Identification, cloning and expression of the mouse *N*-acetylglutamate synthase gene

Ljubica CALDOVIC*, Hiroki MORIZONO*, Xiaolin YU*, Mark THOMPSON*, Dashuang SHI*, Rene GALLEGOS†, Norma M. ALLEWELL‡, Michael H. MALAMY† and Mendel TUCHMAN*[1]

*Children's Research Institute, Children's National Medical Center, George Washington University, 111 Michigan Ave NW, Washington, DC 20010, U.S.A., †Department of Microbiology, Tufts University, 136 Harrison Ave, Boston, MA 02111, U.S.A., and ‡College of Life Sciences, University of Maryland, College Park, MD 20742, U.S.A.

In ureotelic animals, *N*-acetylglutamate (NAG) is an essential allosteric activator of carbamylphosphate synthetase I (CPSI), the first enzyme in the urea cycle. NAG synthase (NAGS; EC 2.3.1.1) catalyses the formation of NAG from glutamate and acetyl-CoA in liver and intestinal mitochondria. This enzyme is supposed to regulate ureagenesis by producing variable amounts of NAG, thus modulating CPSI activity. Moreover, inherited deficiencies in NAGS have been associated with hyperammonaemia, probably due to the loss of CPSI activity. Although the existence of the NAGS protein in mammals has been known for decades, the gene has remained elusive. We identified the mouse (*Mus musculus*) and human NAGS genes using their similarity to the respective *Neurospora crassa* gene. NAGS was cloned from a mouse liver cDNA library and was found to encode a 2.3 kb message, highly expressed in liver and small intestine with lower expression levels in kidney, spleen and testis. The deduced amino acid sequence contains a putative mitochondrial targeting signal at the N-terminus. The cDNA sequence complements an *argA* (NAGS)-deficient *Escherichia coli* strain, reversing its arginine auxotrophy. His-tagged versions of the pre-protein and two putative mature proteins were each overexpressed in *E. coli*, and purified to apparent homogeneity by using a nickel-affinity column. The pre-protein and the two putative mature proteins catalysed the NAGS reaction but one of the putative mature enzymes had significantly higher activity than the pre-protein. The addition of L-arginine increased the catalytic activity of the purified recombinant NAGS enzymes by approx. 2–6-fold.

Key words: *argA*, *ARG2*, arginine metabolism, carbamoylphosphate synthetase I, urea cycle.

INTRODUCTION

N-acetylglutamate (NAG) is an obligatory allosteric activator of carbamoylphosphate synthetase I (CPSI), the first enzyme of the urea cycle (Figure 1) [1,2]. NAG is produced in the mitochondrial matrix of liver and intestinal cells by NAGS (NAG synthase; EC 2.3.1.1) from glutamate and acetyl-CoA (AcCoA) [3]. The enzymic activity of rat NAGS and hNAGS (human NAGS) is markedly enhanced by L-arginine [4]. NAGS has been considered to be a potential regulator of ureagenesis, supplying variable amounts of NAG for modulating CPSI activity [5]. In isolated hepatocytes, ammonia increases the amount of NAG present [6,7], and the mitochondrial level of NAG was found to correlate with the amount of dietary-protein intake [3]. Because L-arginine increases the activity of mammalian NAGS, it has been hypothesized that it is involved in the regulation of NAG production [8]. Intake of dietary protein seems to increase the sensitivity of NAGS to arginine activation, but the mechanism of this effect is unknown [9].

Inherited NAGS deficiency appears to cause hyperammonaemia due to secondary deficiency of CPSI deprived of its cofactor NAG [10]. The diagnosis of this presumably autosomal recessive disorder requires measurement of NAGS enzymic activity in liver tissue. However, the diagnosis of this condition or its inheritance could not be confirmed by DNA testing, as the gene sequence was not available. In addition, secondary reduction in the level of NAG can result either from the inhibition of NAGS activity, or from efflux of NAG from the mitochondria into the cytosol, where it is degraded, or from decreased mitochondrial concentrations of NAGS substrates. These effects can be caused by inherited defects in the metabolism of amino acids and fatty acids or by the administration of valproic acid [11–13].

NAGS has been purified to apparent homogeneity from human and rat liver [4,14,15]. The enzyme appears to be a trimer, but the oligomerization state seems to depend on the concentration of L-arginine [4]. Arginine enhances the activity of mammalian NAGS by 3–5-fold [15]. In addition, protamines and other cationic polypeptides (e.g. polyarginine and polylysine) also increase the activity of NAGS [15].

