Peptidyl-tRNA hydrolase from Sulfolobus solfataricus.

Michel Fromant, Maria-Laura Ferri-Fioni, Pierre Plateau, Sylvain Blanquet

Nucleic Acids Research, 2003, Vol. 31, No. 12 ***3227–3235***
DOI: 10.1093/nar/gkg428

Peptidyl-tRNA hydrolase from *Sulfolobus solfataricus*

Michel Fromant, Maria-Laura Ferri-Fioni, Pierre Plateau* and Sylvain Blanquet

Laboratoire de Biochimie, Unité Mixte de Recherche 7654, CNRS-Ecole Polytechnique, 91128 Palaiseau Cedex, France

Received January 15, 2003; Revised April 1, 2003; Accepted April 23, 2003

ABSTRACT

An enzyme capable of liberating functional tRNA^{Lys} from *Escherichia coli* diacetyl-lysyl-tRNA^{Lys} was purified from the archae *Sulfolobus solfataricus*. Contrasting with the specificity of peptidyl-tRNA hydrolase (PTH) from *E.coli*, the *S.solfataricus* enzyme readily accepts *E.coli* formyl-methionyl-tRNA^{fMet} as a substrate. N-terminal sequencing of this enzyme identifies a gene that has homologs in the whole archaeal kingdom. Involvement of this gene (*SS00175*) in the recycling of peptidyl-tRNA is supported by its capacity to complement an *E.coli* strain lacking PTH activity. The archaeal gene, the product of which appears markedly different from bacterial PTHs, also has homologs in all the available eukaryal genomes. Since most of the eukaryotes already display a bacterial-like PTH gene, this observation suggests the occurrence in many eukaryotes of two distinct PTH activities, either of a bacterial or of an archaeal type. Indeed, the bacterial- and archaeal-like genes encoding the two full-length PTHs of *Saccharomyces cerevisiae*, *YHR189w* and *YBL057c*, respectively, can each rescue the growth of an *E.coli* strain lacking endogeneous PTH. *In vitro* assays confirm that the two enzymes ensure the recycling of tRNA^{Lys} from diacetyl-lysyl-tRNA^{Lys}. Finally, the growth of yeast cells in which either *YHR189w* or *YBL057c* has been disrupted was compared under various culture conditions. Evidence is presented that *YHR189w*, the gene encoding a bacterial-like PTH, should be involved in mitochondrial function.

INTRODUCTION

Ribosomal translation of mRNA open reading frames (ORFs) normally terminates on a stop codon. At this step, several release factors collaborate with ribosome to liberate a newly synthesized polypeptide [for a review, see Kisselev and Buckingham (1)]. However, during elongation of translation, peptidyl-tRNA molecules may prematurely dissociate from the mRNA template. Such abortive events result in accumulation of peptidyl-tRNAs that are left apart from the translation machinery (2). It was recognized early on that *Escherichia coli* and yeast cells possessed a hydrolytic activity capable of recycling such peptidyl-tRNA molecules by removing the peptidyl moiety (3–7). This activity was called peptidyl-tRNA hydrolase (PTH). The *E.coli* PTH was characterized in depth (5,8–12). Provided they are *N*-blocked, aminoacyl-tRNAs are substrates of the hydrolase. However, formyl-methionyl-tRNA^{fMet} appears to be a relatively poor substrate. Resistance to PTH enables the formylated initiator tRNA to participate in the formation of the ribosomal initiation complex. With elongator tRNAs, the action of the hydrolase is facilitated by the 5′-phosphate at the end of a fully base-paired acceptor stem. *Escherichia coli* tRNA^{fMet} lacks base pairing at position 1–72. The resulting mismatch at the top of the acceptor helix is believed to twist the position of the phosphate group so that it can no longer trigger the activity of the enzyme.

The gene encoding PTH was first identified in *E.coli* (13). It is essential to the survival of the bacterium (11,14). Homologs of this gene (*pth*) were then recognized in the genomes of all bacteria and in those of most eukarya. In *Saccharomyces cerevisiae*, the homolog, *YHR189w*, encodes a PTH capable of conferring thermoresistance to a *pth*^{ts} *E.coli* strain (15). Surprisingly, however, analyses of completed archaeal genome sequences did not reveal any *pth* homolog. To examine the possibility that archaea use an alternative enzyme to ensure the recycling of peptidyl-tRNA, we took *Sulfolobus solfataricus* as starting material. A similar search using *Methanocaldococcus jannaschii* has been reported very recently (16). The latter work resulted in the identification of a PTH activity encoded by the *MJ0051* gene. The product of this gene is markedly distinct from the *E.coli* PTH and the yeast *YHR189w* gene product. However, its expression in *E.coli* confers thermoresistance to a *pth*^{ts} strain. Interestingly, the *MJ0051* gene has homologs in all archaeal and eukaryal genomes. The product of *YBL057c*, the ortholog of *MJ0051* in *S.cerevisiae*, cures the thermosensitivity of an *E.coli pth*^{ts} strain. In this case, to obtain complementation, a large part of *YBL057c* had to be deleted on the N-terminal side of the protein product (16).

Here, we isolate PTH activity from *S.solfataricus* crude extracts and identify the corresponding gene. This gene, which is homologous to the *MJ0051* gene, could be over-expressed in an *E.coli* context. Comparison of the biochemical properties of purified *S.solfataricus* PTH with those of *E.coli* PTH indicates distinct mechanisms of substrate recognition. In particular, *S.solfataricus* PTH is only weakly sensitive to the presence of a 5′-phosphate at the top of the acceptor helix of tRNA. As a consequence, the enzyme readily accepts *E.coli*

*To whom correspondence should be addressed. Tel: +33 1 69 33 41 81; Fax: +33 1 69 33 30 13; Email: plateau@botrytis.polytechnique.fr

formyl-methionyl-tRNA^{fMet} as a substrate. This behavior can be related to the special feature of translation in archae, where formylation of methionine esterified to initiator tRNA does not happen. In the second part of the present study, we examine various culture conditions in the hope of revealing phenotypes of *S.cerevisiae* haploid strains defective in either *YHR189w* or *YBL057c*. Finally, *YHR189w* disruption is shown to impair the growth of cells on minimal medium containing a non-fermentable carbon source.

