Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 7, pp 1089–1095, 1996

PII: S0968-0896(96)00098-3
0968-0896/96 $15.00+0.00

The *hemA* Gene Encoding Glutamyl-tRNA Reductase from the Archaeon *Methanobacterium thermoautotrophicum* Strain Marburg

Christoph Hungerer,[a] David S. Weiss,[b] Rudolf K. Thauer[a] and Dieter Jahn[a,*]

[a]*Laboratorium für Mikrobiologie des Fachbereich Biologie der Philipps-Universität and Max-Planck-Institut für Terrestrische Mikrobiologie, Karl-von-Frisch-Strasse, 35043 Marburg, Germany*

[b]*Harvard Medical School, Department of Microbiology, 200 Longwood Avenue, Boston, MA 02115, U.S.A.*

Abstract—In archaea the first general tetrapyrrole precursor 5-aminolevulinic acid (ALA) is formed via the tRNA-dependent five-carbon pathway from glutamate. We have cloned the *hemA* gene encoding the central enzyme of the pathway glutamyl-tRNA reductase from the methanogenic archaeon *Methanobacterium thermoautotrophicum* by complementation of an *Escherichia coli hemA* mutant to ALA prototrophy. An 1194 bp open reading frame that encodes a 398 amino acid polypeptide with the calculated M_r 44,509 was detected. The deduced amino acid sequence showed 20–35% amino acid identity to bacterial HemAs with the highest identity score to the *Pseudomonas aeruginosa* HemA. An identity of approximately 22% was found to plant HemAs. Glutamyl-tRNA reductase activity was shown for the *M. thermoautotrophicum* HemA after overexpression in *E. coli* and partial purification. The enzymatic reaction catalyzed by the partially purified enzyme revealed a temperature optimum of 65 °C at an optimal pH of 7.0. The reductase utilized preferentially NADPH for the reduction of the activated carboxyl group. The presence of ATP and GTP showed no obvious influence on catalysis. Copyright © 1996 Elsevier Science Ltd

Introduction

Tetrapyrroles such as hemes, chlorophylls, corrinoids, and coenzyme F_{430} play an essential role in the metabolism of most organisms as integral parts of electron transport chains and as prosthetic groups of various enzymes.[1–4] They are all synthesized from 5-aminolevulinic acid (ALA), for which two routes of biosynthesis are used in nature. The Shemin pathway utilizes the one step condensation of glycine and succinyl-CoA by ALA synthase and is found in the α-group of the proteobacteria, yeast, avian, and mammalian cells.[1–8] The two-step C_5-pathway starts with an NADPH-dependent reduction of glutamyl-tRNA by the glutamyl-tRNA reductase (HemA) to yield glutamate-1-semialdehyde (Fig. 1).[8–10] ALA is formed in the second step during a pyridoxamine 5′-phosphate-dependent transamination performed by glutamate-1-semialdehyde-2,1-aminomutase (HemL, Fig. 1).[11–13] The C_5-pathway operates in plants, algae, and all analyzed bacteria, with the exception of the α-group of the proteobacteria.[7,8] Interestingly, this biosynthetic route was shown to be present in the archaea *M. thermoautotrophicum* and *Sulfolobus solfataricus*.[14–16]

The genes for glutamyl-tRNA reductase (*hemA*) and glutamate-1-semialdehyde-2,1-aminomutase (*hemL*) have been cloned from various bacteria and plants.[8,17] Recent investigations of the regulation of heme biosynthesis in *E. coli* and *P. aeruginosa* identified the formation of the general precursor 5-aminolevulinic acid (ALA) as one major regulatory point.[18–20]

In archaea a variety of hemes, corrinoids, and the unique nickel-containing porphinoid F_{430} have been described as the central tetrapyrrole cofactors for the various elucidated pathways of energy conservation.[1,4,21,22] In contrast to the information accumulated about the structural and funtional features of some of these molecules, almost nothing is known about the molecular basis of their biosynthesis and its regulation. Only one gene involved in heme biosynthesis (*hemB*), encoding ALA dehydratase, has been cloned from the archeon *Methanothermus sociabilis*.[23]

To initiate a molecular analysis of the enzymes and genes involved in the archaebacterial ALA formation, we cloned and characterized the *hemA* gene from *M. thermoautotrophicum* and investigated some biochemical properties of the encoded glutamyl-tRNA reductase. *M. thermoautotrophicum* is a strictly anaerobic, methanogenic archaeon growing on H_2 and CO_2 with a temperature optimum of 65 °C. The organism contains corrinoids and coenzyme F_{430} but is devoid of cytochrome.[21,22,24] ALA and uroporphyrinogen III have been shown to be intermediates in the biosynthesis of F_{430}.[24]

