Acta Biochimica Polonica

Vol. 46 No. 2/1999

391–403

QUARTERLY

Structure of the arginase coding gene and its transcript in *Aspergillus nidulans*[o]

Piotr Borsuk, Agnieszka Dzikowska, Joanna Empel, Anna Grzelak, Rafał Grześkowiak and Piotr Weglenski[✉]

Department of Genetics, University of Warsaw and Institute of Biochemistry and Biophysics Polish Academy of Science, A. Pawińskiego 5a, 02-106 Warszawa, Poland

Received: 05 May, 1999

Key words: arginase structural gene (*agaA*) from *Aspergillus nidulans*, regulation of gene expression

The arginase structural gene (*agaA*) from *Aspergillus nidulans* has been cloned and characterised. Depending on the growth conditions of the mycelium, transcripts of this gene have different 5′ends. These differences could result either from the presence of multiple transcription initiation sites or from differential processing of mRNA. The *agaA* mRNA has a long 5′UTR with a potentially complex secondary structure. Putative arginine binding aptamers were found in this UTR suggesting interesting possibilities for regulation of the *agaA* expression.

Arginase (EC 3.5.3.1) is an enzyme that catalyses the hydrolysis of arginine to urea and ornithine. It is widely present in the biosphere and was found in representatives of practically all primary kingdoms, from *Archaebacteria* to primates. The enzyme in an essential component of the urea cycle in most higher organisms and plays several other important metabolic functions including involvement in the metabolism of nitric oxide and inhibition of apoptosis (Jenkinson *et al.*, 1996; Esch *et al.*, 1998). Arginase deficiency in humans leads to a severe metabolic disorder (Grody *et al.*, 1992). Arginase synthesis is regulated both during the cell cycle (Skog *et al.*, 1981) and during development and differentiation (Spector *et al.*, 1985; Xu *et al.*, 1993; Patterton & Shi, 1994). Structure and expression of arginase genes were investigated in several organisms including mammals

[o]This work was supported by the State Committee for Scientific Research (KBN, Poland) grant No. GP04A04509, by the University of Warsaw grant BW1420/98/28 and by Polish-French Center of Plant Biotechnology

[✉]To whom correspondence should be addressed.

Abbreviations: OTA, ornithine transaminase; SSC, saline sodium citrate; ssDNA, single-stranded DNA.

(Takiguchi & Mori, 1991; Goodman *et al.*,1994) but most of the information on the regulation of arginase synthesis at the molecular level comes from studies carried out on fungi and bacteria.

Many different microorganisms, including *Aspergillus nidulans,* utilise arginine as a source of carbon and nitrogen. In *A. nidulans* arginine is broken down to glutamate *via* ornithine and Δ^1pyrroline carboxylic acid. Arginase catalyses the first and ornithine transaminase (OTA) the second step of this pathway. Regulation of the synthesis of these two enzymes was intensely studied both in yeast (Bercy *et al.*, 1987) and in *A. nidulans* (Bartnik *et al.*, 1977). Studies in yeast provided one of the most interesting models of eukaryotic gene regulation (Bercy *et al.*, 1987; Smart *et al.*,1996). Models of genetic control of the arginine catabolic pathway in *A. nidulans* were until recently based primarily on the results of classical genetic and biochemical studies. The regulatory system seems to be very complex and in many respects different from that described for yeast. Mutations in at least 10 different genes, unlinked to the loci of structural genes *agaA* (gene for arginase) and *otaA* (gene for ornithine transaminase), affect the synthesis of arginase and OTA. Two of these genes, *areA* (Kudla *et al.*, 1990) and *creA* (Bailey & Arst, 1975; Dowzer & Kelly, 1991) are responsible for general regulation of nitrogen and carbon metabolism, respectively. The remaining genes are specific for the arginine catabolic pathway. Mutations in these genes result either in the derepression or in non inducibility of the arginine catabolic enzymes. One of these genes (*arcA*) was postulated to code an activator protein (Bartnik & Weglenski, 1974) and recently was found to code for zinc finger containing protein (Empel, 1998). The function of the other regulatory genes controlling arginine catabolism is not clear. Some of them seem to participate in formation of the repressor while others influence the sensitivity of arginase and OTA synthesis to carbon and nitrogen regulation (Bartnik & Weglenski, 1974).

To elucidate the mechanism of regulation of arginine catabolism at the molecular level we intend to clone and characterise all participating genes. We have started with structural genes and in this paper we present results of experiments with the gene coding for arginase. We have cloned and sequenced this gene from *A. nidulans* and have found that its promoter has several interesting properties which allow to postulate a novel mechanism of regulation of gene expression in this organism.

MATERIALS AND METHODS

Strains. The following *A. nidulans* strains were used:

paba A12, bi A1

paba A12, bi A1, arcA^{d}47

pro A6, paba A9, bi A1, trpC81, aga A90

Gen libraries. *A. nidulans* chromosome specific (Brody *et al.*, 1991) and cDNA libraries (Aramayo & Timberlake, 1990) were used. The libraries were purchased from Fungal Genetics Stock Center.

Growth conditions. Mycelia for RNA preparations and enzyme assays were grown in standard minimal medium (Pontecorvo *et al.*, 1953) containing 0.1% fructose as a carbon and 5 mM urea or 10 mM nitrate as nitrogen source. For arginine induction 5 mM arginine was added to the medium.

A. nidulans *transformations.* Transformations were done as described by Tilburn *et al.* (1983) and modified by Johnston *et al.* (1985).

RNA isolation. Total RNA was extracted from mycelia of the wild type (*pabaA1, biA1*) *A. nidulans* strain using Tri-Reagent system (Molecular Research Center, Inc., Cincinnati, OH).

RT-PCR analysis. Three primers for RT-PCR reactions were designed:

Prim 2 (-24--4,	5'-GACTCTTGACGG-TGACGACTG-3')
PAB2 (-380--361,	5'-CTTTCCCCTAA-GTACAGGT-3')
PAB4 (-774--755,	5'-GCTGAACCC-GCAAATGGTA-3').

Synthesis of the first strand was carried out as described by McPherson *et al.* (1995), using 10 μg of total RNA isolated from *A. nidulans* mycelium. The reaction mixture in a final volume of 25 μl, also contained Superscript II Reverse Transcriptase (Gibco-BRL), 30 u of RNAsin (Promega, Madison, WI, U.S.A.), 1 × buffer supplied by the manufacturer and 25 pmol of an antisense primer Prim 2. The resulting products served as a template for the PCR reactions carried out with PAB 4 and Prim 2 or PAB 4 and PAB 2 primers. All reactions were performed according to the manufacturer's recommendations.