MATERIALS AND METHODS

Isolation and N-terminal sequencing of peptidyl-tRNA hydrolase from *S.solfataricus*

Cells from *S.solfataricus* strain P2 (DSM1617) were grown at 78°C essentially as in Zillig *et al*. (17). The culture (3.5 l) was quickly cooled and centrifuged for 10 min at 4000 *g*. All buffers used for the purification of PTH contained 10 mM 2-mercaptoethanol and 1 mM EDTA. The cell pellet (21 g, wet weight) was suspended in 100 ml of 50 mM potassium phosphate buffer pH 7.6 containing 0.1 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 µg/ml of each leupeptin, pepstatin A and aprotinin. Cells were disrupted by sonication (10 min, 0°C), and debris removed by centrifugation (60 min, 10 000 *g*). Nucleic acids were precipitated by addition of streptomycin (30 g/l) to the supernatant, which was then centrifuged for 60 min at 10 000 *g*. The resulting supernatant was brought to 70% ammonium sulfate saturation. After centrifugation for 30 min at 10 000 *g*, the protein pellet was dissolved in 15 ml of 20 mM potassium phosphate buffer pH 7.6 containing 0.1 mM PMSF, and dialyzed against 1 l of the same buffer. The resulting solution was applied on a column of Q-Sepharose FastFlow (4.4 × 30 cm; Amersham Biosciences) equilibrated in 20 mM potassium phosphate pH 7.6. The major part of the PTH activity was recovered in the flowthrough and further applied on an SP-Sepharose column (1.1 × 10 cm; Amersham Biosciences) equilibrated in 20 mM potassium phosphate pH 7.6. Elution was carried out at a flow rate of 1 ml/min with a 0.1 l linear gradient of 0–500 mM KCl in the same buffer (200 mM/h). Fractions of 2 ml were collected. PTH activity was recovered at 190 mM KCl. This activity was associated with a material showing a strong light absorbancy at 260 nm. After concentration by ultrafiltration on a Ultrafree 4 centrifugal filter unit 10 K (Millipore), the sample (2 ml) was applied onto a Superdex 75 column (120 ml, 1.6 × 60 cm; Amersham Biosciences) equilibrated in 20 mM potassium phosphate pH 7.6 containing 500 mM KCl and eluted at a flow rate of 0.14 ml/min. PTH activity was recovered at 520 min. Fractions (0.9 ml each) containing activity were pooled and dialyzed against 20 mM potassium phosphate pH 7.6. After addition of 1.2 M ammonium sulfate, the sample (7.4 ml) was applied on a Widepore HI-Propyl column (0.5 × 5.5 cm, particle size 15 µm; Baker) equilibrated in 20 mM potassium phosphate buffer pH 7.6 containing 1.7 M ammonium sulfate. Elution was carried out at a flow rate of 0.15 ml/min with a linear gradient of 1.7–0 M ammonium sulfate (1 M/h). Fractions of 0.2 ml were collected. Enzyme activity free of contaminating nucleic acids was recovered at 95 min. Fractions containing PTH were pooled (1.8 ml) and dialyzed against 20 mM potassium phosphate pH 7.6 containing 60% glycerol, and stored at –20°C. As judged from an SDS–PAGE analysis, the recovered protein (~10 µg) was at least 90% pure.

An aliquot of purified PTH (1 µg) was electrophoresed on an SDS–polyacrylamide gel. After migration, proteins were transferred on a ProBlot membrane (Applied Biosystems) and stained with amido black. The major protein band, corresponding to an M_r of 15 000 Da, was submitted to 10 cycles of Edman degradation on an Applied Biosystems Procise Sequencer.

Cloning of peptidyl-tRNA hydrolase genes

The *SS00175* gene was amplified by PCR using *S.solfataricus* genomic DNA as template, and oligonucleotides CTGCATCATGCCATGGTTAAGATGGT and CGCGGATCCTCACAGTAATTTT as primers. The resulting DNA fragment was purified using the Qiagen PCR Purification Kit 50, digested by both BamHI and NcoI, and inserted into the corresponding sites of plasmid pTrc99-A to give plasmid pTrc-pthS. The *YBL057c* gene was amplified from genomic DNA of *S.cerevisiae* strain YPALS (18), with oligonucleotides CCGAATTCTATGATAACGTCCTTTTTAATGGAAAAGATGACAG and CCAGCCAAGCTTCAATACAATTTCAAATCACCTGTTATTTGATCC as primers. The resulting DNA fragment was purified, digested by both EcoRI and HindIII, and inserted into the corresponding sites of plasmid pKK223-3 to give plasmid pKKpthY2. The *YHR189w* gene was amplified from genomic DNA of *S.cerevisiae* strain YPALS, with oligonucleotides CCATCGATTCTAGAAAGGAGGTACGATCATGTCCGGTAAATGGAGACT and CGCGGATCCCTATGAAATGTACTGAGTCAGAGCACG as primers. The resulting DNA fragment was purified, digested by both BamHI and ClaI, and inserted into the corresponding sites of plasmid pBluescript(+)KS to give plasmid pBSpthY1. In the three cases, the cloned DNAs were verified by DNA sequencing.

To obtain complementation of an *S.cerevisiae* strain where the *YHR189w* gene is disrupted, the HindIII–XbaI polylinker in the expression vector pYES2 (Invitrogen) was replaced by the TCTAGACCCGGGCTCGAGGGTACCCCGCGGGCGGCCGCGTCGAC sequence. The resulting plasmid was called pYES2lpa. Then, the 633 bp XhoI–NotI fragment of plasmid pBSpthY1 was inserted between the corresponding sites of pYES2lpa, to give plasmid pYESpthY1.

Preparation of crude extracts for activity measurements

When PTH activity was assayed in crude *E.coli* extracts, bacteria were grown overnight in 25 ml of 2× TY (1.6% bacto-tryptone, 1% bacto-yeast extract, 0.5% NaCl) medium containing 10 µg/ml of ampicillin. In the case of strain K37ΔpthTr(pBSpthY1), the growth medium also contained 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG). After centrifugation at 8000 *g* for 10 min, bacteria were suspended in 10 mM Tris–HCl pH 7.5 containing 0.1 mM EDTA, 0.1 mM PMSF and 10 mM 2-mercaptoethanol, so that the optical density (OD) of the bacterial suspension was equal to 100 at 650 nm. Cells were disrupted by sonication (3 min, 0°C) and debris removed by centrifugation (15 min, 18 000 *g*). When the assayed enzyme was the *S.solfataricus* PTH, the extract

Table 1. The *E.coli* and *S.cerevisiae* strains used in this study

Strain	Genotype	Reference
E.coli		
K37	*galK rpsL*	(40)
K37ΔpthTr	*galK rpsL* Δ*pth*::*kan* (pMAK705 *pth*)	(11)
XL1-Blue	*endA1 hsdR17 supE thi-1 recA1 gyrA96 relA1 lac* (F′ *proAB lacI*^{q} *lacZ*Δ*M15* Tn*10*)	Stratagene
S.cerevisiae		
BY4741	MAT**a** *his3*Δ1 *leu2*Δ0 *met15*Δ0 *ura3*Δ0	Euroscarf
BY4742	MATα *his3*Δ1 *leu2*Δ0 *lys2*Δ0 *ura3*Δ0	Euroscarf
Y03083	MAT**a** *his3*Δ1 *leu2*Δ0 *met15*Δ0 *ura3*Δ0 *YBL057c*::*kanMX4*	Euroscarf
Y12883	MATα *his3*Δ1 *leu2*Δ0 *lys2*Δ0 *ura3*Δ0 *YHR189w*::*kanMX4*	Euroscarf

Table 2. Purification of *S.solfataricus* PTH from *E.coli* strain XL1-Blue(pTrc-pthS)

Purification step	Protein yield (mg)	Total activity (U)[a]	Specific activity (U/mg)	Yield (%)	Relative purification
Extract	170[b]	56 000	330	100	1
Supernatant after ultracentrifugation	140[b]	54 000	390	96	1.2
Supernatant after heat treatment	7.8[b]	51 000	6500	91	20
Supernatant after ammonium sulfate precipitation	5.5[b]	45 000	8200	80	25
Superdex 75	1.3[b]	34 000	26 000	61	79
HI-Propyl	0.56[b]	26 000	46 000	46	140
SP-Sepharose	0.26[c]	18 000	69 000	32	210

[a]1 U is defined as the amount of enzyme capable of deacylating 1 pmol of diacetyl-[^{14}C]lysyl-tRNA^{Lys} per second in the standard assay conditions (see Materials and Methods).
[b]Protein analysis by the Bio-Rad protein assay, with bovine serum albumin as standard.
[c]Protein amount determined from UV absorbancy, assuming that 1 A_{280} unit corresponds to a protein concentration of 0.788 mg/ml.

was heated at 80°C for 15 min and centrifuged at 18 000 *g* for 15 min. Total amounts of protein in the extracts were determined by the Bio-Rad protein assay, with bovine serum albumin as standard.