Results and Discussion

Cloning of the *M. thermoautotrophicum hemA* gene by complementation of an *E. coli* 5-aminolevulinic acid auxotrophic mutant

Transformation of the ALA-auxotrophic *E. coli* strain GE1387, carrying a mutation in the gene for glutamyl-

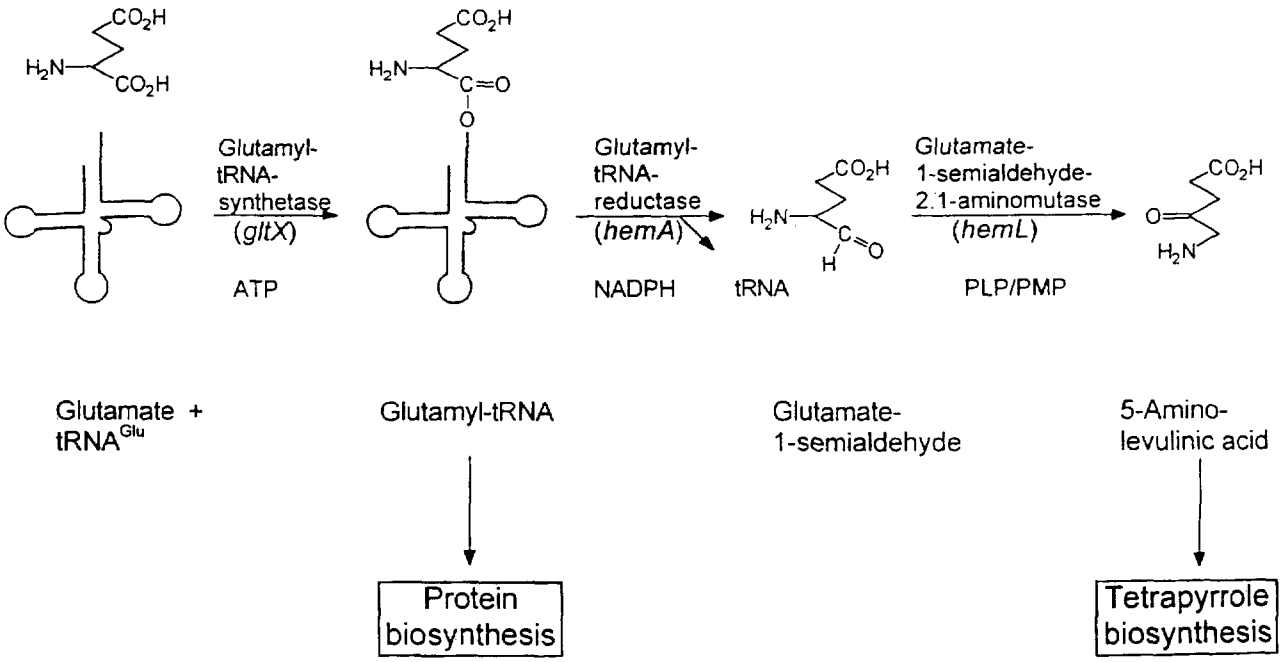

Figure 1. C_5-pathway of 5-aminolevulinic acid formation. The reaction of the glutamyl-tRNA synthetase is common to protein biosynthesis and tetrapyrrole biosynthesis. Therefore, the first step dedicated to tetrapyrrole synthesis is the glutamyl-tRNA reductase reaction. All reactions are given with the currently known cofactors. The abbreviations in brackets represent the genetic symbols for the bacterial genes encoding the respective enzyme. While the identity of the enzymes and the tRNA involved in ALA formation has been established, the precise mechanism of the glutamyl-tRNA reductase reaction is still uncertain, and the chemical nature of the GSA intermediate is still a subject of discussion.[1-13,17,39] The figure shows one of the proposed structures for the intermediate, the linear form.[25]

tRNA reductase (*hemA*) with an *M. thermoautotrophicum* genomic library to ALA prototrophy, yielded approximately 50 slow-growing colonies. Restriction analysis revealed the presence of an identical 3346 bp fragment in nine of the 10 isolated complementing plasmids. One of these nine inserts from the plasmid pMthemA was subjected to complete DNA sequence determination. Two potential open reading frames encoded by the sequenced DNA fragment were identified (Fig. 2). The first 1194 bp open reading frame from position 59 to position 1252 encoded a 398 amino acid protein with a calculated M_r of 44,509, which showed a high degree of amino acid sequence identity to bacterial and plant glutamyl-tRNA reductases (Fig. 3). The highest degree of identity (35%) was found to the enzyme encoded by the *P. aeruginosa hemA* gene. An approximately 22% amino acid sequence identity was detected when the archaebacterial protein was compared to plant analogues (Fig. 3). Several regions of amino acid sequence were found unique to the archaebacterial protein and were found absent in the bacterial and plant enzymes (Fig. 3). Due to the high homology to other known *hemA* genes and to the encoded glutamyl-tRNA reductase activity (see below), the open reading frame was named *hemA*. In vivo protein expression experiments in *E. coli* using the *M. thermoautotrophicum hemA* gene cloned behind the T7 RNA polymerase promoter of pBluescript SK+ (pBlueMthemA) identified a polypeptide with an M_r of approximately 43,000 as the product of the cloned *hemA* gene (Fig. 4, lane 3). The obtained result is in good agreement with the value deduced from the cloned gene. The protein of 43,000 was not detected in control experiments using pBluescript without inserted DNA (Fig. 4, lane 2).