In fungi and prokaryotes, NAGS is the first enzyme of the ornithine and arginine biosynthesis pathway [16–18], and unlike the mammalian enzyme, it is subject to feedback inhibition rather than activation by arginine [17,18]. The arginine biosynthesis pathway in prokaryotes and lower eukaryotes and the mammalian urea cycle share several enzymes and intermediates (Figure 1), leading to the hypothesis that the urea cycle has evolved from the arginine biosynthesis pathway [5,16]. Enzymes of the urea cycle and their counterparts in the arginine biosynthesis pathway share sequence and structural similarities [19]. For example, ornithine transcarbamylase from bacteria to vertebrates has strongly conserved sequence motifs and the same

Abbreviations used: AcCoA, acetyl-CoA; CPSI, carbamoylphosphate synthetase I; EST, expressed sequence tag; IPTG, isopropyl β-D-thiogalactoside; MTS, mitochondrial targeting signal; NAG, *N*-acetylglutamate; NAGS, NAG synthase.

[1] To whom correspondence should be addressed (e-mail mtuchman@cnmc.org).

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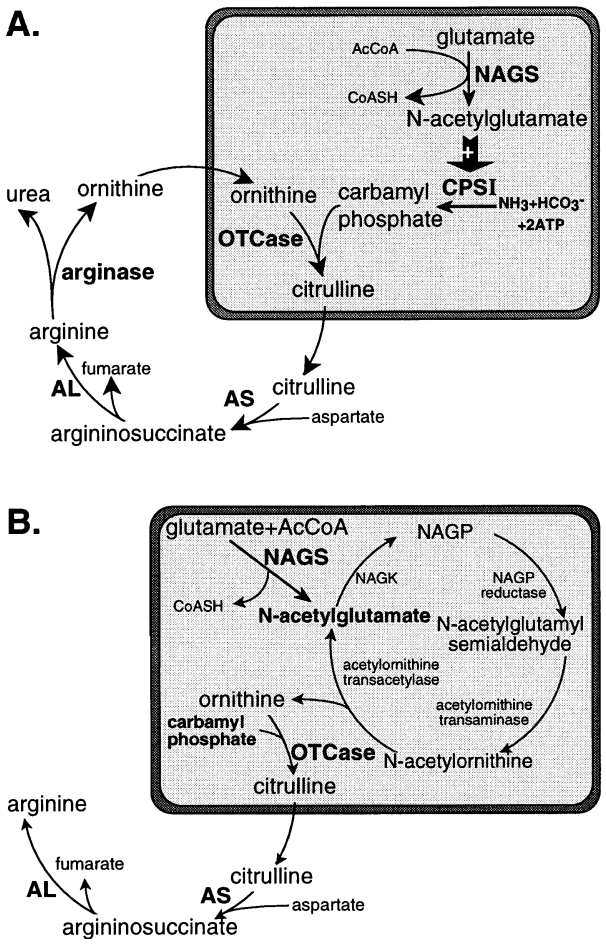

Figure 1 Relationship between the mammalian urea-cycle pathway (A) and the *N. crassa* arginine biosynthesis pathway (B)

The arrow marked with a ' + ' sign indicates the activation of CPSI by NAG. Enzymes shown in bold are common to the urea-cycle pathway and the arginine biosynthesis pathway. AL, argininosuccinate lyase; AS, argininosuccinate synthase; NAGP, *N*-acetylglutamyl phosphate; OTCase, ornithine transcarbamylase.

overall three-dimensional structure [20]. This is not the case for NAGS, where the sequence similarity even between bacteria and fungi is significantly lower [21]. However, we found mammalian sequences with similarity to NAGS from *Neurospora crassa* and used this information to clone the mouse (*Mus musculus*) NAGS gene. This gene, expressed mainly in liver and intestine, complements an NAGS-deficient *Escherichia coli* strain. The purified recombinant protein catalyses the formation of NAG and the activity is enhanced by the addition of L-arginine.