Purification of *S.solfataricus* peptidyl-tRNA hydrolase using expression in an *E.coli* strain

Escherichia coli XL1-Blue (Table 1) transformed with plasmid pTrc-pthS was grown at 37°C in 1 l of 2× TY medium containing 80 µg of ampicillin/ml. When the OD of the culture reached 1 at 650 nm, 0.3 mM IPTG was added, and growth was continued for 4 h. Cells were harvested by centrifugation for 30 min at 4000 *g*. All buffers used for the purification of PTH contained 10 mM 2-mercaptoethanol and 0.1 mM EDTA. The cell pellet was suspended in 50 ml of 50 mM Tris–HCl pH 7.0 containing 0.1 mM PMSF. Cells were disrupted by sonication (3 min, 0°C), and debris removed by centrifugation (60 min, 10 000 *g*). The resulting extract was centrifuged at 100 000 *g* for 90 min. The supernatant was heated at 80°C for 10 min and centrifuged at 10 000 *g* for 30 min. The resulting supernatant was brought to 70% ammonium sulfate saturation. After centrifugation for 30 min at 10 000 *g*, the pellet was dissolved in 20 mM Tris–HCl buffer pH 7.0 and dialyzed overnight against the same buffer. The resulting sample (1.6 ml) was successively chromatographed on a Superdex 75 column and a HI-Propyl column, as described above in the case of the enzyme purified from *S.solfataricus* cells. In the elution profile of the HI-Propyl chromatography, the peak of PTH activity was free of contaminating nucleic acids. Active fractions recovered from the HI-Propyl column (1.8 ml) were pooled, dialyzed against 20 mM Tris–HCl buffer pH 7.0 and applied on an SP-Sepharose column (0.6 × 5.5 cm) equilibrated in the same buffer. This column was eluted with a linear gradient from 0 to 500 mM KCl in the buffer of the column (0.3 ml/min, 200 mM/h). Fractions of 0.45 ml were collected. The peak of activity was recovered at 300 mM KCl. In the course of the isolation of PTH from a *S.solfataricus* extract, the KCl concentration causing elution of activity on the same column was lower. In that case, contaminating nucleic acids may be at the origin of a biased effect of KCl during elution.

The SP-Sepharose column procedure produced 0.26 mg of protein in 5.6 ml. According to an SDS–PAGE analysis, the obtained enzyme was homogeneous. All steps of the purification procedure are summarized in Table 2. Concentration of purified PTH was determined using a light absorption coefficient (1.269 A_{280} U/mg/ml) and a molecular ratio (2 × 13 139) calculated from the amino acid sequence of the protein.

Assay of peptidyl-tRNA hydrolase activity

Escherichia coli tRNA^{Lys}, tRNA^{His}, tRNA^{fMet} and tRNA^{Tyr} were prepared as described previously (11,12,19). These tRNAs were fully aminoacylated with L-[^{14}C]lysine (50 or 317 Ci/mol), L-[^{14}C]histidine (50 Ci/mol), L-[^{14}C]methionine (57.9 Ci/mol) and D-[^{3}H]tyrosine (500 Ci/mol), respectively (11,12,19). L-Lysyl-tRNA^{Lys} and L-histidyl-tRNA^{His} were then acetylated with acetic anhydride (11). Methionyl-tRNA^{fMet} was formylated in the presence of *E.coli* methionyl-tRNA^{fMet} formyltransferase and 10-formyltetrahydrofolate (12). Dephosphorylated diacetyl-[^{14}C]lysyl-tRNA^{Lys} (50 Ci/mol) and formyl-[^{14}C]methionyl-tRNA^{fMet} (57.9 Ci/mol) were obtained as described previously (11,12).

Unless otherwise stated, measurements of *S.solfataricus* PTH activity were performed at 50°C in 100 µl assays containing 20 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT, 0.75 µM diacetyl-[^{14}C]lysyl-tRNA^{Lys} (50 Ci/mol) and catalytic amounts of enzyme (11). The reaction was quenched by the addition of 340 µl of ethanol, 14 µl of sodium acetate (3 M, pH 4.8) and 20 µl of carrier RNA from yeast (4 mg/ml). Samples were then centrifuged. Soluble radioactivity in the supernatant was measured by scintillation counting, as described (10). Data were corrected for spontaneous hydrolysis of the substrate in the absence of PTH. One unit corresponded to the enzyme activity capable of deacylating 1 pmol of diacetyl-[^{14}C]lysyl-tRNA^{Lys} per second in the above conditions.

K_m and k_{cat} values were deduced from measurements of the initial rate of hydrolysis in the presence of a variable concentration of diacetyl-lysyl-tRNA^{Lys} (8–150 nM). To estimate the inhibition constant (K_I) associated with tRNA^{Lys} binding, initial rates of hydrolysis were measured in the presence of variable concentrations of tRNA^{Lys} (0–20 µM) and diacetyl-[^{14}C]lysyl-tRNA^{Lys} (0.2–2 µM). The data verified competitive inhibition by tRNA^{Lys} of diacetyl-lysyl-tRNA^{Lys} hydrolysis. To obtain Michaelian parameters associated with other *N*-blocked aminoacyl-tRNAs, initial rates of hydrolysis were measured at a fixed substrate concentration (~0.75 µM) in the presence of a variable concentration of tRNA^{Lys} (0–20 µM). K_m values for each studied substrate could then be deduced by assuming competitive inhibition by tRNA^{Lys}. To estimate K_I values associated with the other nucleic acids, initial rates of diacetyl-[^{14}C]lysyl-tRNA^{Lys} (0.2 µM) hydrolysis were assayed in the presence of either *E.coli* tRNA^{fMet} (0–20 µM), unfractionated *E.coli* tRNA (0–20 µM), *E.coli* rRNA (0–0.8 mg/ml; Roche) or herring sperm DNA (0–0.8 mg/ml; Sigma).

K_m, k_{cat} and K_I values were derived from iterative non-linear fits of the theoretical Michaelis equation to the experimental values, using the Levenberg–Marquardt algorithm (20).