The *M. thermoautotrophicum hemA* gene encodes glutamyl-tRNA reductase

To identify and initially characterize the enzymatic activity encoded by the cloned gene, the *M. thermoautotrophicum hemA* gene was overexpressed *in E. coli*. The expressed HemA protein was partially purified and analyzed for its catalytic properties. Partial purification was achieved by heating the obtained extracts for 30 min to 65 °C to denature major parts of the host proteins. One major protein with an M_r of approximately 45,000 and some minor contaminants were the result of this procedure (data not shown). This major protein was missing in experiments using only the expression vector without cloned DNA (data not shown). Activity tests under aerobic conditions with the partially purified protein using purified *E. coli* $tRNA^{Glu}$ aminoacylated with [^{14}C]Glu as substrate were performed. The assays yielded radioactive glutamate-1-semialdehyde (GSA) as the product as identified by HPLC chromatography (data not shown). Due to the instable nature of GSA, all other glutamyl-tRNA reductase tests were performed with the addition of purified recombinant *E. coli* glutamate-1-semialdyde-2,1-aminomutase to produce the stable intermediate ALA.[25] The glutamyl-tRNA reductase reaction was found to be clearly RNase sensitive (Table 1). A temperature optimum of 65 °C and a pH optimum of 7.0 were measured for the *M. thermoautotrophicum*

glutamyl-tRNA reductase (Table 1). NADPH was found an essential cofactor for the enzymatic reduction process while the addition of NADH yielded only low enzymatic activity (Table 1). Earlier an enzymatic mechanism for the glutamyl-tRNA reductase reaction similiar to the enzymology of the peptide chain-elongation reaction during protein biosynthesis with a stimulatory role for GTP was proposed.[1] However, similiar to the findings for the *E. coli* and *Chlamydomonas reinhardtii* systems, no additional proteins seemed to be required for the *M. thermoautotrophicum* glutamyl-tRNA reductase reaction, and neither the addition of GTP nor ATP to the enzymatic reaction showed any stimulatory effect (Table 1).[9,10] The possibility to overproduce and purifiy active thermostable glutamyl-tRNA reductase should help to determine the functional and structural basis for glutamyl-tRNA recognition and reduction.