MATERIALS AND METHODS

Identification and cloning of mouse NAGS

GenBank® Nucleotide Sequence Databases were queried with the NAGS protein sequence from *N. crassa*. Several mouse and human expressed sequence tags (ESTs) with sequence similarity were found. Two mouse ESTs (accession nos. AA530535 and BE381620) were purchased from the IMAGE consortium and were sequenced. Further searches of the mouse EST databases with these two ESTs identified two additional matching ESTs (accession numbers BF385477 and AI594902). These allowed the construction of a complete coding sequence for the putative mouse NAGS gene. PCR primers 5′-GGGACGAGTGGGTTTGGTTGTCG-3′ and 5′-AAGGCCGTCTGGTTTGACTTTGGAGC-3′ were used to amplify the NAGS sequence from a mouse liver cDNA library (Lambda TriplEx expression cDNA; ClonTech Laboratories, Palo Alto, CA, U.S.A.). The NAGS sequence was then amplified with the nested primers 5′-AGTGGGTTTGGTTGTCCATATGGCGACGGCG-3′ and 5′-CCGGATCCTTATTATCAGCTGCCTGGGTCAGAAGC-3′. In addition, two different versions (long and short) of the putative mature NAGS, devoid of a mitochondrial targeting sequence (Figure 2), were amplified with the nested primers 5′-GGGGCTCACATATGCTGAGTGGCAGCGCGCGGCGG-3′ or 5′-AGCCCGGGACATATGCTCAGCACCGCCAGGGCTCAC-3′ and 5′-CCGGATCCTTATTATCAGCTGCCTGGGTCAGAAGC-3′. These three amplification products were cloned into PCR®-BluntII-TOPO® plasmids by using the Zero Blunt® TOPO® PCR Cloning Kit (Invitrogen) according to the manufacturer's instructions. The three resulting plasmids were named pTOPONS1 (NAGS pre-protein coding sequence), pTOPONS1L (long mature NAGS coding sequence) and pTOPONS1S (short mature NAGS coding sequence). After cloning into the pTOPO vectors, the inserts were sequenced to confirm the fidelity of the PCR. The coding sequences for the NAGS pre-protein and the long and short mature NAGS were excised from the pTOPO plasmids with *Nde*I and *Bam*HI restriction endonucleases and ligated into pET15b expression vectors. The resulting plasmids pNS1, pNS1L and pNS1S express the pre-protein and the long and short versions of NAGS respectively fused to a N-terminal polyhistidine tag (Figure 2).

Construction of expression plasmids for complementation studies in *E. coli*

NK5992(*argA*::Tn10) is a transposon-mediated *argA* knock-out *E. coli* strain. In order to express NAGS in NK5992(*argA*::Tn10), the genes encoding the His-tagged NAGS pre-protein and putative short mature NAGS were excised from the T7 RNA polymerase-dependent pNS1L and pNS1S plasmids with *Nco*I and *Bam*HI. These fragments were ligated into pTRC99A plasmid to produce pRAG224 and pRAG225. The pRAG224 plasmid expresses the putative long mature NAGS, whereas the pRAG225 plasmid expresses the putative short mature NAGS. The presence of inserts in pRAG224 and pRAG225 was confirmed by restriction analysis. The pRAG224 and pRAG225 plasmids were transformed into NK5992(*argA*::Tn10).

PCR analysis of pNS1L, pRAG224 and pRAG225 plasmids

To rule out the possibility that any contaminating *argA* sequence was present in the NAGS-deficient *E. coli*, the plasmids pNS1L, pRAG224 and pRAG225 were transformed into NK5992(*argA*::Tn10). Individual colonies were assayed by PCR, first with primers 5′-GAGTTGGTCGAGGGATTCC-3′ and 5′-ACACTTTGGATTTACGCTGG-3′ specific for the *argA* gene, then with primers 5′-GCTCACCCAGAAACGCTGG-3′ and 5′-AGCAATAAACCAGCCAGCC-3′ specific for the β-lactamase gene, and finally with primers 5′-CAGCCATATGCTCAGCACC-3′ and 5′-ATGATTGACTAGTTCGTAGG-3′ specific for the mouse NAGS gene.