Aminoacylation of tRNA^{Lys} produced through enzymatic hydrolysis of diacetyl-[^{3}H]lysyl-tRNA^{Lys}

Escherichia coli tRNA^{Lys} was aminoacylated with L-[^{3}H]lysine (320 Ci/mol), then acetylated with acetic anhydride (11), and treated with sodium periodate as described previously (21). A 130 pmol concentration of the resulting diacetyl-L-[^{3}H]lysyl-tRNA^{Lys} sample was incubated in 650 µl at 50°C in the presence of 0.12 U of *S.solfataricus* PTH. At various times (Fig. 1), two 50 µl aliquots were simultaneously withdrawn. The first aliquot was mixed with 1 ml of 5% (w/w) trichloracetic acid (TCA) and 20 µl of carrier RNA from yeast. The second one was diluted in a reaction mixture (50 µl) containing 40 mM Tris–HCl pH 7.6, 19 µM [^{14}C]lysine (317 Ci/mol), 4 mM ATP, 0.1 mM EDTA, 0.1 mM DTT and 2 µM of pure *E.coli* lysyl-tRNA synthetase (22). After 2 min incubation at 28°C, 1 ml of 5 % (w/w) TCA and 20 µl of carrier RNA from yeast were added to the second sample. Insoluble radioactivity (^{3}H in the first sample, ^{3}H and ^{14}C in the second one) was measured by scintillation counting, as described (23). It was systematically verified that ^{3}H radioactivity was the same in each pair of samples, thus showing that diacetyl-L-[^{3}H]lysyl-tRNA^{Lys} did not vary upon incubation in the presence of lysyl-tRNA synthetase.

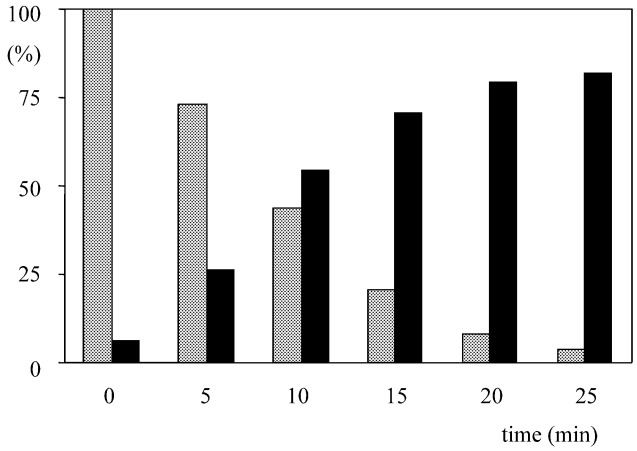

Figure 1. The *SS000175* gene product behaves as a PTH. Diacetyl-L-[^{3}H]lysyl-tRNA^{Lys} was incubated at 50°C in the presence of catalytic amounts of *S.solfataricus* PTH. At various times, aliquots were withdrawn and incubated for 2 min with [^{14}C]lysine, ATP and excess pure *E.coli* lysyl-tRNA synthetase (see Materials and Methods). After quenching of the reaction, TCA-precipitable ^{3}H (open bars) and ^{14}C (closed bars) radioactivities were measured by scintillation counting. Values were normalized to 100% with respect to TCA-precipitable ^{3}H radioactivity at time zero.

Yeast strains and growth media

The *S.cerevisiae* strains used in this work are summarized in Table 1. Rich media contained 1% peptone and 1% yeast extract (Difco) with either 2% glucose or 2% glycerol. Minimal media contained 0.67% yeast nitrogen base (Difco) with either 2% glucose or 2% glycerol. Required amino acids and uracil were added at final concentrations of 50 µg/ml each. Induction of *YHR189w* gene expression from the pYESpthY1 plasmid was obtained by adding 0.1% galactose to the medium.

RESULTS AND DISCUSSION

Identification of the *SS000175* gene and characterization of its product

Upon assaying PTH activity in an *S.solfataricus* cell extract with diacetyl-[^{14}C]lysyl-tRNA^{Lys} as the substrate, dissociation of radioactivity from tRNA could be shown. At 50°C, the reaction occurred at a rate of 8 U/mg of protein in the extract. This assay enabled us to undertake isolation of the archaeal PTH activity through successive chromatographies on Q-Sepharose, SP-Sepharose, Superdex 75 and HI-Propyl columns. At the end of the purification procedure, the PTH specific activity was enriched ~8500-fold if compared with the activity in the crude extract. A nearly homogeneous protein with a 15 ± 2 kDa molecular weight was obtained, as judged by SDS–PAGE analysis. Edman degradation of this protein indicated an N-terminal sequence, MIKMVIVVRS, that unambiguously designated the ORF *SS00175* in the *S.solfataricus* genome. This ORF encodes a short 120 residue polypeptide with a M_w of 13.1 kDa. It belongs to the family of archaeal PTH homologs, as defined by Rosas-Sandoval *et al.*

on the basis of the *MJ0051* gene encoding PTH activity in *M.jannaschii* (16).

To assess whether the *SS00175* ORF actually encoded a protein with a PTH activity, this gene was amplified by PCR and inserted into the expression vector pTrc99-A under control of the *trc* promoter, to give pTrc-pthS. The *E.coli* strain K37ΔpthTr was transformed with the resulting plasmid. In this strain, the chromosomal PTH gene (*pth*) is disrupted while a functional *pth* gene is added *in trans* through a plasmid (pMAKpth) harboring a temperature-sensitive replicon (11). At 42°C, the thermosensitive plasmid is lost and cells die. Upon addition of plasmid pTrc-pthS, K37ΔpthTr acquired the capacity to grow at 42°C, in spite of the loss of the thermosensitive plasmid. Transformation with the control plasmid pTrc99-A did not produce thermoresistant clones. This experiment demonstrates that, similarly to the *MJ0051* gene, the *SS00175* gene can complement for the absence of functional PTH in an *E.coli* cell.

Furthermore, crude extracts of strain K37ΔpthTr and K37ΔpthTr(pTrc-pthS) were exposed to 80°C for 10 min and PTH activity was assayed. With strain K37ΔpthTr, the *E.coli* PTH activity was rapidly lost upon heating. With strain K37ΔpthTr(pTrc-pthS), a PTH specific activity of 600 U/mg persisted at the end of the heat treatment. This thermoresistant PTH activity is likely to originate from the *S.solfataricus* gene introduced *in trans* in the *E.coli* recipient strain.

The *E.coli* strain XL1-Blue transformed with plasmid pTrc-pthS was used to undertake purification of the archaeal PTH. A 0.26 mg aliquot of homogeneous enzyme was obtained from a 1 l culture (Table 2). To determine the molecular mass of the *S.solfataricus* PTH, a sample of this enzyme was chromatographed on a TSK-GEL G3000-SW_{XL} gel filtration column (0.78 × 30 cm; Tosohaas) equilibrated in 20 mM Tris–HCl buffer pH 7.0 containing 150 mM KCl. In this experiment, marker proteins of known molecular mass included carbonic anhydrase, *E.coli* PTH and egg white lysozyme. According to the elution times of the various proteins, an M_r of 25 ± 2 kDa could be estimated for the *S.solfataricus* PTH. Therefore, we concluded that, in contrast to the monomeric *E.coli* enzyme (7), the *S.solfataricus* enzyme behaves as a dimer.