Hybridisation probes. For screening of the *A. nidulans* genomic libraries the 359 nucleotides long fragment was synthesised by PCR. Primers for PCR: aga 1 5'-GGTCTAGA-GGIGCCCAITGIG/AGGTC-3' and aga 4 5'-CTCGAATTCTGGA/GTIGAT/CGCICAT/CA/GC-3' were designed according to known sequences of arginases from other organisms (Sumrada & Cooper, 1984; Kawamoto *et al.*, 1987; Marathe *et al.*, 1993; Schrell *et al.*, 1989; Van Huffel *et al.*, 1994).

Primer extention analysis. Primer extention reactions with total RNA isolated from *A. nidulans paba A12, bi A1* (wild type) and *paba A12, bi A1, arc* A^d47 mycelium were performed according to Graaff *et al.* (1994). The primers used were Prim 2 and PAB3 (5'-AA-AGAACTCCTTTGACACAAGGG-3').

Northern blot analysis. Samples 50 μg of the total *A. nidulans* RNA preparation were resuspended in 1× formaldehyde gel-loading dye. After denaturation (65°C for 15 min) RNA was loaded onto 1% agarose/formaldehyde gel (Sambrook *et al.*, 1989) and run for 10 h at 25 V/cm^2. The gel was soaked in 10 × SSC for 15 min and RNA was transferred to a Nybond-N™ (Amersham-USB) membrane. Northern blots and hybridizations were performed according to Sambrook *et al.* (1989). The hybridised molecules were analysed using either a Molecular Dynamics PhosphoImager or autoradiography.

Sequence analysis. DNA sequencing, using the Sequenase® Version 1.0 DNA Sequencing Kit (Amersham-USB) was carried out according to the manufacturer's instructions.

Computer analysis. Pairwise comparisons were done using BESTFIT program in the sequence analysis software package developed by the Genetics Computer Group (GCG) at the University of Wisconsin (U.S.A.). Prediction of secondary structures and calculation of free energy values of the 5' UTRs were performed with the MFOLD program in the GCG.

RESULTS

Cloning of the *agaA* gene

Arginase from *A. nidulans* was purified to homogeneity and partially sequenced (Dzikowska *et al.*, 1994). This amino-acid sequence as well as the best conserved amino-acid sequences from *Agrobacterium tumefaciens* (Schrell *et al.*, 1989), *Saccharomyces cerevisiae* (Sumrada & Cooper, 1984), *Schizosaccharomyces pombe* (Van Huffel *et al.*, 1994), rat (Kawamoto *et al.*, 1987) and man (Marathe *et al.*, 1993) served to design primers for PCR with the *A. nidulans* DNA used as a template. One pair of primers yielded a PCR fragment 359 bp long which showed strong homology to the C-terminal fragment of the arginase gene from *Neurospora crassa* (GenBank accession number L20687). As the *agaA* locus is located on chromosome VI we have screened 162 cosmids representing this chromosome using the 359 bp fragment as a probe. One of the cosmids (W16H07) gave a positive signal. A 40

kb insert of this cosmid was digested with different restriction enzymes and, after gel electrophoresis and Southern blotting, was hybridised to the same probe. The hybridising 3.6 kb *Eco*RI fragment was selected and cloned into the pBluescript SK^+ plasmid. The resulting pAB93E plasmid was used for further investigations.

To check whether the pAB93E plasmid contains the complete sequence of the *agaA* gene, we have used it for transformation of the *A. nidulans proA6 pabaA9 biA1 trpC81 agaA90* strain. The *agaA90* mutation prevents utilisation of arginine as a sole nitrogen source and as a source of proline in the *proA6* background. However, the selection for the $agaA^+$ phenotype is inconvenient because of relatively high reversion rate of the *agaA90* mutation. Therefore we have cotransformed this strain with the plasmid pAGA22 bearing the *A. nidulans trpC* gene. Plasmids were used in equimolar proportions. Transformants were selected for the trp^+ phenotype and tested for the ability to utilise arginine. The transformation efficiency in this experiment was around 100 transformants per 1 μg of plasmid DNA and the frequency of cotransformation of *trpC* and *agaA* was 59%. Several transformants of the desired phenotype were grown in the presence and absence of arginine in the medium and the arginase activity in the mycelium was assayed. The arginase activity and its rate of induction by arginine in transformants was found to be similar to that in the *A. nidulans* wild type strain, indicating that the transforming plasmid contained the *agaA* gene together with its promoter.

Selection of the *agaA* cDNA clones

cDNA clones were selected from the *A. nidulans* cDNA library constructed on the λZAP vector (see Materials and Methods) using a fragment of the genomic clone (see Fig. 4, probe N) as a probe. Around 50000 phages were screened in the plaque hybridisation test. Nine phages were picked out and four of them with the biggest inserts were restriction mapped and sequenced.

The sequence of the *agaA* gene

The sequence of the arginase genomic clone is shown in Fig. 1. Comparison of this sequence with sequences of the four selected cDNA clones revealed the presence of three introns, 53, 346 and 96 bp long, the first one located upstream from the translation start point. The sequence data containing the *agaA* sequence up to position –310 have been submitted to the GenBank under accession number U62482.