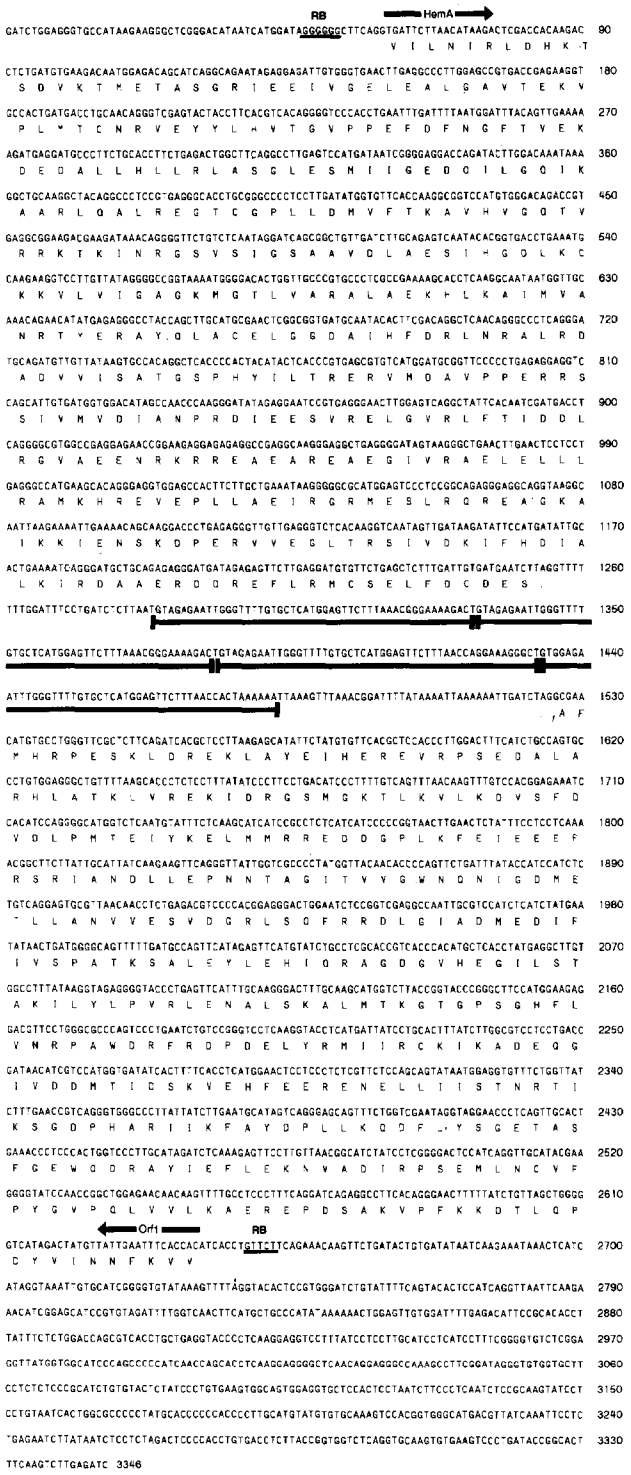

Figure 2. Nucleotide sequence and deduced amino acid sequences of the cloned *M. thermoautotrophicum hemA* gene and *orf1* in its 3′-region. The repeated DNA sequence in the 3′-region of the *hemA* gene are underlined. Please note that *orf1* is orientated in the opposite direction to *hemA*. RB = ribosome binding site.

Analysis of the 3′-region of the cloned *M. thermoautotrophicum hemA* gene

To investigate the existence of a potential *M. thermoautotrophicum hem* operon, as detected in a variety of Gram-positive bacteria, the cloned 5′- and 3′-regions of the *hemA* gene were sequenced.[17] No obvious similarity to currently known *hem* genes was observed. However, the cloned *hemA* gene was directly followed by a four-times repeated, almost identical DNA sequences of 50 bp (Fig. 2). To investigate the potential role of the repeats as repetitive element spread throughout the whole *M. thermoautotrophicum* genome, Southern blot experiments were conducted. However, only one signal with *M. thermoautotrophicum* genomic DNA was visible when the repeated DNA sequence was used as a probe indicating the single site location of the investigated DNA repeats (data not shown). The biological function of the observed DNA repetition remains unclear. A second open reading frame (*orf1*) of 1116 bp from position 2640 to position 1525 orientated in the opposite direction to *hemA* was found in the 3′-region of *hemA* (Fig. 2). The deduced 372 amino acid protein with a calculated molecular weight of 42,691 contains amino acid motifs highly homologous to ATP binding sites found in a variety of other proteins. In vivo protein expression experiments using *orf1* cloned behind the T7 RNA polymerase promoter revealed the presence of a protein with M_r of approximately 43,000 (Fig. 4, lane 1). The highest degree of amino acid identity for Orf1 was found to the cell division control proteins of various bacteria and of yeast, suggesting a possible involvement of the Orf1 protein in the cell cycle of *M. thermoautotrophicum*.[26,27]

Nucleotide sequence accession number

The nucleotide sequence data reported in this paper appeared in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X83691.

Experimental

General

All enzymes employed were purchased from United States Biochemicals, Bad Homburg, Germany, unless

