The *Aspergillus nidulans* *cysA* gene encodes a novel type of serine O-acetyltransferase which is homologous to homoserine O-acetyltransferases

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The *Aspergillus nidulans* *cysA* gene was cloned by functional complementation of the *cysA1* mutation that impairs the synthesis of O-acetylserine. The molecular nature of *cysA1* and *cysA103* alleles was characterized; a nucleotide substitution and a frame shift were found in the former and a deletion mutation in the latter. The CYSA protein is 525 amino acids long and is encoded by an uninterrupted open reading frame. Expression of the *cysA* gene appears not to be regulated by sulfur, carbon and nitrogen sources. Protein sequence analysis reveals extensive similarity to homoserine O-acetyltransferases, particularly the bacterial ones, and no homology with known serine O-acetyltransferases. The authors propose that the CYSA protein is analogous to serine O-acetyltransferases, i.e. it catalyses the same reaction but has an independent evolutionary origin.

Keywords: *A. nidulans*, cysteine synthesis, analogous genes

INTRODUCTION

The synthesis of cysteine in *Aspergillus nidulans*, like that in many bacteria and plants, involves the acetylation of serine followed by its thiolation (Fig. 1, steps 1 and 2). If this pathway is impaired, the fungus can synthesize cysteine by an alternative pathway comprising homocysteine synthase, cystathionine β -synthase and cystathionine γ -lyase (Fig. 1, steps 5, 6 and 7). In consequence, two mutations, impairing both pathways, are required for cysteine auxotrophy. In addition, mutations affecting the main pathway, like *cysA* or *cysB*, suppress the mutations impairing cysteine γ -synthase and cystathionine β -lyase (Fig. 1, steps 3 and 4) since homocysteine synthesized from O-acetylhomoserine, the product of homoserine O-acetyltransferase (HATase) (Fig. 1, step 8), is utilized for both cysteine and methionine synthesis (Paszewski & Grabski, 1974, 1975).

cysA, *cysD* double mutants grow on cysteine as well as on O-acetylserine (OAS); this differentiates them clearly from *cysB*, *cysD* double mutants, which require cysteine (both strains grow also on methionine). OAS also restores growth of *cysA* single mutants inhibited by γ -propargylglycine, which strongly blocks cystathionine γ -lyase (Fig. 1, step 7) (Piotrowska & Paszewski, 1986). Pieniążek *et al.* (1974) found no serine O-acetyltransferase (SATase) activity in the *cysA1* strain. These data strongly suggest that OAS is an intermediate in the main cysteine-synthesizing pathway in *A. nidulans*.

In the present report, we describe the cloning and characterization of the *A. nidulans* *cysA* gene located on chromosome V (Cybis *et al.*, 1988). The gene encodes a protein which is homologous to HATases, but not to any of the known SATases from bacteria and plants.

METHODS

Strains, media and growth conditions. The following strains of *A. nidulans* from our collection, which carry standard markers (see Clutterbuck, 1994) were used: *cysA1*, *proA2* *pabaA2*, *yA2*; *cysA1*, *cysD11*, *yA2*, *phenA2*; *cysB102*, *pyroA4*, *yA2*; *cysC103*, *choA1*, *chaA1*; *cysC103*, *cysD11*, *choA1*, *chaA1*; *cysD11*, *phenA2*, *yA2*; *metE31*, *nicA2*, *biA1*; *sconB2*,

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Abbreviations: HATase, homoserine O-acetyltransferase; OAS, O-acetylserine; SATase, serine O-acetyltransferase.

The GenBank accession number for the sequence reported in this paper is AF029885.