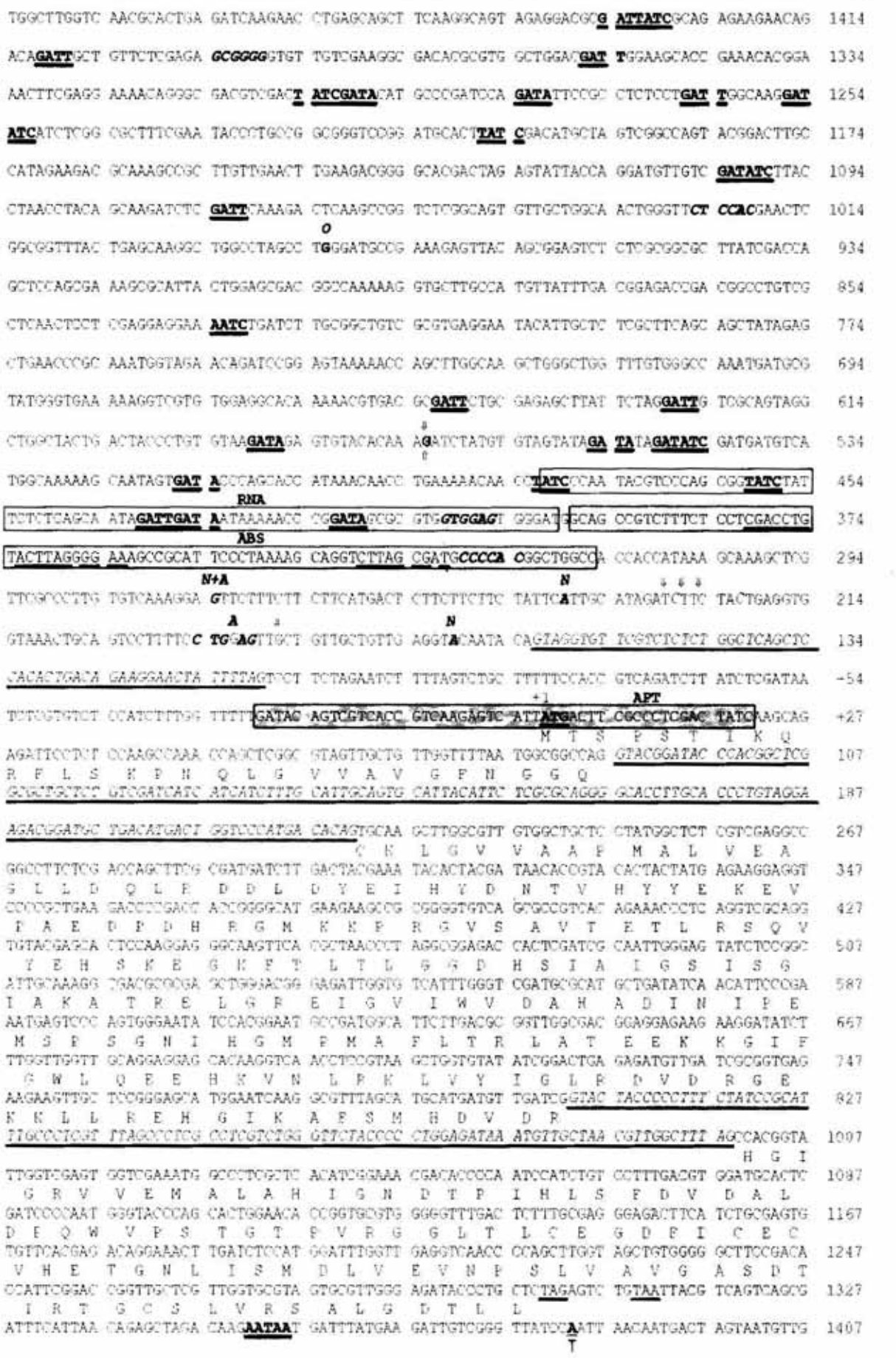
The open reading frame of the *agaA* transcript potentially encodes an acidic protein of 323 amino acids, with the predicted isoelectric point of 9.5 and molecular mass of 36 kDa.

Structure of the promoter and transcription of the *agaA* gene

RNA was isolated from the mycelium of the *A. nidulans* wild type strains grown in the presence and absence of arginine. Northern blot analysis with the DNA fragment of the genomic clone used as a probe (probe N, see

Figure 1. Sequence of the *agaA* gene.

Putative AREA and CREA binding sites are marked with bold letters and AREA sites are additionally underlined. Transcription initiation sites determined by primer extension are marked O, N and A, similarly as in Fig. 3. The 5′ ends of the *agaA* cDNA clones are marked with arrows. The 3′end is marked with the letter T. Introns in the 5′ UTR and in coding sequences are underlined. The polyadenylation site (AATAA) is also marked. The sequences ABS and APT (see Discussion) of potential importance for regulation of *agaA* expression are boxed.


RNA
ABS
APT

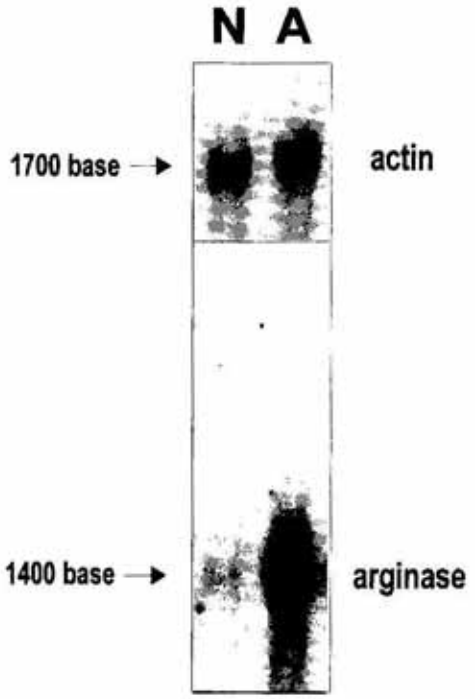


Figure 2. Northern blot analysis of the *agaA* transcripts.

RNA was isolated from the mycelium of the *proA6 paba A9 biA1* strain grown on nitrate (N) or arginine (A) as a nitrogen source.

Fig. 4) revealed a single class of mRNA molecules approximately 1400 bases long (Fig. 2). The amount of mRNA was standardised against mRNA of actin present in the same preparations and was shown to be 10-fold greater in the mycelium grown in the presence than in the absence of arginine. This result correlates reasonably well with the results of arginase activity assays which gave the result of 0.2 and 1.5 units respectively. One can therefore postulate that regulation of arginase synthesis proceeds at the level of transcription or stability of the mRNA.