Tissue expression

A nylon membrane blotted with a panel of polyadenylated [poly(A)$^+$] RNA from 12 adult mouse tissues was purchased from

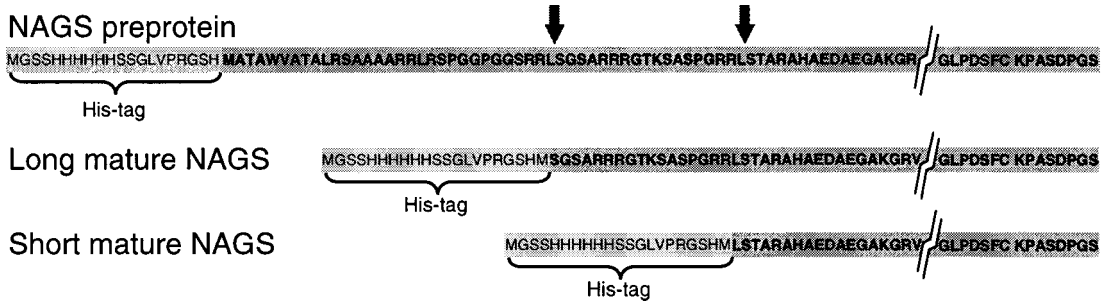

Figure 2 Recombinant NAGS proteins used in the present study

The arrows indicate potential cleavage sites for the mitochondrial processing peptidase, and this information was used to create the long and short versions of the putative mature mitochondrial enzymes. All three proteins were fused to a polyhistidine tag at the N-terminus.

OriGene Techologies (Rockville, MD, U.S.A.). Each lane contained approx. 2 μg of RNA. A probe was excised from the plasmid containing a part of the NAGS cDNA (EST; accession no. AA530535) by using *Eco*RI and *Xho*I, and it was then purified from an agarose gel. The probe (25 ng) was labelled with [α-^{32}P]dATP by random priming using a StripEZ™ DNA kit (Ambion, Austin, TX, U.S.A.). The radiolabelled probe was hybridized on the membrane and washed under stringent conditions using a NorthernMax™ kit (Ambion). Probe preparation, hybridization and washing of the membrane were performed according to the instructions provided with the kit.

Protein purification

The NAGS pre-protein and the two putative mature proteins (Figure 2) were overexpressed in *E. coli* BL21star(DE3). Expression was induced at mid-exponential phase with 0.2 mM isopropyl β-D-thiogalactoside (IPTG), and the cultures were maintained overnight at room temperature and then harvested. Frozen cell pellets were resuspended in 50 mM Tris buffer (pH 8.5) containing 1 mM EDTA, an equal concentration of dithiothreitol and 15 % glycerol, and were sonicated for 1 min. The NAGS pre-protein and the two mature proteins were purified from the soluble fraction using Qiagen nickel-affinity spin columns according to the manufacturer's instructions.

Enzyme assays

The substrate concentrations used to measure NAGS enzyme activity were as described previously [22]. Briefly, samples were assayed in 50 mM Tris buffer (pH 8.5), with 10 mM L-glutamate, 2.5 mM AcCoA and 20 mg/ml BSA in a final reaction volume of 100 μl. The reaction mixture was incubated at 30 °C for 5 min. Initially, the amount of NAG produced by NAGS was followed for 15 min and was found to be linear with respect to time for the first 6 min of the assay (results not shown). The same was true when arginine was added to the assay mixture. Where applicable, 1 mM L-arginine was added to the assay mixture. Protein concentrations were 12–19 mg/ml in cell lysates and 0.17–0.19 mg/ml in purified NAGS preparations. The reaction was quenched with 100 μl of 30 % trichloroacetic acid. Denatured control samples were prepared by heating at 100 °C for 5 min before assaying. The precipitated proteins were removed by micro-centrifugation for 5 min. Glutamate and NAG in the reaction supernatant fluid were separated by reverse-phase HPLC (Hewlett–Packard 1100 series; Boise, ID, U.S.A.). The mobile phase was 93 % solvent A (1 ml of trifluoroacetic acid in 1 litre of water) and 7 % solvent B (1 ml of trifluoroacetic acid in a 1 litre mixture of water and acetonitrile in a ratio 1:9); flow rate was 0.6 ml/min. Glutamate and NAG were detected and quantified by MS (1100 MSD, Hewlett–Packard) using selective ion monitoring. Some of the measurements of NAGS activity are less reproducible than others due to the use of external standardization. We are presently developing a stable isotope-dilution method for quantifying NAG that would be more precise.