Fig. 1. An outline of sulfur amino acid metabolism in *A. nidulans*: 1, serine O-acetyltransferase (SAT); 2, cysteine synthase; 3, cystathionine γ -synthase; 4, cystathionine β -lyase; 5, homocysteine synthase; 6, cystathionine β -synthase; 7, cystathionine γ -lyase; 8, homoserine O-acetyltransferase (HAT). The biochemical nature of the lesions in the *metA*, *metB* and *cysE* mutants has not yet been established. PG (propargylglycine) is a strong inhibitor of cystathionine γ -lyase.

nicA2, *biA1*; *sconC3*, *pyroA4*, *yA2*; *metR1*, *pyroA4*, *biA1*. *pyroA4*, *yA2* was used as a reference wild-type strain. The genetic status of the *cysC103* mutation was revised in the course of this work (see below). The *sconB* (Natorff *et al.*, 1998) and *sconC* (Piotrowska *et al.*, 1997; Natorff *et al.*, 1993) genes encode sulfur regulatory proteins. The *metR* gene encodes a sulfur-specific transcriptional factor (Natorff *et al.*, 1999).

Strains were grown in minimal medium (MM), containing 2 mM sulfate, as described previously (Paszewski & Grabski, 1973) or in MM-S (minimal minus sulfur medium) with sulfates substituted with corresponding chlorides. These media were supplemented according to the auxotrophic requirements of the strains employed. The complete medium was according to Cove (1966). The cultures were grown with shaking (200 r.p.m.) at 37 °C for 12–16 h. Mycelia were harvested by filtration, blotted on filter paper and used, depending on the experiment, for cell-free extracts, protoplast preparation or nucleic acid isolation.

To test the effect of carbon catabolite repression and nitrogen metabolite repression on *cysA* gene expression, the wild-type mycelia were grown in media containing either sulfate (0.25 mM or 2 mM) or methionine (5 mM) and non-repressing carbon and nitrogen sources (0.1% fructose, 5 mM urea) for 8 h at 37 °C. The cultures were then supplemented with different combinations of carbon and nitrogen sources as follows: 0.1% fructose, 5 mM urea (non-repressed); 1% glucose, 5 mM urea (carbon repressed); 0.1% fructose, 20 mM ammonium tartrate (nitrogen repressed); 1% glucose, 20 mM ammonium tartrate (carbon and nitrogen repressed) for 2 h at 37 °C (Dzikowska *et al.*, 1999; Gonzalez *et al.*, 1997).

Escherichia coli XL-1 Blue (Stratagene) was used for cloning and plasmid propagation. *E. coli* strains were grown in standard media, LB, 2 $\times$ TY and NZY (Sambrook *et al.*, 1989), as required. Antibiotics were added in the following concentrations: ampicillin 50 μ g ml⁻¹, kanamycin 50 μ g ml⁻¹, tetracycline 35 μ g ml⁻¹.

Plasmids, gene libraries and synthetic primers. The cloning vectors were pBluescript II KS and SK (Stratagene) and pUC19 (Yanisch-Perron *et al.*, 1985). The pHELP1 plasmid (Gems *et al.*, 1991), supplied by J. Clutterbuck (University of Glasgow, UK), contains the AMA1 sequence, which enables autonomous replication. Co-transformation with this plasmid results in up to 200-fold increase of transformation efficiency

(Gems & Clutterbuck, 1993). The pIC19R plasmid, containing a fragment of the *A. nidulans* γ -actin gene (Fidel *et al.*, 1988), was obtained from R. Bradshaw (Massey University, Palmerston North, New Zealand). The *A. nidulans* chromosome-specific cosmid pWE15 and pLORIST2 gene libraries (Brody *et al.*, 1991) and the *A. nidulans* λ ZAPII 24 h developmental cDNA library, constructed by R. Aramayo, were obtained from the Fungal Genetics Stock Center, Kansas City, KS, USA.

The following oligonucleotides were used as primers for PCR and primer extension: CYSCATG, 5'-GGAATTCCAAATGAGTCCGCTGAACGGCGTCGCT-3' [384+extension]; CYSCNFLG, 5'-TCCCCCGGGCTTGTTCATCGTCGTCCTTGTAGTCTTGTACAATTATGTGCGA-3' [1958(C)+extension]; MGPR3, 5'-GATCGGCGATAGAGACGAGC-3' [196(C)]; MGPR4, 5'-CGTTCAGCGGACTCATTTCAGCAGGCAC-3' [372(C)]; ANAKT1032, 5'-GTGATGAGGCACAGTCCA-3' [1032]; ANAKT1669, 5'-TACCACCGCTTCCAGACCA-3' [1669(C)]. The numbers in square brackets correspond to the first nucleotide (in bold) from the 5' end of the primer which hybridizes with the template sequences found in the GenBank. (C) designates the complementary strand; 'extension' denotes nucleotide stretches added for various purposes, e. g. cloning sites.