Primer extension experiments (Fig. 3) have shown that arginase mRNA is initiated in the region approximately 200 bp upstream from the codon AUG. Different transcription start points are utilised depending on the presence or absence of arginine. When arginase synthesis is induced by arginine, the arginase mRNA starts at the position -191 (A) while in the absence of arginine the main starting point (N1) is located at the position -238 and

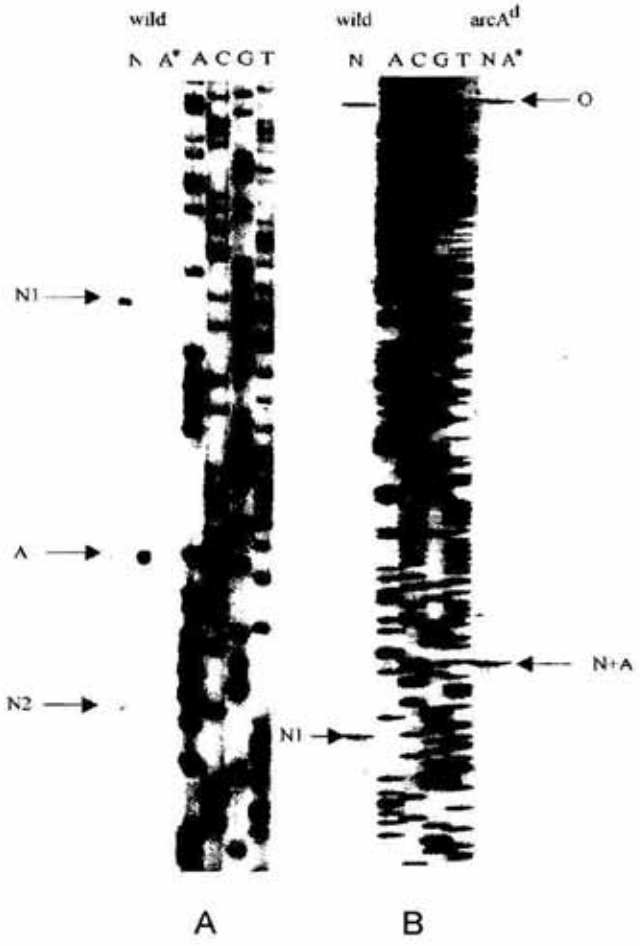


Figure 3. Primer extension experiments.

Primer PRIM2 (see Fig. 4) was used. A. RNA was isolated from mycelia of the wild type and the *areA*^{d}*47* strains grown on nitrate (N) or arginine (A*) as nitrogen sources. On the autoradiogram, bands corresponding to the messengers with the 5′ ends located at the positions -238 (N1), -191 (A) and -169 (N2) in respect to the AUG are visible. B. Messengers with the 5′ ends located in the position -982 (O) in the *arcA*^{d}*47* and in the wild type strains grown on nitrate. The N1 and N+A bands seen on the autoradiogram A are also visible. The weak N2 band could not be detected in this experiment.

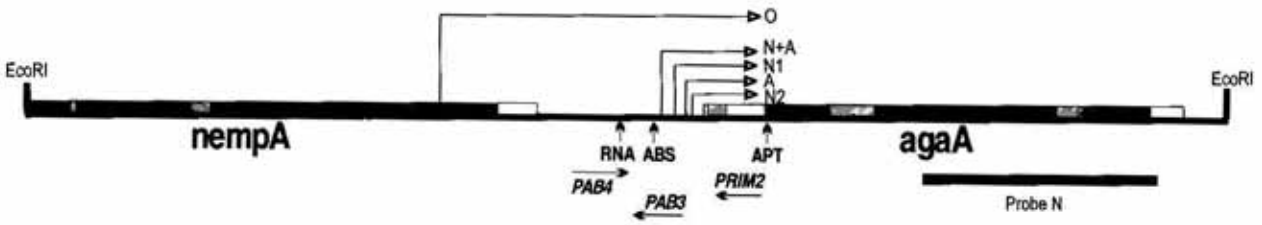


Figure 4. Structure of the *agaA* gene.

Arrows show the 5′ ends of mRNA as revealed by primer extension. The positions of putative recognition sequences of the arcA product (ABS) and of the arginine aptamer (APT) are marked. The positions of primers used for RT-PCR experiments and of the probe used for hybridisation experiments (probe N) are indicated.

a minor one (N2) at the position −169. The fourth starting point (N+A, −272) was observed in the case of mRNA isolated from the *arcA* ^{d}*47* mutant grown either in the presence or in the absence of arginine. The *arcA* gene codes for the transcriptional activator of the arginine catabolic pathway (Empel, unpublished results). In the *arcA47* mutant arginase is synthesised at the derepressed level.

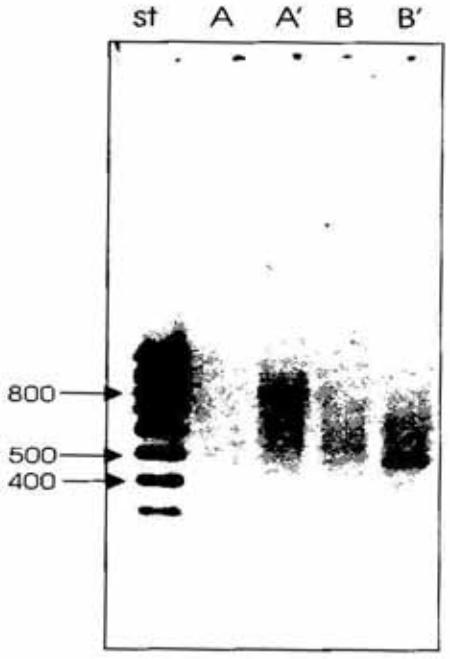


Figure 5. RT-PCR of the 5′UTR of the *agaA* mRNA.

Total RNA preparation from *A. nidulans* wild type served as a template for the ssDNA synthesis primed with PRIM2. The PCR reaction was carried out with primers PRIM2 and PAB4 (A and A′) and PAB3 and PAB4 (B and B′). The location of primer binding sequences is shown in Fig. 4. RNA preparations used in A and B were treated with RNAse prior to the RT-PCR reactions. St – relative molecular mass standards.

Our primer extension studies revealed, both in the wild type and *arcA*^{d}*47* strains, one additional transcription start point (0) located within the last exon of the neighbouring *nempA* gene over 750 bp from the three start points mentioned above. We have repeated primer extension assays using primers designed for the region upstream to these transcription starting points and to the 5′ end of the longest arginase cDNA we have obtained. The results of these tests (not shown) confirmed the presence of the mRNA molecule with the 5′ ends at the position −982. mRNA of the size corresponding to the molecules initiated at this position was not detected in our Northern blots experiments.