RESULTS

Identification and cloning of mouse NAGS

When GenBank® Nucleotide Sequence Databases were queried with the amino acid sequence of NAGS (Arg-14) from *N. crassa*, a region of sequence similarity was identified in human chromosome 17, band 17q21.31 (Figure 3). The deduced amino acids from this sequence showed regions of conservation with other fungal NAGS (Figure 3). The gene-finding program GRAIL (http://compbio.ornl.gov) was used to predict the exons and a putative hNAGS open reading frame was assembled. This sequence was then used to search the EST databases and several mouse- and human-matching ESTs were identified. From these ESTs, the complete coding sequence was assembled for mouse NAGS. The most upstream sequence of the human gene is still unknown, as there are no human ESTs or genomic sequences matching this region. We therefore proceeded with cloning of the mouse NAGS gene.

The overall similarity between mouse and *Neurospora* NAGS is relatively low, with 26 % identity and 33 % similarity. However, there are conserved sequence stretches within the C-terminal region of the mouse NAGS that show higher degrees of similarity to the available fungal protein sequences (boxed sequences in Figure 3).

The sequence and composition of the mouse NAGS N-terminal amino acids are consistent with the known properties of a mitochondrial targeting signal (MTS) [23]. Two potential cleavage sites for mitochondrial processing peptidase were identified in that region (Figure 3) [24].

Expression pattern of mouse NAGS

Tissue expression of mouse NAGS is shown in Figure 4. The gene is highly expressed in liver and small intestine but is expressed to a much lesser degree in kidney, spleen and testis. Expression in the liver and intestine was expected, because CPSI