Because of their role in tRNA folding, various compounds such as $MgCl_2$, KCl or polyamines were varied in the assay of *S.solfataricus* PTH activity. Data in Table 3 indicate that, at their optimal concentration, each of these compounds stimulated the rate of diacetyl-lysyl-$tRNA^{Lys}$ hydrolysis ~80-fold. We concluded that, similarly to the case of the *E.coli* enzyme (11), the archaeal PTH requires its substrate to adopt a compact 3D structure.

Table 3. Activity of *S.solfataricus* PTH under various ionic conditions

Compound added	Specific activity (s^{-1})[a]
None	0.026
5 mM $MgCl_2$	1.3
10 mM $MgCl_2$	1.8
40 mM $MgCl_2$	2.3
150 mM KCl	1.8
300 mM KCl	2.0
500 mM KCl	1.1
0.03 mM spermidine-HCl	1.6
0.1 mM spermidine-HCl	1.9

[a]Specific activities were measured at 50°C in the presence of 20 mM Tris–HCl pH 7.5, 0.1 mM EDTA, 0.1 mM DTT, 0.75 μM diacetyl-[^{14}C]lysyl-$tRNA^{Lys}$ and catalytic amounts of enzyme (between 0.1 and 10 nM, depending on the magnitude of the rate to be measured), as described in Materials and Methods. Standard errors on the values shown are <20%.

Substrate specificity of the *SS00175* protein product

PTHs purified from bacteria or yeast transform *N*-acyl-aminoacyl-tRNA into *N*-acyl-amino acid and tRNA (3,5–7). However, it was reported earlier that rabbit reticulocytes contain a nuclease activity capable of converting *N*-acyl-aminoacyl-tRNA into *N*-acyl-aminoacyl-AMP plus truncated tRNA lacking the 3′-AMP terminus (24,25). To examine whether the *S.solfataricus* enzyme carried such an activity, we determined whether the product of the reaction catalyzed by this protein remained aminoacylatable. A sample of diacetyl-[^{3}H]lysyl-$tRNA^{Lys}$ was treated with sodium periodate, in order to oxidize, and thus inactivate, any uncharged tRNA molecules. Then, during incubation of this sample in the presence of homogeneous *S.solfataricus* PTH, pairs of aliquots were withdrawn as a function of time to measure (i) the decline of the diacetyl-[^{3}H]lysyl-$tRNA^{Lys}$ substrate; and (ii) the appearance of $tRNA^{Lys}$ aminoacylatable by [^{14}C]lysine upon addition of [^{14}C]lysine, ATP and unlimiting amounts of *E.coli* lysyl-tRNA synthetase. Along the kinetics, the amount of [^{14}C]lysine that could be esterified with $tRNA^{Lys}$ exactly paralleled the amount of deacylated diacetyl-[^{3}H]lysyl-$tRNA^{Lys}$ (Fig. 1). Upon omission of PTH from the reaction mixture, the deacylation rate of diacetyl-[^{3}H]lysyl-$tRNA^{Lys}$ was strongly reduced (16% deacylation in 25 min), as was the capacity to transfer [^{14}C]lysine onto tRNA (<0.2 pmol of [^{14}C]lysine per pmol of initial diacetyl-[^{3}H]lysyl-$tRNA^{Lys}$). These experiments enabled us to conclude that the *S.solfataricus* enzyme behaves as a hydrolase capable of liberating functional tRNA from an *N*-blocked aminoacyl-tRNA substrate. Such a hydrolase activity fully accounts for the capacity of the archaeal protein to compensate for the lack of endogeneous PTH activity in an *E.coli* cell.

In *E.coli* and in yeast, a tRNA-specific hydrolase distinct from PTH ensures the deacylation of mischarged D-amino-acyl-tRNAs (19,26,27). The genes encoding these D-amino-acyl-tRNA deacylases have homologs in the genomes of all bacteria and eukarya, but not in those of archaea. Therefore, we wondered whether the new PTH isolated from *S.solfataricus*, which is markedly different from a bacterial PTH, could have a specificity large enough to also catalyze deacylation of D-aminoacyl-tRNAs. Actually, the results shown in Table 4 exclude this possibility. The efficiency of D-aminoacyl-tRNA hydrolysis by the archaeal PTH is poor compared with the transformation of an *N*-blocked aminoacyl-tRNA. The value of the ratio between the two reaction rates is close to that measured with the *E.coli* PTH.

Escherichia coli PTH has a specificity large enough to recycle any peptidyl-tRNA species (28). However, formyl-methionyl-$tRNA^{fMet}$ escapes hydrolysis by the bacterial enzyme (5,8,10,29). Resistance of formyl-methionyl-$tRNA^{fMet}$ can be accounted for by the absence of a canonical base pair at the top of its acceptor stem. In archaea, formylation of methionyl-$tRNA_i^{Met}$ is supposed not to happen, and nucleotides 1 and 72 of initiator tRNA are correctly base

Table 4. Hydrolysis of diacetyl-L-[^{14}C]lysyl-tRNA^{Lys} and D-[^{3}H]tyrosyl-tRNA^{Tyr} by either *S.solfataricus* or *E.coli* PTH

	Specific activity of deacylation (s^{-1})		
	Diacetyl-L-[^{14}C]lysyl-tRNA^{Lys}	D-[^{3}H]tyrosyl-tRNA^{Tyr}	Ratio value
Purified *S.solfataricus* PTH (37°C)	0.53	0.0013	410
Purified *E.coli* PTH (28°C)	0.50	0.0025	200

Specific activities were measured in 20 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM DTT, in the presence of either 0.75 µM diacetyl-[^{14}C]lysyl-tRNA^{Lys} and 1 nM PTH (*S.solfataricus* or *E.coli*), or 0.1 µM D-[^{3}H]tyrosyl-tRNA^{Tyr} and 400 nM PTH. Standard errors on the values shown are <20%.

Table 5. Catalytic parameters of various *N*-blocked tRNAs in the hydrolysis reaction catalyzed by *S.solfataricus* PTH

tRNA	k_{cat} (s^{-1})	K_m (nM)	Relative k_{cat}/K_m
Diacetyl-lysyl-tRNA^{Lys}	1.8	11	1
Formyl-methionyl-tRNA^{fMet}	3.0	12	1.6
Acetyl-histidyl-tRNA^{His}	3.4	16	1.3
Diacetyl-lysyl-tRNA^{Lys} (dephosphorylated)	0.86	2.8	1.9
Formyl-methionyl-tRNA^{fMet} (dephosphorylated)	3.0	30	0.62

Michaelian k_{cat} and K_m parameters were measured as described in Materials and Methods. Relative k_{cat}/K_m values were given an arbitrary value of 1 for the measurement in the presence of diacetyl-lysyl-tRNA^{Lys}. Standard errors on the values shown are <20%.