DNA isolation. *A. nidulans* DNA was isolated by grinding mycelia frozen in liquid nitrogen in a mortar as described by Yelton *et al.* (1984). Plasmid DNA from *E. coli* was isolated by alkaline lysis (Sambrook *et al.*, 1989) or using a QIAGEN Plasmid Kit.

Transformation of *A. nidulans* and cloning of the *cysA* gene. Preparation of protoplasts from the *cysA1*, *cysD11*, *choA1*, *chaA1* strain and subsequent transformation were performed as described by Sieńko *et al.* (1998) except for the amount of Novozyme 234 (5–6 mg ml⁻¹) and helicase (2.5 mg ml⁻¹) used. The protoplasts were co-transformed to the wild-type phenotype (Cys⁺) with DNA isolated from subpools of chromosome V cosmid clones and the pHELP1 plasmid. The subpool that produced Cys⁺ transformants was further subdivided in order to identify the cosmid able to rescue the *cysA1* mutation. To identify the region of the cloned cosmid complementing the mutation we used the 'instant gene bank' method of Gems *et al.* (1994). The method is based on the observation that co-transformation of a strain with the restricted pHELP1 plasmid and transforming DNA leads to *in vivo* ligation of DNA fragments with the vector. This method allows the selection of transformants harbouring plasmids carrying required DNA

fragments, which can be then rescued into *E. coli* (Ballance & Turner, 1985). The *A. nidulans* insert which reversed auxotrophy was used as a probe to screen the cosmid digests. All the digests were transferred to a membrane and after Southern hybridization (Sambrook *et al.*, 1989) the smallest transforming restriction fragment was cloned into a sequencing vector and subsequently checked for complementation of the *cysA1* mutation. This last step was necessary to sequence the original copy of *cysA*, excluding any rearrangements caused by *in vivo* ligation in *A. nidulans*.

A *cysA* cDNA copy was isolated from the λ ZAPII library according to the Stratagene protocol using a digoxigenin-labelled probe (Boehringer Mannheim Kit).

PCR reactions. PCR reactions were performed in an Air Thermocycler (Idaho Technology) according to the supplier's instructions. The Promega *Taq* DNA polymerase was used. The cycling conditions were: 94 °C/20 s + (94 °C/1 s, 50–58 °C/1 s, 72 °C/20–35 s) $\times$ 30 + 72 °C/30 s. At least three independent clones of each allele were sequenced on both strands.

DNA sequencing. DNA sequence was determined using an ALF automatic sequencer (Pharmacia) or an ABI310 sequencer (Perkin Elmer).

Mapping of the 5' and 3' *cysA* mRNA termini. The *cysA* transcription initiation site was mapped by primer extension analysis. MGPR3 or MGPR4 oligonucleotides were hybridized to total RNA isolated from the wild-type strain grown in minimal medium. The primer was then extended with 200 units M-MLV reverse transcriptase (Promega) at 42 °C for 2 h, using [α -³²P]dCTP according to the Gibco-BRL protocol. The reaction products were fractionated by 8% denaturing gel electrophoresis in parallel with DNA sequencing reactions initiated with the same primer. The 3' end of *cysA* mRNA was determined by sequencing cDNA clones of the *cysA* gene.

RNA isolation and Northern analysis. Total RNA was isolated using TRI Reagent (Molecular Research Center) according to the manufacturer's instructions (Chomczynski, 1993). A sample containing 20 μ g total RNA was glyoxylated at 50 °C for 1 h followed by fractionation in 1% agarose and overnight capillary transfer to a nylon membrane (Hybond-N, Amersham). Filters were prehybridized for 1 h at 65 °C. DNA probes were α -³²P-labelled by random priming with a DNA labelling kit (MBI Fermentas). Hybridization was carried out overnight at 65 °C. Filters were washed for 1 h with 2 $\times$ SSC, 0.1% SDS at 50 °C and then with 1 $\times$ SSC, 0.1% SDS. Standard autoradiography with intensifying screens was performed at –80 °C. All the procedures were according to Sambrook *et al.* (1989).