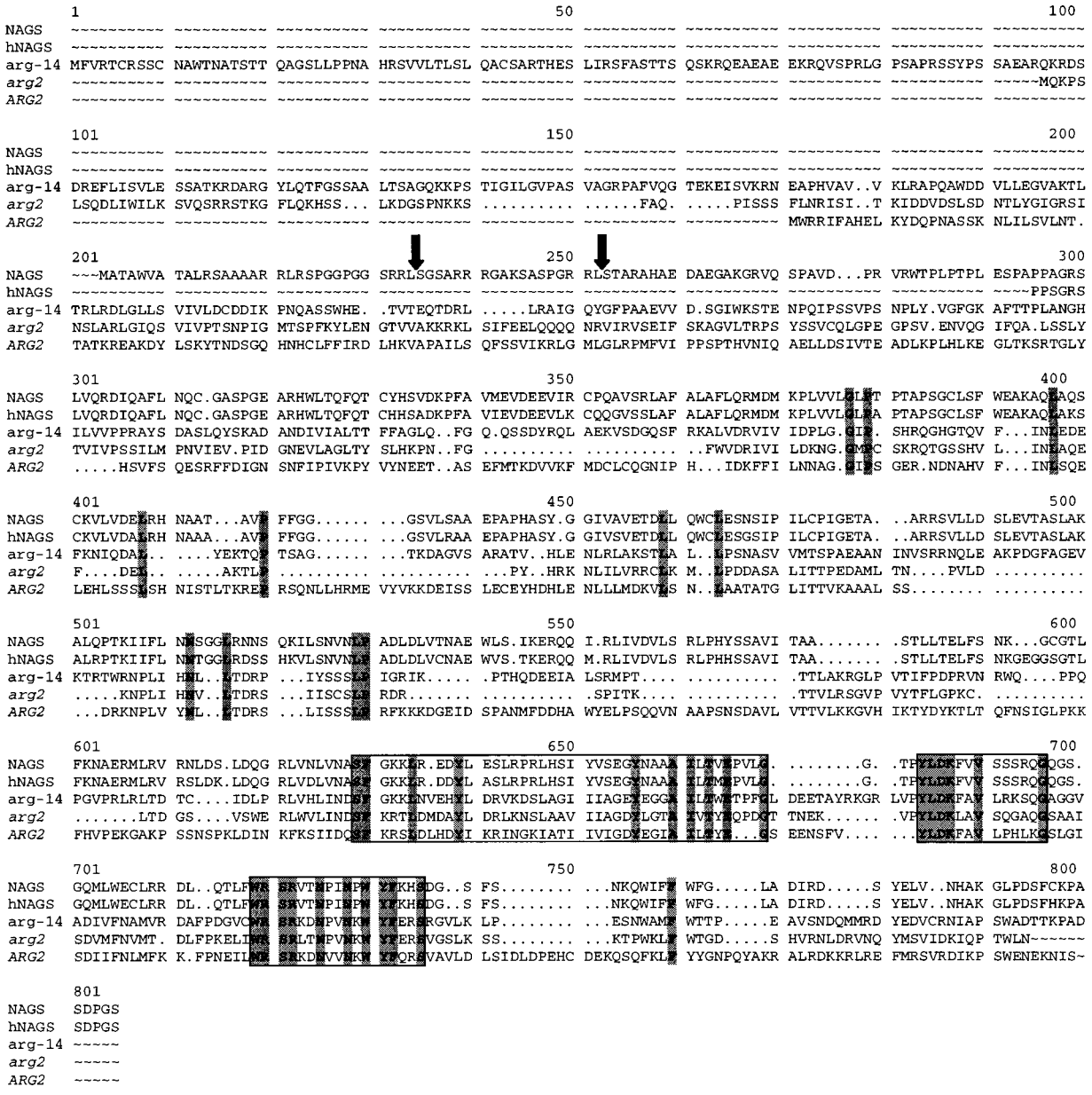

Figure 3 Alignment of mouse NAGS, hNAGS, *N. crassa* NAGS (Arg-14), *S. pombe* NAGS (*arg2*) and *S. cerevisiae* NAGS (*ARG2*)

The regions with the highest similarity are boxed. The predicted protein sequence of hNAGS was generated with the gene-finding program GRAIL; it lacks approx. 90 amino acids of the N-terminal region. Amino acids that are identical in all five enzymes are highlighted in grey. Arrows indicate potential cleavage sites for the mitochondrial-processing peptidase.

is also expressed exclusively in these two tissues [19]. Expression in testis, spleen and kidney raises the possibility that NAGS has additional functions besides catalysing production of the allosteric activator for CPSI.

Enzymic activity of recombinant NAGS

Recombinant mouse NAGS pre-protein and the two versions of the putative mature protein were expressed in *E. coli*. An arginine feedback-insensitive NAGS from *E. coli* (*argA17*) [25] was also overexpressed in the same strain as a positive control. A human gene (glutamine synthase), cloned downstream from the same promoter as the mouse NAGS [26], was used as a negative control. NAGS enzymic activity of the cell extracts and purified recombinant NAGS proteins is shown in Table 1. Substantial NAGS activity was present in cells expressing all three versions of mouse and *E. coli* NAGS, whereas the negative controls had undetectable activity. NAGS activity was dependent on the presence of AcCoA and was absent from cell extracts denatured by heat. Since mammalian NAGS is activated by arginine [15], we also performed the assay in the presence of this amino acid. The addition of 1 mM L-arginine increased mouse NAGS enzyme activity by 2–6-fold, whereas it had no effect on *E. coli* feedback-insensitive NAGS (Table 1).

The activity of the three mouse NAGS enzymes in cell extracts varied considerably and the short mature version showed the

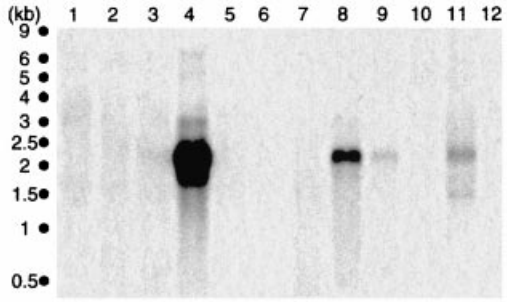

Figure 4 Expression pattern of NAGS mRNA in mouse tissues

Poly(A)$^+$ RNA from 12 tissues was hybridized with a NAGS cDNA probe. Lane 1, brain; lane 2, heart; lane 3, kidney; lane 4, liver; lane 5, lung; lane 6, muscle; lane 7, skin; lane 8, small intestine; lane 9, spleen; lane 10, stomach; lane 11, testis; lane 12, thymus.

Table 1 NAGS activity of cell lysates and purified protein

One unit of activity is defined as 1 nmol of NAG produced in 1 min. n.d., not detectable.