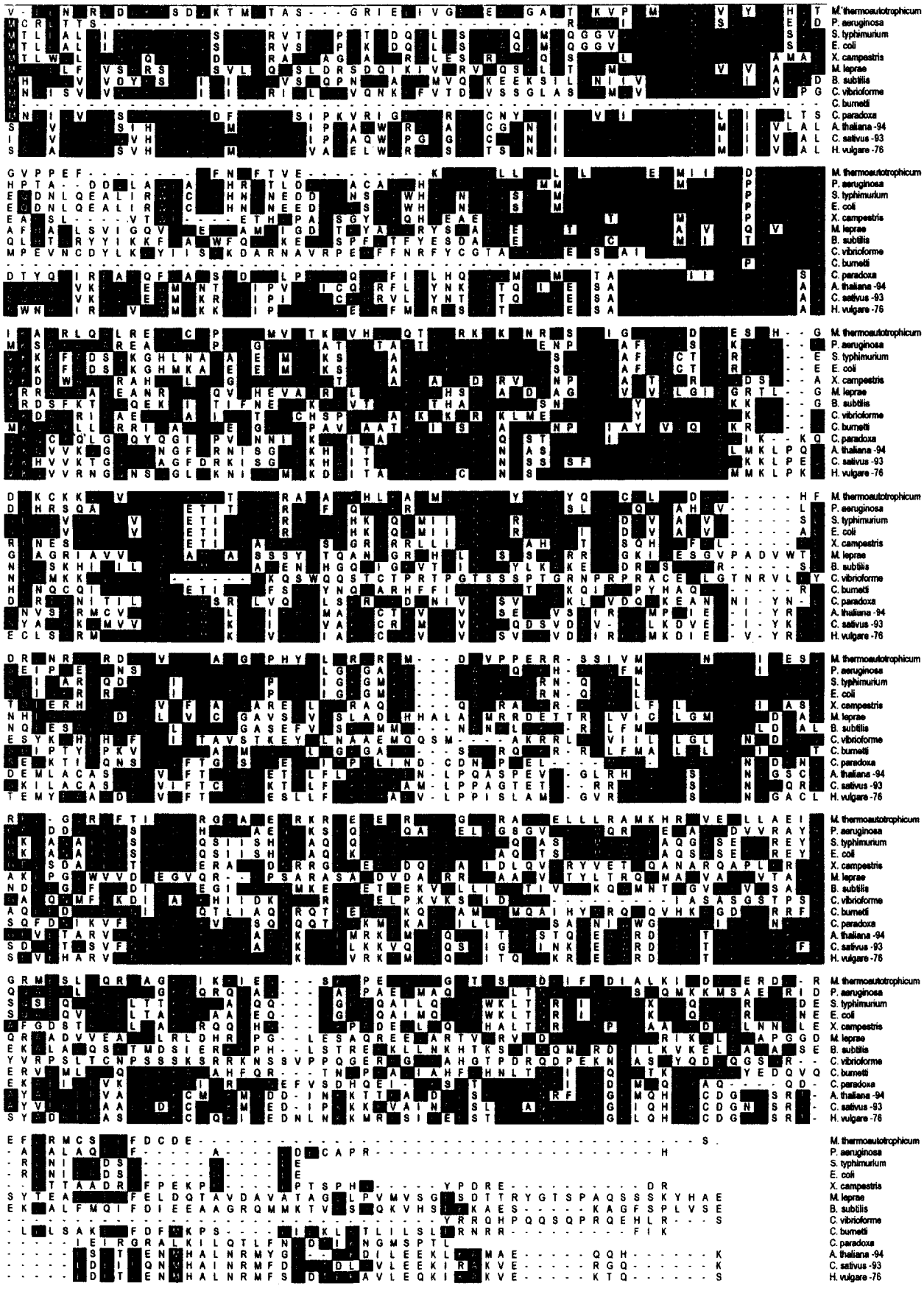

Figure 3. Amino acid sequence comparison of the proteins deduced from the currently known *hemA* genes.[17,19]

stated otherwise. Chemicals were reagent grade from Merck, Darmstadt, Germany. Nucleotides, 5-aminolevulinic acid, and antibiotics were from Sigma, Deisenhofen, Germany. Growth media were obtained from Difco, Augsburg, Germany. Oligonucleotides were purchased from Roth, Karlsruhe, Germany. Radioisotopes were supplied by Amersham, Braunschweig, Germany. *Methanobacterium thermoautotrophicum* strain Marburg was cultured and harvested as outlined before.[28] *Escherchia coli* strain DH5α was cultured on LB medium at 37 °C unless stated otherwise.[29] Growth media for the ALA-auxotrophic mutant of *E. coli* GE1387 were supplemented with 50 μg/mL ALA.[30] Ampicillin was used at a concentration of 100 μg/mL. Genomic DNA from *M. thermoautotrophicum* was prepared as outlined before and partially digested with *Sau*3A, sized, fractionated, and ligated into the *Bam*HI site of pUCBM20.[29,31,32] The genomic library was amplified in the *E. coli* DH5α. The ALA-auxotrophic strain GE1387 (*hemA*-) was transformed via electroporation with an *M. thermoautotrophicum* genomic library prepared in pUCBM20.[30,32] Complementing plasmids were selected by cultivating the transformed bacteria on LB medium without ALA.[19,20] One complementing plasmid termed pMthemA was subjected to further analysis. All DNA manipulations were carried out as described, unless stated otherwise.[29] The structures of pUCBM20 and pBluescript SK+ have been described before.[32] DNA sequencing was performed with denatured double-stranded plasmid DNA by the dideoxy method with Sequenase version 2.0.[33] In vivo translation and analysis of the obtained products was performed as described earlier.[34–36] The complete DNA sequence of the insert of pMthemA was determined via primer walking. *Escherichia coli* BL21 (DE3), which carries T7 RNA polymerase under control of the *lac*UV5 promoter, was transformed with the plasmids pBlueMthemA and pBlueMtorf1 and as a control pBluescript SK+.