Arginase synthesis is under general nitrogen and carbon regulation (Bartnik *et al.*, 1977). Analysis of the nucleotide sequence upstream from the arginase open reading frame reveals the presence of two clusters of sequences recognised by the AREA activator and CREA repressor proteins (Fig. 1). These sequences – GATA for AREA and SYGGRG for CREA are located upstream from the transcription start points in all *A. nidulans* genes regulated by *areA* and *creA* (Pateman *et al.*, 1983; Cove, 1979; Sophianopoulou *et al.*, 1993). The positions of two clusters of these sequences in the *agaA* gene correspond with the 5′ ends of mRNA's as revealed by primer extension experiments. To get additional evi-

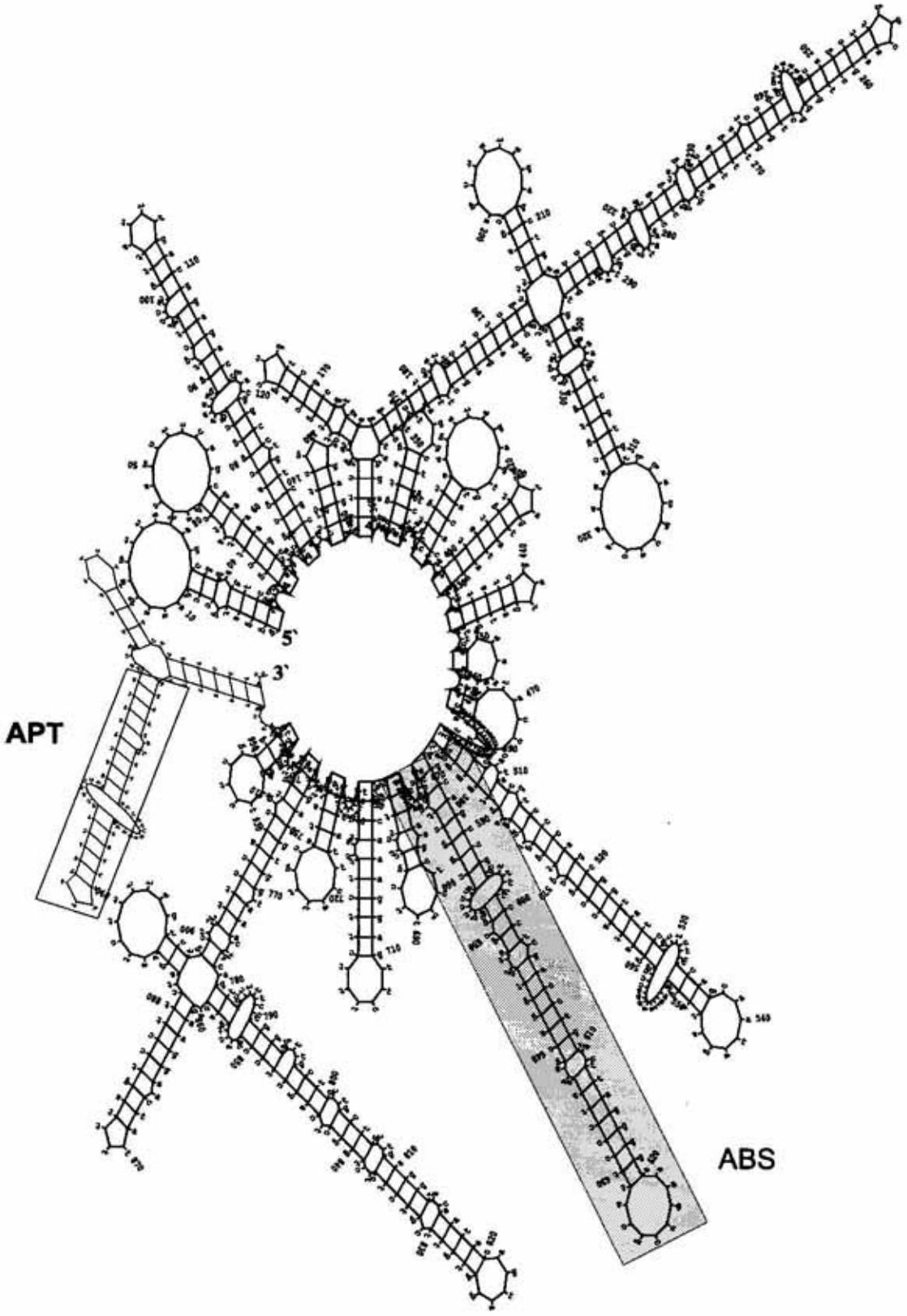


Figure 6. Putative secondary structure of the 5′ UTR of the *agaA* mRNA.

ABS, the putative binding sequence of the *arcA* product; APT, arginine aptamer.

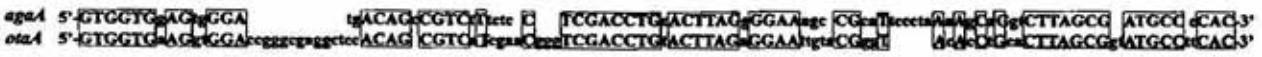


Figure 7. Common putative regulatory sequences (ABS) found in promoters of the *agaA* and *otaA* genes.

dence that at least some of the mRNA molecules are initiated at the "upper" promoter we have performed the RT-PCR experiments. Two pairs of primers were employed. One pair was designed for the region between the two promoters. The second pair of primers flanked the downstream promoter (see Fig. 4). In both cases the RT-PCR products of the expected size were obtained (Fig. 5). To prove that these products represented sequences located upstream to the second promoter we have probed them with the labelled oligonucleotide PRIM2. Both RT-PCR products hybridised to this probe (results not shown).

Putative secondary structure of 5′ UTR of the *agaA* mRNA

We have applied the MFOLD program (see Materials and Methods) to obtain putative secondary structure of the 5′ UTR. The results are shown in Fig. 6. There are at least two interesting elements found within this structure. First, the loop marked "APT" bears significant resemblance to the arginine aptamers found in several other RNAs (Tao & Frankel, 1996). Second, the loop marked "ABS" contains the DNA sequence found also in the promoter of the *otaA* gene (Dzikowska *et al.*, 1999), the gene which is regulated in the same fashion as the *agaA*. Alignment of the ABS sequences from the *agaA* and *otaA* genes is shown in Fig. 7.