Table 6. K_I values for various nucleic acids in the hydrolysis reaction catalyzed by *S.solfataricus* PTH

Compound	K_I[a] (nM)	(mg/ml)
Escherichia coli tRNA^{Lys}	50	1.2
Escherichia coli tRNA^{fMet}	110	2.7
Unfractionated *E.coli* tRNA	95	2.4
Escherichia coli rRNA		>2000
Herring sperm DNA		>2000

[a]Initial rates of hydrolysis of 0.2 µM diacetyl-[^{14}C]lysyl-tRNA^{Lys} in 20 mM Tris–HCl pH 7.5, 0.1 mM EDTA and 0.1 mM DTT were measured at 50°C in the presence of 0.075 nM *S.solfataricus* PTH. Concentrations of the studied inhibitors were varied as described in Materials and Methods. Standard errors on the values shown are <20%.

paired (30). Therefore, we wondered whether the archaeal PTH exhibited any particular sensitivity to the C_1–A_{72} mismatch in *E.coli* tRNA^{fMet}. To answer this question, Michaelian parameters of the reaction catalyzed by *S.solfataricus* PTH were measured in the presence of either *E.coli* diacetyl-lysyl-tRNA^{Lys} or *E.coli* formyl-methionyl-tRNA^{fMet} (Table 5). Comparison of the k_{cat} and K_m values obtained clearly shows that the archaeal enzyme does not discriminate between the two tRNAs. With *E.coli* PTH, under the same experimental conditions, the catalytic efficiencies measured with the two substrates differ by a factor of nearly 20 (12).

As explained in the Introduction, the activity of the bacterial PTH towards elongator tRNAs is facilitated by the 5′-terminal phosphate at the end of a fully base-paired acceptor stem. Indeed, the catalytic efficiency of the *E.coli* enzyme is reduced upon removal of the 5′-phosphate in acetyl-phenylalanyl-tRNA^{Phe} or diacetyl-lysyl-tRNA^{Lys} (8,11). On the other hand, the activity of the bacterial enzyme does not significantly depend on the dephosphorylation of formyl-methionyl-tRNA^{fMet} (12). Therefore, we also measured the activity of the *S.solfataricus* PTH in the presence of diacetyl-lysyl-tRNA^{Lys} or formyl-methionyl-tRNA^{fMet} devoid of 5′-phosphate. As shown in Table 5, upon dephosphorylation, the catalytic efficiencies with the two substrates varied by factors <2.5-fold. The above results force us to conclude that, in an archaeal context, PTH uses neither the 1–72 matching nor the 5′-phosphate as identity elements in the tRNA molecule. We also verified that *E.coli* acetyl-histidyl-tRNA^{His} behaved as a substrate of the *S.solfataricus* PTH (Table 5). Indeed, in all living kingdoms, tRNA^{His} has the peculiarity of possessing an extra base at its 5′ end. The activity of *E.coli* PTH is not sensitive to this feature (31).

With *E.coli* PTH, recognition of the substrate is believed to involve the peptidyl moiety and the acceptor end of the esterified tRNA molecule (9,11). K_m values for the various studied substrates are in the micromolar range (9–11). Here, the data in Table 5 show K_m values for *N*-acetyl-aminoacyl-tRNAs in the nanomolar range. Such a difference suggests that the *S.solfataricus* PTH recognizes additional features in the tRNA structure. In agreement with this view, we observed a strong inhibition of the hydrolytic reaction catalyzed by the archaeal enzyme upon addition of non-esterified tRNA^{Lys} or tRNA^{fMet}. The K_I constants with these tRNAs (50 and 110 nM, respectively) are indicative of markedly stable complexes (Table 6). Formation of these complexes is specific for tRNA. Indeed, unfractionated tRNA from *E.coli* also inhibited the reaction, with a K_I value of ~100 nM. On the other hand, *E.coli* rRNA or herring sperm DNA could be added in great excess without significant consequences on the velocity of the reaction. Such a behavior of the *S.solfataricus* PTH in the presence of tRNA is in contrast to that of *E.coli* PTH. Indeed, uncharged tRNA had to be added at concentrations in the range of 10 µM to observe inhibition of the bacterial PTH (9).

Presence of homologs of the *SS00175* gene in archaeal and eukaryal genomes

Searches of the sequence databases using the BLAST program (32) indicated the presence of *SS00175* homologs in all sequenced archaeal and eukaryal genomes, as well as in the fowlpox virus genome. *MJ0051* belongs to this family of homologs. In *S.cerevisiae*, the homolog is the *YBL057c* gene. However, homologs could not be found in any of the available bacterial genomes. On the other hand, homologs of the *E.coli pth* gene are detected in bacteria and eukarya, but not in

Table 7. Comparison of the hydrolysis of *E.coli* diacetyl-L-[^{14}C]lysyl-tRNA^{Lys} and formyl-[^{14}C]methionyl-tRNA^{fMet} by the various PTHs (*S.solfataricus*, *S.cerevisiae* or *E.coli*)

	Initial rate of deacylation (pmol/s/mg)		
	Diacetyl-[^{14}C]lysyl-tRNA^{Lys}	Formyl-[^{14}C]methionyl-tRNA^{fMet}	Ratio value
Crude extract of K37ΔpthTr(pTrc-pthS)	600	1300	0.46
Purified *S.solfataricus* PTH	69 000	140 000	0.49
Crude extract of K37ΔpthTr(pKKpthY2)	48	22	2.2
Crude extract of K37ΔpthTr(pBSpthY1)	83	480	0.17
Purified *E.coli* PTH	24 000	1200	20

Initial rates were measured in 20 mM Tris–HCl pH 7.5, 10 mM $MgCl_2$, 0.1 mM EDTA and 0.1 mM DTT, containing catalytic amounts of the PTH assayed (0.02–1 μg of total proteins in the case of the crude extracts, 0.2–0.5 ng of purified *S.solfataricus* PTH and 1–20 ng of purified *E.coli* PTH), and either 0.75 μM diacetyl-[^{14}C]lysyl-tRNA^{Lys} or 0.57 μM formyl-[^{14}C]methionyl-tRNA^{fMet}. Activities were measured at 50°C in the case of the *S.solfataricus* PTH and at 28°C in all other cases. Plasmids pTrc-pthS, pKKpthY2 and pBSpthY1 harbor the *SS000175*, *YBL057c* and *YHR189w* genes, respectively. Standard errors on the values shown are <20%.

archaea. Therefore, as recently proposed (16), both bacterial- and archaeal-like PTH enzymes are likely to be encoded in eukarya.

The *YHR189w* gene product in *S.cerevisiae* corresponds to a bacterial-like PTH. This gene has already been shown to complement an *E.coli* thermosensitive mutant deficient in PTH activity (15,16). We could reproduce this experiment with a *pth*-null mutant, K37ΔpthTr, and a pBluescript derivative carrying *YHR189w*. To know whether the *YBL057c* gene product could also function as a PTH, we inserted this gene inside pKK233-3 and transformed the *E.coli* strain K37ΔpthTr with the resulting plasmid (pKKpthY2). Transformants grew at 42°C despite the loss of the thermosensitive pMAKpth plasmid at this temperature. To confirm that the yeast bacterial-like and archeal-like gene products carry a hydrolytic activity resembling that of the *E.coli* enzyme, we assayed PTH activity in crude extracts of K37ΔpthTr(pBSpthY1) and K37ΔpthTr(pKKpthY2). Measured rates with diacetyl-lysyl-tRNA^{Lys} as substrate were 83 and 48 pmol/s/mg, respectively (Table 7). With a crude extract of strain K37, which possesses an intact *pth* gene, the rate of hydrolysis was only 8 pmol/s/mg. The large discrepancy between the two former and the latter rate values indicates that most if not all activity in K37ΔpthTr(pBSpthY1) and K37ΔpthTr(pKKpthY2) must originate from the *YHR189w* and *YBL057c* genes, respectively. We may conclude that yeast cells benefit from two types of PTH at a same time. At first sight, this property explains why inactivation of *YHR189w* had no effect on yeast growth (15) while a *pth* gene is essential for *E.coli* (11,14). Indeed, the presence of the archaeal-like PTH is likely to compensate for deprivation of the bacterial-like enzyme. In fact, the *YBL057c* and *YHR189w* genes could both be destroyed without noticeable effect on the growth of *S.cerevisiae* (16). This finding may reflect the occurrence in yeast of additional PTH-like activities.

