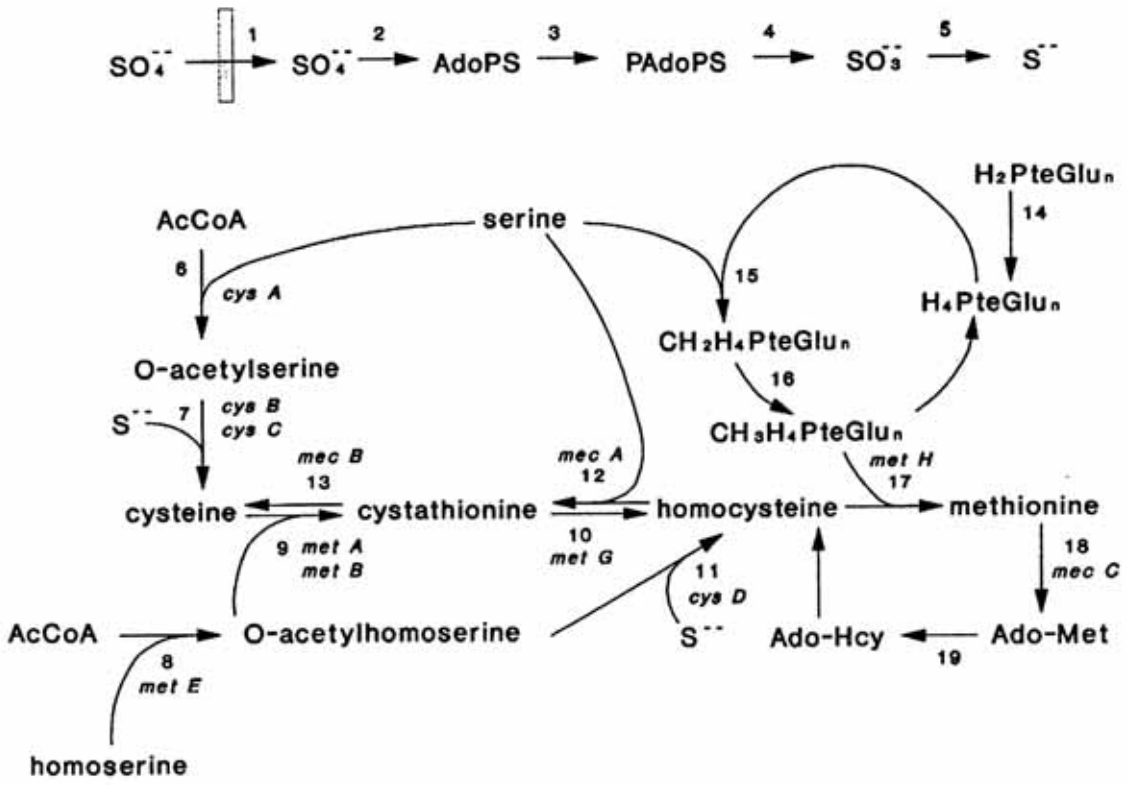

Fig. 1. An outline of sulfur-containing amino acid metabolism and folate metabolism in Aspergillus nidulans. Enzymes: (1) sulphate permease, (2) ATP-sulfurylase, (3) AdoPS-kinase, (4) PAdoPS-reductase, (5) sulfite reductase, (6) serine transacetylase, (7) cysteine synthase, (8) homoserine transacetylase, (9) cystathionine γ-synthase, (10) cystathionine β-lyase, (11) homocysteine synthase, (12) cystathionine β-synthase, (13) cystathionine γ-lyase, (14) dihydrofolate reductase, (15) serine hydroxymethyltransferase, (16) methylenetetrahydrofolate oxidoreductase, (17) methionine synthase, (methyltetrahydrofolate:homocysteine methyltransferase), (18) methionine *S*-adenosyltransferase, (19) various transmethylases.
Abbreviations used: AdoMet, *S*-adenosylmethionine; Ado-Hcy, *S*-adenosylhomocysteine; AdoPS, adenosine 5-phosphosulate; PAdoPS, adenosine 3-phosphate 5-phosphosulate; H_2PteGlu, dihydrofolate; H_4PteGlu, tetrahydrofolate; C_2H_4PteG1u, methylenetetrahydrofolate; CH_3H_4PteGlu_n, 5-methyltetrahydrofolyl-polygultamate. Mutations leading to particular metabolic lesions are indicated.

enzyme derepression confirming this conclusion [1].

The physiological role of homocysteine synthase in the wild-type strain is not quite clear as mutants defective in this enzyme are prototrophs growing on mnimal medium at wild-type rate. We have shown, however, that the enzyme is necessary for recyclization of the methylthio group from 5-methylthioadenosine (a by-product of polyamine synthesis from *S*-adenosylmethionine) [6] and this may be its major role.