	Specific activity (units/mg of protein)†	
Samples	Without arginine	With 1 mM arginine
Pre-NAGS* cell lysate	49.6 ± 3.7	63.8 ± 0.8
NAGS (long)‡ cell lysate	47.9 ± 3.6	57.8 ± 2.2
NAGS (short)§ cell lysate	77.9 ± 6.4	88.2 ± 3.6
argA17‖ cell lysate	3.0 ± 1.0	2.8 ± 0.6
Glutamine synthase cell lysate	n.d.	n.d.
NAGS (short) heated cell lysate	n.d.	n.d.
NAGS (short) cell lysate – AcCoA	n.d.	n.d.
Pure pre-NAGS	70.1 ± 15.5	234.4 ± 23.5
Pure NAGS (long)	31.3 ± 12.0	125.7 ± 7.7
Pure NAGS (short)	346.5 ± 42.2	803.6 ± 54.4

* NAGS pre-protein.
† Values are the means ± S.E.M. for three measurements.
‡ Putative mature NAGS, long version.
§ Putative mature NAGS, short version.
‖ Feedback-resistant NAGS from *E. coli*.

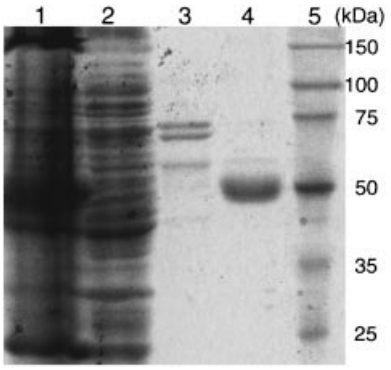

Figure 5 Purification of recombinant NAGS

The short version of the putative mature enzyme with a N-terminal polyhistidine tag was overexpressed in *E. coli* and purified on a nickel-affinity column. Lane 1, cell lysate; lane 2, flow-through; lane 3, wash fraction; lane 4, elution fraction; lane 5, molecular-mass markers.

highest activity. These three NAGS proteins were subsequently purified by nickel-affinity chromatography. A representative purification of the short mature NAGS is shown in Figure 5. All three purified proteins showed enzymic activity, which increased in the presence of arginine; the short mature NAGS again showed the highest activity (Table 1).

Complementation of the *argA* deficiency in NK5992(*argA*::Tn10)

The plasmids pNS1L and pNS1S were introduced into the NAGS-deficient *E. coli* strain NK5992(*argA*::Tn10). PCR of individual colonies confirmed the presence of the mouse NAGS and the β-lactamase genes and the absence of an intact *argA* gene.

NK5992(*argA*::Tn10) cells containing pNS1L (T7-NAGS putative long mature protein), pRAG224 (trc-NAGS putative long mature protein) or pRAG225 (trc-NAGS putative short mature protein) were plated on minimal medium plates containing tetracycline (for the selection of cells containing Tn10) in the presence or absence of arginine. IPTG was used to induce the expression of the mouse NAGS gene. NK5992(*argA*::Tn10) containing pRAG224 or pRAG225 grew with the same efficiency on media containing or lacking arginine. Although IPTG was not absolutely required in the absence of arginine, the colonies grew much slower without it. NK5992(*argA*::Tn10) containing pNS1L, which requires T7 RNA polymerase for transcription, grew on plates with arginine as expected, but not on plates without arginine, irrespective of the presence or absence of IPTG ($10 < 10^{-5}$ reversion frequency). Since NK5992(*argA*::Tn10) does not contain a gene for T7 RNA polymerase, only low levels of basal transcription are expected irrespective of the presence or absence of IPTG.

NAGS activity was measured in NK5992(*argA*::Tn10) cells containing plasmids pRAG224 and pRAG225. Much higher levels of NAGS activity were found in cells induced by IPTG compared with the basal levels of expression seen in the cells that were not induced (results not shown). This explains why cells that contain pRAG224 and pRAG225 grow more slowly in the absence of arginine and IPTG.

DISCUSSION

In the present study, we describe the identification and cloning of the NAGS gene from mouse, based on its similarity to the NAGS gene from *N. crassa*. The overall sequence similarity between mouse and *Neurospora* NAGS is relatively low compared with other genes in the arginine biosynthesis/urea-cycle pathways of these two organisms. However, the C-terminal region has a higher degree of similarity, suggesting that this domain probably has an important role either in substrate recognition or in catalytic function. At the beginning of this project, the draft of the human genome was not available, and although we found a bacterial artificial chromosome that contained most of the exons encoding the putative gene for hNAGS, the 5′ flanking sequence was not present in the bacterial artificial chromosome. This upstream sequence is also missing in the recently published draft of the human genome.