DISCUSSION

Comparative studies on arginases and related enzymes have been recently published by several authors (Ouzounis & Kyrpides, 1994; Jenkinson *et al.*, 1996; Perozich *et al.*, 1998). In *A. nidulans* all of the 32 amino acids of arginase are conserved in all species studied with the exception of proline at position 24 which is sometimes replaced by glycine. Studies on amino-acid sequences of arginases from many organisms clearly indicate that this enzyme can serve well as a phylogenetic marker and one can follow the evolution of this enzyme starting from *Archaebacteria* and

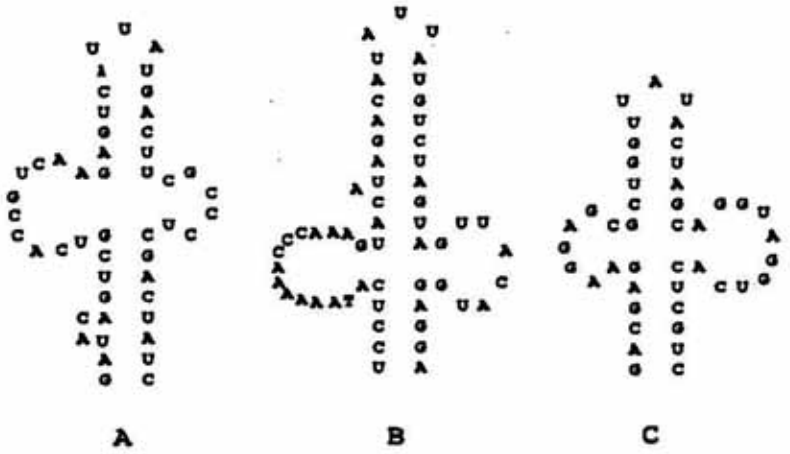


Figure 8. Putative arginine binding sequences found in: A, *A. nidulans agaA* gene (this work); B, *Sch. pombe* arginase structural gene CAR1 (GenBank.X75559) (Yarus *et al.*, 1991; C, synthetic arginine aptamer (Famulok, 1996).

ending with man. It would be even more interesting to compare regulatory devices and mechanisms employed in regulation of arginase synthesis in various organisms. In all cases studied so far arginase regulation appeared to be surprisingly complex. As it was mentioned earlier, in *A. nidulans* there are over 10 genes which seem to control the expression of the arginase structural gene. In *S. cerevisiae* three regulatory genes were found to control arginine specific activation of the arginase gene (Bercy *et al.*, 1987; Qiu *et al.*, 1990; 1991; Messenguy & Dubois, 1993) while other genes (GLN3, GAT1 and URE2) are responsible for nitrogen metabolite repression of this gene (Minehart & Magasanik, 1991; Coschigano & Magasanik, 1991; Coffman *et al.*, 1994; 1996; Stanbrough *et al.*, 1995). In addition, in *S. cerevisiae*, arginase expression is regulated by general yeast transcription activators, the products of ABF1 an RAP1 genes (Kovari & Cooper, 1991; Kovari *et al.*, 1992). Functional analysis of the *S. cerevisiae* promoter reveals 11–13 *cis*-acting elements which bind products of the genes listed above and participate in the arginase gene expression and regulation (Smart *et al.*, 1996). Analysis of the liver arginase promoters in man and *Macaca fascicularis* (Goodman *et al.*, 1994) has shown binding of multiple protein complexes both liver specific and shared among other tissues. In rat at least three transcription factors, CTF/NF1, Sp1 and C/EBP, were found to bind to arginase promoter (Takiguchi & Mori, 1991). The 5′ flanking regions of human and rat arginase genes up to position −105 are in 84% identical (Takiguchi *et al.*, 1988).

Analysis of the nucleotide sequence of the *A. nidulans agaA* promoter revealed the presence of several putative GATA sites for binding of AREA activator protein and several SYGGRG sites for binding the CREA repressor. AREA and CREA binding sites form two clusters separated by approximately 400 bp. Two pairs of GATA sites and one CREA site located proximally to the AUG (see Fig. 1) were found to bind to the purified proteins in the gel retardation experiments (Weglenski, results not shown). The 83 bp long sequence named ABS (see Fig. 7) was found to be very similar to that present in the promoter of *otaA* (Dzikowska *et al.*, 1999). It is plausible that this sequence or its portion is a target for the transcriptional activator specified by the *arcA* gene which controls expression of both *agaA* and *otaA* genes.

Primer extension and RT-PCR experiments presented in this paper indicate that transcription of arginase mRNA starts at several different positions (Figs. 1 and 3). The longest mRNA molecules start at the position −982 in respect to AUG codon. mRNA molecules of this size were not detected by the Northern technique, nor they were found among the four cDNA clones. It seems that the relative abundance of these molecules is very low as compared to those initiated in the region −238–−169.

Our results do not allow to decide whether arginase transcripts are initiated at several positions or at a single position well within the reading frame of the neighbouring *nempA* gene and then processed at one of the positions closer to the arginase AUG codon. The *nempA* gene seems to have nothing to do with arginase and arginine catabolism it is a homologue of a *S. cerevisiae* gene involved in mitochondrial biogenesis.

The idea of a single arginase transcript processed at different points in relation to the metabolic state of the mycelium becomes appealing when one looks at the putative secondary structure of the arginase mRNA. The 5′ UTR of this mRNA shows an extensive secondary structure which usually indicates the possibility of post-transcriptional regulation (Sachs, 1993; Dandekar & Hentze, 1995; Klaff *et al.*, 1966; Duret *et al.*,1993) and contains two structural elements which might be significant for regulation of arginase synthesis. One of these elements is a hairpin closely resembling an arginine aptamer. It is shown in Fig. 8 together with two arginine binding ap-

tamers, one from the TAR mRNA of HIV (Yarus *et al.*, 1991) and the second obtained by *in vitro* selection (Famulok, 1996). We have found that structures resembling arginine aptamers are present in two other genes involved in arginine metabolism: the arginase structural gene (CAR1) from *Sch. pombe* (Van Huffel *et al.*, 1994) and the *otaA* gene from *A. nidulans*, coding for the second enzyme of the arginine catabolic pathway. Arginine aptamers were found to play a role in several biological systems (Draper, 1995). The best known examples are the self-splicing of the group I intron in *Tetrahymena* (Sachs, 1993) and the process of recognition of HIV-1 TAT protein by TAR mRNA (Draper, 1995). The presence of the arginine aptamers in the mRNA of genes involved in arginine metabolism and expressed upon arginine induction can be hardly considered as purely coincidental. Two types of experiments are, however, necessary to prove the role of those aptamers in the regulation of expression of the *agaA* gene. One is a functional analysis of the *agaA* promoter, and the second direct analysis of processing of the arginase mRNA and of its ability to bind arginine. Experiments of both types are now in progress.