Sulfur metabolite repression

The addition of methionine to the growth medium results in drastically reduced levels of several coordinately regulated enzymes involved in the utilization of sulfate and other less favorable sulfur sources. This is a manifestation of sulfur metabolite repression (SMR) – a system analogous to carbon and nitrogen metabolite repression systems. Although repression is usually exerted most effectively by exogenous methionine, experiments with mutants impaired in its conversion to cysteine and experiments with cysteine-requiring mutants mentioned ealier point to the latter compound as a low molecular weight effector in this regulatory system [4]. We have identified four genes involved in this system: *sconA*, *sconB*, *sconC* and *sconD* (*scon* – sulfur controller) [7]. Mutations in any of these genes render a group of sulfur amino acid biosynthetic enzymes insensitive to SMR and suppress lesions in the cystathionine pathway of methionine synthesis indicating the presence of functional homocysteine syn-

Table 1

Activities of some enzymes of sulfur metabolism in wild type and cys strains of Aspergillus nidulans

Enzyme activities are expressed as per cent of wild type activity. All strains were grown in minimal mineral medium. Data adapted from [1] and [3]

Strain	ATP-sulfurylase 2*	Sulfite reductase 5	Homocysteine synthase 11	Cystathionine β-synthase 12	Cystathionine γ-lyase 13
Wild type	100	100	100	100	100
cysB	185	171	324	286	269
cysC	168	200	350	302	278

*Numbers refer to Fig. 1.

thase. Exogenous methionine has little or no effect on sulfate assimilation in the *scon* mutants, in stark contrast to the wild type strain where sulfate assimilation was almost eliminated (Table 2). Similarly, three enzymes shown in the table as examples were releaved (completely or partially) from SMR in the mutant strains.

Interrelated regulation of sulfur and folate metabolizing enzymes

The final step of methionine synthesis involves methylation of homocysteine by methionine synthase with methyltetrahydrofolate as the methyl donor. The levels of this enzyme as well as of other enzymes of folate metabolism are elevated in the *scon* mutants grown in minimal medium as compared with the wild-type strain.

However, in contrast to the enzymes of sulfur metabolism which are under SMR control (Table 2) the presence of methionine in the medium brings the folate enzymes to the same, repressed level in wild type and mutant strains. This implies that folate enzymes are not regulated directly by the SMR system.

The finding that homocysteine induces folate enzymes in the wild type (Table 3) suggests that a high level of these enzymes observed in the *scon* mutants is a secondary consequence of an increased in size of the homocysteine pool observed in them. Since the *scon* mutants accumulate about four times more homocysteine and less than twice as much methionine than wild type strain [7], it appears that the inductive effect of homocysteine overcomes the repressive effect of methionine. Recently, we have described two methionine-requiring mutants in which homocysteine-mediated induction of folate enzymes is impaired [8].

Table 2

Effect of sulfur metabolite repression on sulfate assimilation and some enzymes of sulfur metabolism in A. nidulans (adapted from [7])

The strains were grown in minimal medium supplemented with $^{35}SO_4$, with or without L-methionine

Strain	L-Methionine	Sulfate assimilation*	Homocysteine synthase	ATP-sulfurylase	Aryl-sulfatase
Wild type	–	100	100	100	100
	+	7	56	37	65
sconB	–	263	246	148	430
	+	294	258	127	430
sconC	–	137	155	145	206
	+	95	105	77	127
sconD	–	231	204	143	270
	+	147	131	121	143

*Numbers refer to radioactivtiy in cell extracts which retained on Dowex 50 (H^+)

Table 3

Regulation of folate metabolizing enzymes in wild type and scon strains of A. nidulans

All strains were grown in minimal mineral medium supplemented with L-methionine (5 mM) or DL-homocysteine (3 mM) as indicated. Results are expressed as per cent of wild-type activities in cells grown in minimal medium (adapted from [7]). MS, methionine synthase; OMTF, methylenetetrahydrofolate dehydrogenase; HMTS, serine hydroxymethyltransferase

Strain	L-Methionine (+m) DL-homocysteine (+h)	MS	OMTF	HMTS
Wild type	–	100	100	100
	+m	45	57	74
	+h	280	177	169
sconB	–	160	204	120
	+m	50	96	80
sconC	–	160	154	131
	+m	54	57	74
sconD	–	154	142	133
	+m	45	61	80

Concluding remarks

The sulfur amino acid metabolism we have described in *A. nidulans* represents a rich repertoire of metabolic options, sharing features of enterobacterial and mammalian systems in being able to interconvert cysteine and methionine. Besides this, the fungus possesses two pathways for *de novo* cysteine synthesis. A number of enzymes of sulfur metabolism are subject to regulation by the sulfur metabolite repression system which involves at least four regulatory genes. Cysteine seems to play the role of a small molecular weight effector in this system.

The regulation of folate enzymes which provide methyltetrahydrofolate for methionine synthesis is linked with the regulation of sulfur metabolism through mediation of homocysteine and methionine. The levels of folate enzymes are determined by relative pool size of these two amino acids.

It is worth noting that a pattern of sulfur amino acid metabolism similar to that found in *A. nidulans* was described in *Neurospora crassa* [9], *Cephalosporium acremonium* [10] and *Yarrowia lipolytica* [11] but not in *Saccharomyces cerevisiae*. In the latter organism homocysteine synthase is an essential enzyme [12] and the reverse transsulfuration pathway appears to be the main (in some strains the only) pathway of cysteine synthesis [13, 14]. On the other hand no reverse transsulfuration pathway enzymes have been detected in two yeast species, *Candida valida* [15] and *Schizosaccharomyces pombe* [16]. These findings indicate that there is considerable variety in sulfur metabolism among fungi.

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