In addition to its low similarity to fungal and bacterial NAGS, the mouse gene shows a weak similarity to fungal and bacterial NAGK (NAG kinase), which catalyses the second step of ornithine and arginine biosynthesis. Previous studies [27] have found that *E. coli* NAGS and *N. crassa* NAGK have some similarity, suggesting that NAGS arose by tandem duplication of the NAGK gene. However, there is very little sequence con-

servation even between bacterial and fungal NAGS (our observations and [21]). This suggests that eukaryotic NAGS arose independently during evolution, or that bacterial and fungal NAGS sequences began diverging long ago. Without additional information on the structure of their proteins, it is difficult to assess whether bacterial NAGS and eukaryotic NAGS represent convergent or divergent evolution.

Like NAGS from lower eukaryotes, mammalian NAGS is localized in the mitochondrial matrix [19]; we found a putative MTS and two potential cleavage sites for the mitochondrial processing peptidase at the N-terminus of the protein [24]. Our experiments reveal that the NAGS pre-protein and the putative mature proteins possess enzymic activity that is increased by L-arginine (Table 1). Since the N-terminus of *S. cerevisiae* NAGS is not removed when imported into mitochondria [21], it remains to be seen whether the putative MTS of mouse NAGS is cleaved after mitochondrial import. Although the enzymic activity of mouse NAGS increases after removal of this predicted MTS, we cannot rule out the possibility that this region of the protein functions as a negative regulatory domain. Experiments to address these questions are in progress.

We showed that mouse NAGS mRNA is highly expressed in the liver and small intestine and is expressed to a much lesser degree in kidney, spleen and testis. Expression in the liver and small intestine was expected, as it mirrors the expression pattern of CPSI. However, the presence of NAGS mRNA in kidney, testis and spleen was unexpected and it may reflect additional functions of NAGS beyond ureagenesis. An additional role for NAGS has been suggested before, based on the fact that it appears to be active even before CPSI activity is detected in embryonic rat liver [28]. Moreover, the enzymic activity of NAGS is enhanced by protamines, which are arginine-rich proteins involved in maintaining the compacted structure of spermatozoa chromatin [15]. Alternatively, it is possible that NAG is a precursor for ornithine and polyamine biosynthesis in the testis. NAGS enzymic activity in tissues other than the liver and intestine needs to be confirmed before additional hypothetical functions of NAGS are explored.

The genes encoding NAGK and the next two enzymes of the ornithine biosynthesis pathway (Figure 1B) have not been found in mammals. However, as was the case for NAGS, the gene sequences of these enzymes may differ markedly from their lower eukaryotic counterparts, making them hard to identify. Cross-comparative searches of the rapidly increasing number of the available genomes are needed, to identify these genes in higher eukaryotes if they exist.

Modulation of NAGS activity can be expected to regulate ureagenesis, since CPSI requires NAG for its catalytic activity. As seen with other urea-cycle enzymes, NAGS activity also seems to be subject to regulation by dietary-protein intake and by hormones such as glucagon [29,30]. Arginine levels in the liver could reflect the amount of dietary-protein intake, and thus the enhanced production of NAG in response to arginine is a potential mechanism for increasing nitrogen flux by the urea cycle. There has been a long-standing debate over the role of NAG in the regulation of ureagenesis; this debate primarily stems from a disagreement on the physiological concentrations of NAG in the liver [8]. If the concentration of NAG falls within a range close to the affinity constant for CPSI, a case could be made for its function as a regulator. Several investigators tried to address the above question by modulating CPSI activity with *N*-carbamoylglutamate, a structural analogue of NAG that is non-toxic and is able to cross the plasma and mitochondrial membranes [5,31]. Now that the mouse NAGS gene has been cloned, it could be used to study the effect of NAG directly by modulating NAGS expression levels in liver cells and the effect this has on nitrogen flux through the urea cycle.

Inherited NAGS deficiency is a disorder whose diagnosis has been hampered by lack of specific biochemical markers [19]. At present, the only method for diagnosis is liver biopsy, but it is complicated by low specific activity of NAGS and the possibility of secondary reduction of catalytic activity. With the cloning of the mouse NAGS and the identification of the human genomic sequence, cloning of hNAGS is expected in the near future. Subsequently, molecular diagnosis of NAGS deficiency would be possible, leading to verification of the genetic basis and inheritance of this disorder and determination of the correlation between the genotype and phenotype.

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