REFERENCES

Aramayo, R. & Timberlake, W.E. (1990) Sequence and molecular structure of the *Aspergillus nidulans yA* (laccase I) gene. *Nucleic Acids Res.* **18**, 3415.

Bailey, C. & Arst, H.N., Jr. (1975) Carbon catabolite repression in *Aspergillus nidulans*. *Eur. J. Biochem.* **51**, 573–577.

Bartnik, E., Guzewska, J., Klimczuk, J., Piotrowska, M. & Weglenski, P. (1977) in *Genetics and Physiology of Aspergillus* (Smith, J.E. & Pateman, J.A., eds.) pp. 243–254, Academic Press.

Bartnik, E. & Weglenski, P. (1974) Regulation of arginine catabolism in *Aspergillus nidulans*. *Nature* (London) **250**, 591–592.

Bercy, J., Dubois, E. & Messenguy, F. (1987) Regulation of arginine metabolism in *Saccharomyces cerevisiae*: Expression of the three ARGR regulatory genes and cellular localization of their products. *Gene* **55**, 277–285.

Brody, H., Griffith, J., Cuticchia, J., Arnold, J. & Timberlake, W.E. (1991) Chromosome-specific recombinant DNA libraries from the fungus *Aspergillus nidulans*. *Nucleic Acids Res.* **19**, 3105–3109.

Coffman, J.A., El Berry, H.M. & Cooper, T.G. (1994) The URE2 protein regulates nitrogen catabolic gene expression through the GATAA-containing UASNTR element in *Saccharomyces cerevisiae*. *J. Bacteriol.* **176**, 7476–7483.

Coffman, J.A., Rai, R., Cunningham, T., Svetloy, V. & Cooper, T.G. (1996) Gat1p, a GATA family protein whose production is sensitive to nitrogen catabolite repression, participates in transcriptional activation of nitrogen-catabolic genes in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **16**, 847–858.

Coschigano, P.W. & Magasanik, B. (1991) The URE2 gene product of *Saccharomyces cerevisiae* plays an important role in the cellular response to the nitrogen source and has homology to glutathione *S*-transferases. *Mol. Cell. Biol.* **11**, 822–832.

Cove, D.J. (1979) Genetic studies on nitrate assimilation in *Aspergillus nidulans*. *Biol. Rev.* **54**, 291–327.

Dandekar, T. & Hentze, M.W. (1995) Finding the hairpin in the haystack: Searching for RNA motifs. *Trends Genet.* **11**, 45–50.

Dowzer, C.E.A. & Kelly, J.M. (1991) Analysis of the *creA* gene, a regulator of carbon catabolite repression in *Aspergillus nidulans*. *Mol. Cell. Biol.* **11**, 5701–5709.

Draper, D.E. (1995) Protein-RNA recognition. *Annu. Rev. Biochem.* **64**, 593–620.

Duret, L., Dorkeld, F. & Gautier, C. (1993) Strong conservation of non-coding sequences during vertebrates evolution: Potential involvement in post-transcriptional regulation of gene expression. *Nucleic Acids Res.* **21** 2315–2322.

Dzikowska, A., Le Caer, J.P., Jończyk, P. & Weglenski, P. (1994) Purification of arginase

from *Aspergillus nidulans*. *Acta Biochim. Polon.* **41**, 467–471.

Dzikowska, A., Swianiewicz, M., Talarczyk, A., Wisniewski, M., Goras, M., Scazzocchio, C. & Weglenski, P. (1999) Cloning, characterisation and regulation of the ornithine transaminase (*otaA*) gene of *Aspergillus nidulans*. *Curr. Genet.* **35**, 118–126.

Empel, J. (1998) Klonowanie i charakterystyka genu *arcA* z *Aspergillus nidulans*. Ph.D. Thesis, University of Warsaw (in Polish).

Esch, F., Lin, K.I., Hills, A., Zaman, K., Baraban, J.M., Chatterjee, S., Rubin, L., Ash, D.E. & Ratan, R.R. (1998) Purification of a multipotent antideath activity from bovine liver and its identification as arginase: Nitric oxide-independent inhibition of neuronal apoptosis. *J. Neurosci.* **18**, 4083–4095.

Famulok, M. (1996) Combinatorial references. *Chem. Biol.* **3**, 815–816.

Goodman, B.K., Klein, D., Tabor, D.E., Vockley, J.G., Cederbaum, S.D. & Grody, W.W. (1994) Functional and molecular analysis of liver arginase promoter sequences from man and *Macaca fascicularis*. *Somat. Cell Mol. Genet.* **20**, 313–325.

Grody, W.W., Klein, D., Dodson, A.E., Kern, R.M., Wissmann, P.B., Goodman, B.K., Bassand, P., Marescau, B., Kang, S.S. & Leonard, J.V. (1992) Molecular genetic study of human arginase deficiency. *Am. J. Hum. Genet.* **50**, 1281–1290.

Graaff, L.H. de, van den Broeck, H.C., van Ooijen, J.J. & Visser, J. (1994) Regulation of the xylanase-encoding *xlnA* gene of *Aspergillus tubigensis*. *Mol. Microbiol.* **12**, 479–490.

Jenkinson, C.P., Grody, W.W. & Cederbaum, S.D. (1996) Comparative properties of arginases. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **114**, 107–132.

Johnston, I.L., Hughes, S.G. & Clutterbuck, A.J. (1985) Cloning an *Aspergillus nidulans* developmental gene by transformation. *EMBO J.* **4**, 1307–1311.

Kawamoto, S., Amaya, Y., Murakami, K., Tokunaga, F., Iwanaga, S., Kobayashi, K., Saheki, T., Kimura, S. & Mori, M. (1987) Complete nucleotide sequence of cDNA and deduced amino acid sequence of rat liver arginase. *J. Biol. Chem.* **262**, 6280–6283.

Klaff, P., Riesner, D. & Steger, G. (1996) RNA structure and the regulation of gene expression. *Plant Mol. Biol.* **32**, 89–106.

Kovari, L.Z. & Cooper, T.G. (1991) Participation of ABF-1 protein in expression of the *Saccharomyces cerevisiae* CAR1 gene. *J. Bacteriol.* **173**, 6332–6338.