Table 1. Functional properties of the partially purified glutamyl-tRNA reductase (HemA) from *M. thermoautotrophicum*

Changes to normal assay conditions[a]	Incubation (°C)	pH value	Glutamyl-tRNA reductase activity[b]
—	65	7.0	420
RNase treatment[c]	65	7.0	35
No NADPH addition	65	7.0	52
NADH instead of NADPH	65	7.0	71
Addition of 2 mM GTP	65	7.0	405
Addition of 2 mM ATP	65	7.0	395
—	30	7.0	45
—	40	7.0	102
—	55	7.0	255
—	65	7.0	420
—	75	7.0	270
—	65	5.0	45
—	65	6.0	127
—	65	6.5	390
—	65	7.0	420
—	65	7.5	402
—	65	8.0	105
—	65	9.0	32

[a]Enzyme assays were carried out as detailed in the Experimental.
[b]Glutamyl-tRNA reductase activity is expressed as pmol of [^{14}C]ALA synthesized from *E. coli* [^{14}C]Glu-tRNA^{Glu} per mg protein fraction. 50 μL of the heat treated column fraction containing partially purified *M. thermoautotrophicum* glutamyl-tRNA reductase were used for each assay.
[c]RNase A (10 mg) treatment was for 30 min at 30 °C.

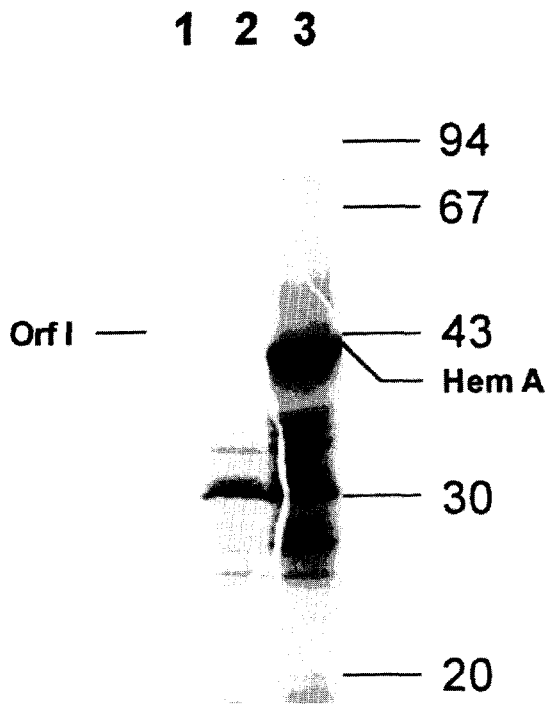

Figure 4. In vivo expression of the *hemA* gene and *orf1* residing on the cloned *M. thermoautotrophicum* genomic DNA fragment. The plasmid-encoded gene products were labeled with [^{35}S]methionine as described in the text and detected by autoradiography after separation of proteins on SDS-polyacrylamide gels.[35,36] The plasmids pBlueMthemA (lane 3), pBluescript (lane 2), and pBlueMtorf1 (lane 1) used in these experiments are described in the text. The position and apparent molecular weights of protein standards run on the same gel are indicated.

Overexpression and purification of the *M. thermoautotrophicum hemA*

Escherichia coli GE1387 transformed with pGP1-2 (encoding the thermo-inducable T7 RNA polymerase gene) and pBlueMthemA or as a control with pBluescript SK+ were grown overnight in LB media at 30 °C containing 50 μg kanamycin/mL and 75 μg ampicillin/mL to an OD_{600} of 0.8 to 1.2, and T7 RNA polymerase was induced by a temperature shift to 42 °C for 30 min.[29] Incubation was continued for a further 1 h at 37 °C before the bacteria were harvested by centrifugation. The cells were resuspended in extraction buffer (50 mM Hepes, pH 7.9, 10 mM EDTA, 5 mM DTT, 20% v/v glycerol) including 10 mM KCl, sonicated and centrifuged for 90 min at 100,000 × *g*. Obtained cell free extracts were heated for 30 min to 65 °C. Resulting debris of denatured proteins was removed by centrifugation. Subsequently, the cleared supernatant was loaded directly onto a DEAE cellulose column at a concentration of 10 mg protein per mL column

volume. After extensive washing with extraction buffer, proteins were eluted with extraction buffer containing 300 mM KCl. The tRNAs which were still bound to the ion exchanger at 300 mM KCl, were eluted with extraction buffer containing 1 M KCl. The protein (300 mM KCl elution step) and nucleic acid (1 M KCl elution step) fractions were dialyzed extensively against assay buffer (20 mM Hepes, pH 7.0 or pH values as indicated, 10 mM KCl, 10 mM $MgCl_2$, 3 mM DTT, 10% v/v glycerol).[10,19,20,37]