Computer sequence analysis. Computer analysis of DNA and protein sequences was performed using GENEPRO 4.20 (Riverside Scientific), GCG software (version 8.1) (Genetics Computer Group) on a Silicon Graphics Challenge computer, AllAll (Computational Biochemistry Research Group Server) (Gonnet, 1994), PSORT II (Nakai & Kanehisa, 1992), BOXSHADE 3.21, PRODOM 99.1 and BLAST (Altschul *et al.*, 1990) using the GenBank, EMBL, PIR(R), PDB and SWISS-PROT databases.

Protein structure predictions were obtained with the position-specific iterative BLAST algorithm (PSI-BLAST) (Altschul *et al.*, 1997) and FFAS, a proprietary profile–profile alignment program, developed at the authors' laboratory (Rychlewski *et al.*, 2000), followed by homology modelling using MODELLER (Sali & Blundell, 1994). In both prediction algorithms, the pre-

diction reliability is expressed as an e-value, which can be interpreted as the probability that the prediction is false. e-values of 0.001 and less are normally associated with very reliable predictions (Altschul *et al.*, 1997; Rychlewski *et al.*, 2000).

RESULTS

Isolation of *cysA* clones

Since the *cysA1* mutant is a prototroph we used a double mutant *cysA1*, *cysD11*, having both pathways of cysteine synthesis impaired, for the *cysA* gene cloning. This strain was transformed with a chromosome V specific library. As the *cysA* gene is located on this chromosome and the *cysD* gene on chromosome II, prototrophic transformants could only arise by acquisition of the *cysA* gene. This led to the identification of one cosmid, W05C10, which complemented the *cysA1* mutation. This cosmid contained an *A. nidulans* insert of about 43 kb (data not shown). Applying the 'instant gene bank' procedure (see Methods) we co-transformed the mutant strain with pHELP1 linearized with *Bam*HI and the W05C10 cosmid digested with *Eco*RI. Cys⁺ transformants were selected. The transforming recombinant plasmids were rescued in *E. coli*. One of them, containing an 8 kb *A. nidulans* insert, was used as a probe to identify the shortest transforming fragment of the cosmid digests. It turned out to be a 3.9 kb *Eco*RI–*Hind*III fragment which was cloned into pBluescript KS.

To identify the cDNA copy of the *cysA* gene, about 10⁵ phage clones from the cDNA library were screened by plaque hybridization with the digoxigenin-labelled *Bam*HI–*Hind*III fragment from the 5' region of the *cysA* gene. Five positive plaques were found. Three of them, giving the strongest hybridization signals, were purified and amplified. pBluescript SK(–) plasmids containing cDNA inserts were excised *in vivo* according to the Stratagene protocol. Only one cDNA insert extended (6 bp) beyond the putative translation initiation codon.

Nucleotide sequence analysis

The 3.9 kb *Eco*RI–*Hind*III fragment was sequenced on both strands (GenBank accession number AF029885). DNA sequence analysis revealed one major open reading frame corresponding to the *cysA* gene. This gene has no introns as shown by cDNA sequencing. The main transcription start point, determined by 5' extension, is positioned at –161 from the putative ATG codon assumed to be the start point of translation. Although there is another in-frame ATG located 45 nt upstream from the former one, we stress that the cDNA we have isolated, which contains only 6 nt upstream from the initiation codon, complements both the *cysA1* and *cysA103* (deletion) mutations. This shows that the translation product beginning with the downstream ATG is functional. Upstream from the transcription start site there is a putative CAAT-box (–71). Furthermore, at a typical distance (–34) from the transcription start point there is a CT-rich region which has

Fig. 2. For legend see facing page.

been noted in other fungal genes at, or near, transcription start sites. In the promoter region many putative CREA and AREA binding sites were found. The UAG translational termination codon is followed by a purine (A), which is typical for eukaryotic genes (Unkles, 1992). The putative polyadenylation signal (AUAA) is located 10 nt after the stop codon. Sequencing of cDNA indicated polyadenylation sites 28 nt and 35 nt downstream from the UAG.