Kovari, L.Z., Kovari, I. & Cooper, T.G. (1992) Participation of RAP1 protein in expression of the *Saccharomyces cerevisiae* arginase (CAR1) gene. *J. Bacteriol.* **175**, 941–951.

Kudla, B., Caddick, M.X., Langdon, T., Martinez-Rossi, N.M., Bennett, C.F., Sibley, S., Davis, R.W. & Arst, H.N., Jr. (1990) The regulatory gene *areA* mediating nitrogen metabolite repression in *Aspergillus nidulans*: Mutations affecting specificity of gene activation alter a loop residue of a putative zinc finger. *EMBO J.* **9**, 1355–1364.

Marathe, S.V., Becker, E., Yu, Y.-G. & Weiss, R.L. (1993) Direct submission to the GenBank database (accession L20687).

McPherson, M.J., Hames, E.F. & Taylor, G.R. (1995) in: *PCR: A Practical Approach*. IRL Press, Oxford.

Messenguy, F. & Dubois, E. (1993) Genetic evidence for a role for MCM1 in the regulation of arginine metabolism in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **13**, 2586–2592.

Minehart, P.L. & Magasanik, B. (1991) Sequence and expression of GLN3, a positive nitrogen regulatory gene of *Saccharomyces cerevisiae* encoding a protein with a putative zinc finger DNA-binding domain. *Mol. Cell. Biol.* **11**, 6216–6228.

Ouzounis, C.A. & Kyrpides, N.C. (1994) On the evolution of arginases and related enzymes. *J. Mol. Evol.* **39**, 101–104.

Pateman, J.A., Doy, C.H., Olsen, J.E., Norris, U., Creaser, E.H. & Hynes, M. (1983) Regulation of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AldDH) in *Aspergillus nidulans*. *J. Proc. R. Soc. Lon.* **B217**, 243–264.

Patterton, D. & Shi, Y.B. (1994) Thyroid hormone-dependent differential regulation of multiple arginase genes during amphibian metamorphosis. *J. Biol. Chem.* **269**, 25328–25334.

Perozich, J., Hempel, J. & Morris, S.M., Jr. (1998) Roles of conserved residues in the arginase family. *Biochim. Biophys. Acta* **1382**, 23–37.

Pontecorvo, G., Roper, J.A., Hemmons, L.M., MacDonald, R.D. & Bufton, A.W.J. (1953) The genetics of *Aspergillus nidulans*. *Adv. Genet.* **5**, 141–238.

Qiu, H.F., Dubois, E., Broen, P. & Messenguy, F. (1990) Functional analysis of ARGR1 and ARGRIII regulatory proteins involved in the regulation of arginine metabolism in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* **222**, 192–200.

Qiu, H.F., Dubois, E. & Messenguy, F. (1991) Dissection of the bifunctional ARGRII protein involved in the regulation of arginine anabolic and catabolic pathways. *Mol. Cell. Biol.* **11**, 2169–2179.

Sachs, A.B. (1993) Messenger RNA degradation in eukaryotes. *Cell* **74**, 413–421.

Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989) in *Molecular Cloning: A Laboratory Manual.* (Nolan, C., ed.) CSHL Press, New York.

Schrell, A., Alt-Moerbe, J., Lanz, T. & Schroeder, J. (1989) Arginase of Agrobacterium Ti plasmid C58. DNA sequence, properties, and comparison with eucaryotic enzymes. *Eur. J. Biochem.* **184**, 635–641.

Skog, S., Eriksson, V. & Eliasson, E. (1981) Arginase, an S-phase enzyme in human cell line. *Biochim. Biophys. Acta* **672**, 33–44.

Smart, W.C., Coffman, J.A. & Cooper, T.G. (1996) Combinatorial regulation of the *Saccharomyces cerevisiae* CAR1 (arginase) promoter in response to multiple environmental signals. *Mol. Cell. Biol.* **16**, 5876–5887.

Sophianopoulou, V., Suarez, T., Diallinas, G. & Scazzocchio, C. (1993) Operator derepressed mutations in the proline-specific upstream activation sequences. *Mol. Gen. Genet.* **236**, 209–213.

Spector, E.B., Kern, R.M., Haggerty, D.F. & Cederbaum, S.D. (1985) Differential expression of multiple forms of arginase in cultured cells. *Mol. Cell. Biol.* **66**, 45–53.

Stanbrough, M., Rowen, D.W. & Magasanik, B. (1995) Role of the GATA factors GLN3p and Nil1p of *Saccharomyces cerevisiae* in the expression of nitrogen-regulated genes. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 9450–9454.

Sumrada, R.A. & Cooper, T.G. (1984) Nucleotide sequence of the *Saccharomyces cerevisiae* arginase gene (CAR1) and its transcription under various physiological conditions. *J. Bacteriol.* **160**, 1078–1087.

Tao, J. & Frankel, A.D. (1996) Arginine-binding RNAs resembling TAR identified by *in vitro* selection. *Biochemistry* **35**, 2229–2238.

Takiguchi, M., Haraguchi, Y. & Mori, M. (1988) Human liver-type arginase gene: Structure of the gene and analysis of the promoter region. *Nucleic Acids Res.* **16**, 8789–8802.

Takiguchi, M. & Mori, M. (1991) *In vitro* analysis of the rat liver-type arginase promoter. *J. Biol. Chem.* **266**, 9186–9193.

Tilburn, J., Scazzocchio, C., Taylor, G.G., Zabicky-Zissman, J.H., Lockington, R.A. & Davies, R.W. (1983) Transformation by integration in *Aspergillus nidulans*. *Gene* **26**, 205–221.

Van Huffel, C., Dubois, E. & Messenguy, F. (1994) Cloning and sequencing of *Schizosaccharomyces pombe car1* gene encoding arginase. Expression of the arginine anabolic and catabolic genes in response to arginine and related metabolites. *Yeast* **10**, 923–933.

Xu, Q., Baker, B.S. & Tata, J.R. (1993) Developmental and hormonal regulation of the *Xenopus* liver-type arginase gene. *Eur. J. Biochem.* **211**, 891–898.

Yarus, M., Illangesekare, M. & Christian, E. (1991) Selection of small molecules by the *Tetrahymena* catalytic center. *Nucleic Acids Res.* **19**, 1297–1304.