Glutamyl-tRNA reductase assay

Protein extracts (50 µL each) dialyzed against assay buffer were incubated in the presence 10 mM levulinic acid, pH 7.0, 10 µM pyridoxal 5′-phosphate, 3 mM NADPH, 10 mM Glu, 20 units RNAsin, 20.000 cpm [^{14}C]Glu-tRNA^{Glu} (41 cpm/pmol) at 65 °C (or temperatures as indicated) for 15 min. Subsequently, the assays were supplemented with 30 µg/mL *E. coli* glutamate-1-semialdehyde-2,1-aminomutase purifed to apparent homogeneity, as described earlier and incubation was continued for another 20 min at 30 °C.[10,19,20] Aminoacylated tRNA^{Glu} was prepared using purified *E. coli* Glutamyl-tRNA synthetase, as outlined earlier.[9,10,19] The reactions were stopped by the addition of 400 µL 20 mM KOH. Samples were boiled for 5 min and subsequently neutralized by the addition of 65–80 µL 100 µM HCl (the exact amount HCl was determined in pilot experiments with assay buffer) and 100 µL 0.5 M tris–HCl, pH 7.0. Samples were applied to 300 µL Dowex-1 columns equilibrated with H_2O. The formed [^{14}C]ALA, found exclusively in the flow through fraction, was directly quantitated by liquid scintillation counting. All [^{14}C]Glu derived from the deacylation of [^{14}C]Glu-tRNA^{Glu} was retained by the column material.[10,19,38] HPLC analysis of the enzymatic product was performed as outlined earlier.[10,13]

Acknowledgments

This work was supported by grants of the Deutsche Forschungsgemeinschaft, the Max-Planck-Gesellschaft, Fonds der Chemischen Industrie and the Graduiertenkolleg Enzymchemie of the Philipps-Universität Marburg. We are indebted to G. Eggertson (University of Reykjavik) for the gift of the *E. coli* strain GE1387. We thank E. Bremer, P. Dersch, T. Hoffmann, B. Kempf, A. Szabo, and B. Troup (Laboratorium für Mikrobiologie, Universität Marburg) for the gift of *E. coli* strains and plasmids and many helpful discussions.

References

1. *Ciba Foundation Symposia 180. The Biosynthesis of the Tetrapyrrole Pigments*; Chadwick, D. J.; Ackrill, K., Eds.; Wiley: Chichester, 1994.

2. *Biosynthesis of Heme and Chlorophylls*; Dailey, H. A., Ed.; McGraw-Hill: Hightstown, 1990.

3. Jordan, P. M. In *New Comprehensive Biochemistry*; Neuberger, A.; van Deenen, L. L. M., Eds.; Elsevier: Amsterdam, 1991; Vol. 19.