In order to analyse the mutated form we cloned the *cysA1* allele and found four mutations, all except one located at the 3' end of the gene. In the case of the *cysA* allele, three independent PCR products were sequenced on both strands. The wild-type allele PCR products obtained with the same polymerase confirmed the previously established genomic and cDNA sequences. Three of the mutated sites are clustered. Two of the four mutations are substitutions (A784C, T1547G) and two are insertions (1516A and 1545T [the numbers represent the nucleotides situated upstream of the insertion]). These changes in DNA sequence lead to an amino acid substitution (S262R) and frameshifts (the first at position 506), in the latter case causing premature protein chain termination at amino acid 518.

Protein sequence analysis and structure predictions

The *cysA* gene encodes a peptide of 525 amino acids with a calculated molecular mass of 57 kDa. Contrary to expectations from the *cysA1* phenotype the CYSA protein exhibited a very high degree of identity to the family of HATases, not to SATases. The protein shows a high degree of identity (52.5%) to a putative HATase-like protein from *Schizosaccharomyces pombe* (Fig. 2) and a lower but significant identity (23–29%) to other HATases, including those encoded by the *metE* gene of *A. nidulans* and its *Schiz. pombe* homologue, the *met2* gene (see Fig. 5). This homology is spread all along the protein sequence. The PSORT II analysis indicated the presence of a putative leader sequence (47 amino acids) targeting the CYSA protein to mitochondria (probability 73.9%) (Fig. 2).

Analysis of the structure of CYSA to uncover a possible similarity with SATases was performed with PSI-BLAST. This procedure identifies all members of a family within a given sequence similarity criterion. This analysis showed that SATases and HATases form distinct families. The three-dimensional structures of CYSA and the proteins from the SATase family could be reliably predicted to be dramatically different. By the high-reliability [PSI-BLAST (Altschul *et al.*, 1997) e-value of $4e-19$] match to iminopeptidase from *Xanthomonas campestris*, PDB code 1azw, CYSA can be predicted to have an α/β hydrolase fold with the characteristic central

β -sheet surrounded on both sides by α -helices. In contrast, by the high-reliability (PSI-BLAST e-value of $2e-17$) match to *Mycobacterium bovis* N-succinyltransferase, PDB code 3tdt, the SATase family can be predicted to have a fold of a left-handed β -helix. These predictions, and thus the ensuing lack of structural similarity between CYSA and the SATase family, can be further corroborated by markedly different secondary structure predictions for the two proteins (Rost & Sander, 1993) and by the presence of a characteristic hexapeptide repeat with a consensus sequence of {V,L,I}-G-X-X-X in the proteins from the SATase family (Vaara, 1992) and its absence in CYSA.

The CYSA protein is clearly not a typical HATase. As seen in Fig. 2, the main distinctive feature of this protein is the lack of the 70 amino acid loop in the C-terminal half found in classical eukaryotic HATases.

Regulation of *cysA* expression

cysA expression was assayed in the wild-type, *sconB2*, *sconC3* and *metR1* strains under repressing and derepressing conditions (i.e. high and low levels of sulfur source). Total RNA was isolated and Northern blots were prepared and probed with an α - 32 P-labelled fragment of the *cysA* gene amplified with CYSCATG and CYSCNFLG primers. As a control, the same blots were hybridized with an α - 32 P-labelled fragment of the γ -actin gene amplified with ANAKT1032 and ANAKT1669 primers. No significant difference in the level of the *cysA* transcript was found in mycelia grown in the presence of a high or low level of sulfur source (Fig. 3).

The *cysA* transcript did not show any regulation by nitrogen or carbon source regardless of the sulfur source used in the experiment (data not shown).

Revised status of the *cysC103* mutation

The *cysC103* as well as *cysB102* mutations were originally isolated as suppressors of the *metB3* mutation (Ayling, 1969) and later found to impair the main pathway of cysteine synthesis (Paszewski & Grabski, 1975). Since the cysteine-requiring double mutant *cysC103*, *cysD11* grew well on cysteine but weakly and with some delay on OAS, it was suggested that the mutation interfered in the conversion of OAS to cysteine and represents a separate locus named *cysC*. Its biochemical nature remained unknown (Paszewski *et al.*, 1994).