In contrast to our study, Rosas-Sandoval *et al*. did not succeed in complementing an *E.coli pth*^{ts} strain with *YBL057c* inserted in the pKQV4 vector (16). To eventually cure the thermosensitive phenotype of the strain, the *YBL057c* ORF had to be truncated by 258 nucleotides on the 5′ side. To explain this experiment, Rosas-Sandoval *et al.* considered the possibility that the N-terminal domain of the *YBL057c* product is a transit signal for mitochondrial localization. The demonstration, in the present study, that the full-length product of *YBL057c* readily complements an *E.coli* strain lacking endogeneous PTH forces a rediscussion of this speculation. Actually, we observed that excessive overproduction of *S.solfataricus* PTH from plasmid pTrc-pthS impairs the growth of *E.coli* strain XL1-Blue. On this plasmid, the *S.solfataricus* PTH gene is placed under the control of the inducible *trc* promoter. Growth of the transformed strain was possible only in the absence of the IPTG inducer. In Table 5, we show that the *S.solfataricus* enzyme readily accepts *E.coli* formyl-methionyl-tRNA^{fMet} as a substrate. Consequently, it is tempting to associate the toxicity of *SS00175* in an *E.coli* context with the capacity of the archaeal enzyme to scavenge formyl-methionyl-tRNA^{fMet}. The two yeast PTHs also show efficiency in the hydrolysis of *E.coli* formyl-methionyl-tRNA^{fMet} (Table 7). Possibly, in the experiments of Rosas-Sandoval *et al*. (16), truncation of the *YBL057c* ORF was necessary to reduce the specific activity of the plasmid-encoded yeast PTH to a value supportable by the *E.coli* translation apparatus.

The capacity of *S.solfataricus* PTH to use formyl-methionyl-tRNA^{fMet} as substrate *in vitro* may be explained by the special feature of translation in archaea where formylation of methionyl-tRNA$_i^{Met}$ does not occur (30). In the cytoplasm of eukarya, like in an archaeal cell, methionylated initiator tRNA is not formylated. On the other hand, translation initiation in the mitochondrial compartment involves a formylatable tRNA^{fMet} species. In yeast, mitochondrial tRNA^{fMet} displays a mismatch (U_1–U_{72}) at the top of its acceptor stem (33). This defect resembles the C_1–A_{72} mispairing in *E.coli* tRNA^{fMet}. Therefore, in view of their harmful behavior toward *E.coli* formyl-methionyl-tRNA^{fMet} (Table 7), the two PTHs encoded by *YBL057c* and *YHR189w* can be suspected to stay in the cytoplasm of yeast. In agreement with the idea of cytosolic enzymes, the double inactivation of the *YBL057c* and *YHR189w* ORFs was reported not to impair the growth of *S.cerevisiae* on solid medium containing glycerol (16). However, in contrast to this result, another study performed in liquid medium claimed that *YHR189w* disruption reduces the growth rate on several non-fermentable carbon sources (34).

In this context, one must take care that small variations in the culture conditions may be enough to succeed in revealing mitochondrial dysfunction in response to gene inactivation. For instance, recent reports indicated that the respiratory

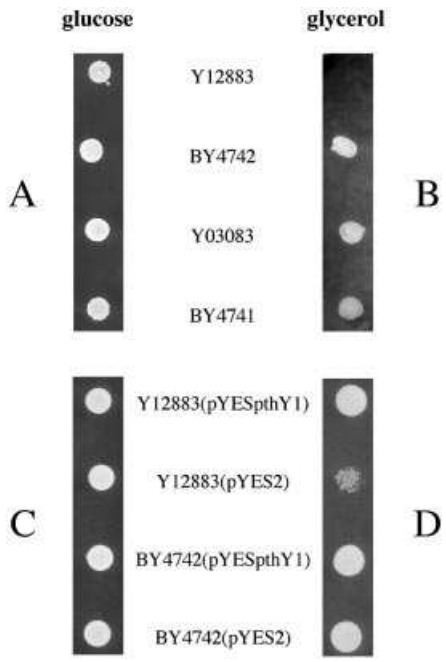

Figure 2. Growth of *S.cerevisiae* strains BY4742, BY4741, Y12883 (*YHR189w*::*kanMX4*), Y03083 (*YBL057c*::*kanMX4*), Y12883(pYESpthY1), Y12883(pYES2), BY4742(pYESpthY1) and BY4742(pYES2) on various solid media. Exponentially growing cultures in minimal fermentative medium were diluted in 10 mM Tris–HCl pH 8.0 containing 1 mM EDTA, to obtain suspensions with an OD of 1 at 650 nm. Then, 4 µl aliquots were spotted on minimal medium plates containing either glucose (**A** and **C**) or glycerol (**B** and **D**) and supplemented with either His, Ura, Lys, Leu and Met (A and B) or His, Lys, Leu and 0.1% galactose (C and D). Plates containing glucose or glycerol were incubated at 30°C for 4 or 7 days, respectively.

growth of *S.cerevisiae* was insensitive to deletion of mitochondrial methionyl-tRNA^{fMet} formyltransferase (35,36). Nevertheless, by changing the growth conditions, a selective advantage could eventually be associated with mitochondrial formylation (36).

To possibly associate phenotypes with *YBL057c* and *YHR189w* products, we compared the growths of strains Y12883 (*YHR189w*::*kanMX4*) and Y03083 (*YBL057c*::*kanMX4*) with those of their parental strains (Table 1). In agreement with previous studies (15,16), all cells grew normally on rich solid medium containing either glucose or glycerol, or on minimal medium containing glucose. On the other hand, a growth defect of Y12883 became clearly visible on minimal medium containing glycerol (Fig. 2). To establish that this defect was directly caused by the disruption of the *YHR189w* gene, we cloned this gene into the pYES2 expression vector under the control of the inducible *GAL1* promoter and introduced the resulting plasmid (pYESpthY1) in the Y12883 mutant. As shown in Figure 2, the growth defect of the mutant was cured by the transformation. We verified that the parental strain BY4742 was insensitive to the transformation. A residual growth of the strain Y12883 carrying the control plasmid pYES2 can be accounted for by the presence of 0.1% of the fermentable sugar galactose in the medium. Addition of this compound was necessary to induce expression of the plasmid-encoded PTH. These results indicate that an intact *YHR189w* gene contributes to yeast mitochondrial function provided that the cells are grown on minimal medium.