4. Friedmann, H. C.; Thauer, R. T. *Encyclopedia of Microbiology*; Academic: New York, 1992; Vol. 3, pp 1–19.

5. Kikuchi, G.; Kumar, A.; Talmage, P.; Shemin, D. *J. Biol. Chem.* **1958**, *233*, 1214.

6. Gibson, K. D.; Laver, W. G.; Neuberger, A. *Biochem. J.* **1958**, *70*, 71.

7. Avissar, Y. J.; Ormerod, J. G.; Beale, S. I. *Arch. Microbiol.* **1989**, *151*, 513.

8. Jahn, D.; Verkamp, E.; Söll, D. *Trends Biochem. Sci.* **1992**, *17*, 215.

9. Chen, M.-W.; Jahn, D.; O'Neill, G. P.; Söll, D. *J. Biol. Chem.* **1990**, *265*, 4058.

10. Jahn, D.; Michelsen, U.; Söll, D. *J. Biol. Chem.* **1991**, *266*, 2542.

11. Ilag, L. L.; Jahn, D.; Eggertsson, G.; Söll, D. *J. Bacteriol.* **1991**, *173*, 3408.

12. Ilag, L. L.; Jahn, D. *Biochemistry* **1992**, *31*, 7143.

13. Jahn, D.; Chen, M.-W.; Söll, D. *J. Biol. Chem.* **1991**, *266*, 161.

14. Friedmann, H. C.; Thauer, R. K. *FEBS Lett.* **1986**, *207*, 84.

15. Friedmann, H. C.; Thauer, R. K.; Gough, S. P.; Kannangara, C. G. *Carlsberg Res. Commun.* **1987**, *52*, 363.

16. Matters, G. L.; Beale, S. I. *Arch. Microbiol.* **1994**, *161*, 272.

17. Asahara, N.; Marakami, K.; Korbrisate, S.; Hashimoto, Y.; Murooka, Y. *Appl. Microbiol. Biotech.* **1994**, *40*, 846; Avissar, Y. J.; Beale, S. I. *J. Bacteriol.* **1990**, *72*, 1656; Drolet, M.; Peloquin, I.; Echeland, Y.; Cousineau, I.; Sasarman, A. *Mol. Gen. Genet.* **1989**, *216*, 347; Elliott, T. *J. Bacteriol.* **1989**, *171*, 3948; Ilag, L. L.; Kumar, A. M.; Söll, D. *Plant Cell* **1994**, *6*, 265; Li, J. M.; Russell, C. S.; Cosloy, S. D. *Gene* **1989**, *82*, 209; Majumdar, D.; Avissar, Y. J.; Wyche, J. H.; Beale, S. I. *Arch. Microbiol.* **1991**, *156*, 281; Verkamp, E.; Chelm, B. K. *J. Bacteriol.* **1989**, *171*, 4728; Verkamp, E.; Jahn, M.; Jahn, D.; Kumar, M.; Söll, D. *J. Biol. Chem.* **1992**, *267*, 8275; Petricek, M.; Rutberg, L.; Schröder, I.; Hederstedt L. *J. Bacteriol.* **1990**, *172*, 2250. The not yet published HemA sequences were obtained from the database genpep with the accession numbers X92403, X86101, D67088, U30821, X78969, and from swiss prot with the accession number P46724.

18. Darie, S.; Gunsalus R. P. *J. Bacteriol.* **1994**, *176*, 5270.

19. Hungerer, C.; Troup, B.; Römling, U.; Jahn, D. *J. Bacteriol.* **1995**, *177*, 1435.

20. Hungerer, C.; Troup, B.; Römling, U.; Jahn, D. *Mol. Gen. Genet.* **1995**, *248*, 375.

21. Heiden, S.; Hedderich, R.; Setzke, E.; Thauer, R. K. *Eur. J. Biochem.* **1994**, *221*, 855.

22. Weiss, D. S.; Thauer, R. K. *Cell* **1993**, *72*, 819.

23. Bröckl, G.; Berchtold, M.; Behr, M.; König, H. *Gene* **1992**, *119*, 151.

24. Gilles, H.; Thauer, R. K. *Eur. J. Biochem.* **1983**, *135*, 109.

25. Gough, S. P.; Kannangara, C. G.; Bock, K. *Carlsberg Res. Commun.* **1989**, *54*, 99.

26. Fröhlich, K. U.; Fries, H. W.; Rüdiger, M.; Erdmann, R.; Botstein, D.; Mecke, D. *J. Cell Biol.* **1991**, *114*, 44.

27. Tomayasu, T.; Yuki, T.; Morimura, S.; Mori, H.; Yamanaka, K.; Niki, H.; Hiraga, S.; Ogura, T. *J. Bacteriol.* **1993**, *175*, 1344.

28. Schönheit, P.; Moll, J.; Thauer, R. K. *Arch. Microbiol.* **1980**, *127*, 59.

29. *Current Protocols in Molecular Biology*; Ausubel, F. M.; Brent, R.; Kingston, R. E.; Moore, D. D.; Seidman, J. D.; Smith, J. A.; Struhl, K., Eds.; Greene Publishing and Wiley Interscience: New York, 1987. *Molecular Cloning: a Laboratory Manual*; Sambrook, J.; Fritsch, E. F.; Maniatis, T., Eds.; Cold Spring Harbor Laboratory: Cold Spring Harbor, 1989; 2nd edn.

30. Verkamp, E.; Backman, V. M.; Björnsson, J. M.; Söll, D.; Eggertsson, G. *J. Bacteriol.* **1993**, *175*, 1452.

31. Kiener, A.; König, H.; Winter, J.; Leisinger, T. *J. Bacteriol.* **1987**, *169*, 1010.

32. pUCBM20 is a product of Boehringer Mannheim, pBluescript is a product of Stratagene.

33. Chen, E. Y.; Seeburg, P. H. *DNA* **1985**, *4*, 165.

34. Studier, F. W.; Rosenberg, A. H.; Dunn, J. J.; Dudendorff, J. W. *Methods Enzymol.* **1990**, *185*, 60.

35. Troup, B.; Jahn, M.; Hungerer, C.; Jahn, D. *J. Bacteriol.* **1994**, *176*, 673.

36. Troup, B.; Hungerer, C.; Jahn, D. *J. Bacteriol.* **1995**, *177*, 3326.

37. Chen, M.-W.; Jahn, D.; Schön, A.; O'Neill, G. P.; Söll, D. *J. Biol. Chem.* **1990**, *265*, 4054.

38. Jahn, D.; Kim, Y.-C.; Ishino, Y.; Chen, M.-W.; Söll, D. *J. Biol. Chem.* **1990**, *265*, 8059.

39. O'Neill, G. P.; Jahn, D.; Söll, D. *Subcell. Biochem.* **1991**, *17*, 235.

(*Received in U.S.A.* 23 *January* 1996; *accepted* 15 *April* 1996)