In the present work we have transformed the *cysC103*, *cysD11* mutant with the *cysA* gene to Cys⁺ phenotype, finding that this gene complements the *cysC103* mu-

Fig. 2. Alignment of the *A. nidulans* CYSA protein with representatives of prokaryotic and eukaryotic HATases. Black boxes indicate identical residues, frames indicate similar ones. Putative cleavage sites for mitochondrial presequences are marked by a bigger arrow for the CYSA sequence and a smaller arrow for the HAT-like *Schiz. pombe* sequence. Abbreviations: *cysA*, *A. nidulans* CYSA; *spombe*, HAT-like SATase from *Schiz. pombe*; *hat-sp*, *Schiz. pombe* MET2; *hat-ye*, *Sacch. cerevisiae* MET2; *hat-cg*, *Corynebacterium glutamicum* META; *hat-ml*, *Mycobacterium leprae* HATase; *hat-hi*, *Haemophilus influenzae* MET2; *hat-lm*, *Leptospira meyeri* METX.

Fig. 3. Analysis of *cysA* expression in the wild-type and sulfur regulatory mutant strains grown in various sulfur conditions (Met, methionine). Total RNA (20 µg) was subjected to electrophoresis, blotted to a nylon membrane and hybridized with α - 32 P-labelled DNA probes.

Fig. 4. (a) Southern analysis of DNAs from the wild-type (*pyroA4*, *yA2*) (1) and *cysA103* (*cysA103*, *choA1*, *chaA1*) (2) strains digested with *Bam*HI and hybridized with a *cysA* probe. Hybridization with a *cysB* probe was used as a control. (b) Analysis of *cysA* expression in the *cysA103* (*cysA103*, *choA1*, *chaA1*) and *cysD11* (*cysD11*, *phenA2*, *yA2*) strains grown on different sulfur sources (Met, methionine). Experimental conditions as in Fig. 3.

tation as well as *cysA1*. Molecular analysis revealed that the whole *cysA* gene is deleted in the *cysC103* mutant (Fig. 4). These data forced us to revise the genetic status of the *cysC103* mutation. It had previously been located in chromosome V, as was *cysA1*, but direct crosses between strains carrying these mutations were infertile except for one which suggested a loose linkage between them (Cybis *et al.*, 1988). We find now that the strain that crossed with *cysA1* carried the *cysB102* mutation and was erroneously labelled as *cysC103*. This mistake must have taken place earlier when *cys* mutants were designated only with their suppressor numbers: *su102* and *su103*, respectively. Although in the present work we failed again in crossing strains carrying the *cysA1* and *cysC103* mutations, this time we got clear results from heterokaryon tests: the *cysA1* and *cysC103* mutations did not complement each other on medium supplemented with γ -propargylglycine, which strongly

inhibits cystathionine γ -lyase (Fig. 1, step 7) by blocking the only pathway of cysteine synthesis left in these strains. OAS enabled growth of both mutants and the heterokaryon in the presence of the inhibitor. Also heterokaryons between the *cysA1*, *cysD11* and *cysC103*, *cysD11* strains required OAS, cysteine or methionine to grow. Therefore, both the genetic and molecular data indicate that *cys103* is a mutation affecting the *cysA* gene. The mutation is now designated *cysA103* and *cysC* is considered not to be a valid locus.

DISCUSSION

The *cysA* gene of *A. nidulans* was cloned by functional complementation of the *cysA1* mutation, and analysed. Since *cysA* mutants respond to OAS, which appears to be an intermediate in the main pathway of cysteine synthesis, it was expected that the gene would encode a SATase. It turned out, however, that the CYSA protein is highly similar to HATases and not to any of 22 bacterial and plant SATases whose sequences are available in databases. All SATases represent a group of homologous proteins. The gene was found mutated in the *cysA1* and deleted in the *cysA103* (formerly *cysC103*) strain, indicating that the cloned gene is not a suppressor of these mutations. There is a possibility that the CYSA protein does not function as a SATase itself but is its activator, which happens to be similar to HATase. This seems unlikely since the search in the *A. nidulans* genome for sequences potentially encoding SATases (SATases from watermelon and *E. coli* were used as references), kindly performed at our request by Cereon Genomics, LLC, Cambridge, MA, USA, did not reveal such a sequence (personal communication). Therefore, both the nutritional and molecular data strongly suggest that *cysA* encodes a HATase-like SATase. This conclusion is supported by the fact that *A. nidulans* possesses a *bona fide* HATase encoded by *metE* (GenBank accession number AF162658). *metE* mutations render strains OAH dependent, which indi-

Fig. 5. Molecular phylogenetic tree of HATases constructed with the use of the ALLALL program. The length of each branch is proportional to the evolutionary distance between the nodes. The black dot indicates the weighted centroid of the tree. Database accession numbers for the proteins are as follows: a, AAD43584; b, AAA32681; c, CAA18890; d, CAA05109; e, AAB04108; f, AAD22947; g, AAD22446; h, P39058; i, AAD30471; j, AAC06035; k, P45131; l, AAB86286; m, CAB10992; n, CAA17113; o, AAD03540; p, CAA71733; q, AAB84208; r, CAA93795.

cates that CYSA does not have a HATase activity, at least *in vivo*.

Interestingly, *Schiz. pombe* possesses a protein (GenBank accession number CAB53733), described as HATase-like, which is highly similar to CYSA. We believe that it also functions as a SATase. The two proteins appear to be phylogenetically closely related (Fig. 2) and, having overall homology with HATases, they are clearly closer to the bacterial than the eukaryotic ones (Fig. 5). It should be noted that *Schiz. pombe* also has a HATase encoded by *met2*, homologous to the *A. nidulans* METE. These two enzymes are typical eukaryotic HATases.

It would be worthwhile to characterize the CYSA protein biochemically. Since the SATase activity measured by acetylation of [14 C]serine observed in earlier experiments by Pieniżek *et al.* (1974) was extremely low in the wild-type strain (but totally missing in *cysA1*), we have attempted to apply the spectrophotometric assay used for bacteria and plants in order to characterize the enzyme biochemically. Unfortunately this did not work, even though we observed SATase activity in control assays with bacterial and plant extracts (data not shown). Possibly, because of differences between the CYSA protein and classical SATases, different assay conditions and purification procedures are required.

The *cysA* gene, similarly to *cysB* and *metG* (Topczewski *et al.*, 1997; Sienko & Paszewski, 1999) appears not to be transcriptionally regulated. Since mutations in this gene lead to a derepression of some enzymes of sulfur metabolism (Paszewski & Grabski, 1975), OAS does not seem to play the role of an inducer in this metabolism as found in enterobacteria (Kredich, 1992) and as postulated in plants (Neuenschwander *et al.*, 1991; Smith *et al.*, 1997) and some *Saccharomyces cerevisiae* strains which have SATase activity (Ono *et al.*, 1996). In addition, the *cysA* gene was found not to be regulated by

carbon or nitrogen sources, although some putative AREA and CREA binding sites are found in the *cysA* promoter region.

Since the HATase and SATase structures are markedly different, it can be concluded that CYSA is analogous, but not homologous, to SATases. Such proteins of independent evolutionary origin catalysing the same reaction are not uncommon, but are relatively rare (Galperin *et al.*, 1998). Most likely the *cysA* gene has differentiated from an ancestor gene encoding a HATase of bacterial type which acquired a novel function. In this connection it is worth noting that O-acetylserine thiolase, encoded by *cysB* (Topczewski *et al.*, 1997), is also closely related to bacterial and not to its eukaryotic counterparts. In addition, both genes have a very low frequency of 'optimal' codons: 0.37 and 0.4 for *cysA* and *cysB*, respectively (Lloyd & Sharp, 1991; Nakamura *et al.*, 1999). It is therefore tempting to speculate that the *cysA* and *cysB* genes are both of prokaryotic origin, especially as the proteins they encode are likely to be localized in the mitochondrion.

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