By using predictive computational methods [(37), http://mips.gsf.de/cgi-bin/proj/medgen/mitoprot_yeast], the *YHR189w* product is given only a probability of 0.111 to be mitochondrial. Therefore, the possibility that the *YHR189w* product remains cytosolic but indirectly contributes to respiration must not be ruled out. Care must be taken, however, in extrapolating this idea to other eukaryotes. For instance, the *Arabidospis thaliana* genome (http://mips.gsf.de/proj/thal/db/index.html) indicates four bacterial-like PTHs and three archaeal-like PTHs. Using the TargetP algorithm (38), two of the bacterial-like PTHs are predicted to be chloroplastic, whereas one of the bacterial-like PTHs and all three archaeal-like PTHs are predicted to be mitochondrial. Clearly, more work will be necessary to unambiguously assign the cell compartments where the PTH-like products exert activity in eukaryotes. In this search, additional functions will have to be considered. Maize nuclear gene *crs2* illustrates this need. This gene has been reported to encode a PTH homolog (39). However, it fails to rescue an *E.coli pth*^{ts} mutant. Instead, the product of *crs2* has been proposed to participate in group II intron splicing (39).

ACKNOWLEDGEMENTS

We thank Jacques d'Alayer and Peggy Chrisment for determining the N-terminal sequence of *S.solfataricus* PTH, and Marc Nadal for providing us with *S.solfataricus* strain P2.

REFERENCES

1. Kisselev,L.L. and Buckingham,R.H. (2000) Translational termination comes of age. *Trends Biochem. Sci.*, **25**, 561–566.
2. Menninger,J.R. (1976) Peptidyl transfer RNA dissociates during protein synthesis from ribosomes of *Escherichia coli*. *J. Biol. Chem.*, **251**, 3392–3398.
3. Cuzin,F., Kretchmer,N., Greenberg,R.E., Hurwitz,R. and Chapeville,F. (1967) Enzymatic hydrolysis of *N*-substituted aminoacyl-tRNA. *Proc. Natl Acad. Sci. USA*, **58**, 2079–2086.
4. Atherly,A.G. and Menninger,J.R. (1972) Mutant *E.coli* strain with temperature sensitive peptidyl-transfer RNA hydrolase. *Nature*, **240**, 245–246.
5. Kössel,H. and RajBhandary,U.L. (1968) Studies on polynucleotides. LXXXVI. Enzymic hydrolysis of *N*-acylaminoacyl-transfer RNA. *J. Mol. Biol.*, **35**, 539–560.
6. Jost,J.P. and Bock,R.M. (1969) Enzymatic hydrolysis of *N*-substituted aminoacyl transfer ribonucleic acid in yeast. *J. Biol. Chem.*, **244**, 5866–5873.
7. Kössel,H. (1970) Purification and properties of peptidyl-tRNA hydrolase from *Escherichia coli*. *Biochim. Biophys Acta*, **204**, 191–202.
8. Schulman,L.H. and Pelka,H. (1975) The structural basis for the resistance of *Escherichia coli* formylmethionyl transfer ribonucleic acid to cleavage by *Escherichia coli* peptidyl transfer ribonucleic acid hydrolase. *J. Biol. Chem.*, **250**, 542–547.
9. Shiloach,J., Bauer,S., de Groot,N. and Lapidot,Y. (1975) The influence of the peptide chain length on the activity of peptidyl-tRNA hydrolase from *E.coli*. *Nucleic Acids Res.*, **2**, 1941–1950.
10. Dutka,S., Meinnel,T., Lazennec,C., Mechulam,Y. and Blanquet,S. (1993) Role of the 1–72 base pair in tRNAs for the activity of *Escherichia coli* peptidyl-tRNA hydrolase. *Nucleic Acids Res.*, **21**, 4025–4030.
11. Schmitt,E., Mechulam,Y., Fromant,M., Plateau,P. and Blanquet,S. (1997) Crystal structure at 1.2 Å resolution and active site mapping of *Escherichia coli* peptidyl-tRNA hydrolase. *EMBO J.*, **16**, 4760–4769.
12. Fromant,M., Plateau,P., Schmitt,E., Mechulam,Y. and Blanquet,S. (1999) Receptor site for the 5′-phosphate of elongator tRNAs governs

substrate selection by peptidyl-tRNA hydrolase. *Biochemistry*, **38**, 4982–4987.
13. García-Villegas,M.R., De La Vega,F.M., Galindo,J.M., Segura,M., Buckingham,R.H. and Guarneros,G. (1991) Peptidyl-tRNA hydrolase is involved in λ inhibition of host protein synthesis. *EMBO J.*, **10**, 3549–3555.
14. Menninger,J.R. (1979) Accumulation of peptidyl tRNA is lethal to *Escherichia coli*. *J. Bacteriol.*, **137**, 694–696.
15. Menez,J., Buckingham,R.H., de Zamaroczy,M. and Campelli,C.K. (2002) Peptidyl-tRNA hydrolase in *Bacillus subtilis*, encoded by *spoVC*, is essential to vegetative growth, whereas the homologous enzyme in *Saccharomyces cerevisiae* is dispensable. *Mol. Microbiol.*, **45**, 123–129.
16. Rosas-Sandoval,G., Ambrogelly,A., Rinehart,J., Wei,D., Cruz-Vera,L.R., Graham,D.E., Stetter,K.O., Guarneros,G. and Söll,D. (2002) Orthologs of a novel archaeal and of the bacterial peptidyl-tRNA hydrolase are nonessential in yeast. *Proc. Natl Acad. Sci. USA*, **99**, 16707–16712.
17. Zillig,W., Kletzin,A., Schleper,C., Holz,I., Janekovic,D., Hain,J., Lanzendorfer,M. and Kristjansson,J.K. (1994) Screening for *Sulfolobales*, their plasmids and their viruses in Icelandic solfataras. *Syst. Appl. Microbiol.*, **16**, 609–628.
18. Plateau,P., Fromant,M., Schmitter,J.M. and Blanquet,S. (1990) Catabolism of bis(5′-nucleosidyl) tetraphosphates in *Saccharomyces cerevisiae*. *J. Bacteriol.*, **172**, 6892–6899.
19. Soutourina,J., Plateau,P., Delort,F., Peirotes,A. and Blanquet,S. (1999) Functional characterization of the D-Tyr-tRNA^{Tyr} deacylase from *Escherichia coli*. *J. Biol. Chem.*, **274**, 19109–19114.
20. Dardel,F. (1994) MC-Fit: using Monte-Carlo methods to get accurate confidence limits on enzyme parameters. *CABIOS*, **10**, 273–275.
21. Lazard,M., Mirande,M. and Waller,J.P. (1987) Expression of the aminoacyl-tRNA synthetase complex in cultured Chinese hamster ovary cells: specific depression of the methionyl-tRNA synthetase component upon methionine restriction. *J. Biol. Chem.*, **262**, 3982–3987.
22. Brevet,A., Chen,J., Lévêque,F., Blanquet,S. and Plateau,P. (1995) Comparison of the enzymatic properties of the two *Escherichia coli* lysyl-tRNA synthetase species. *J. Biol. Chem.*, **270**, 14439–14444.
23. Lawrence,F., Blanquet,S., Poiret,M., Robert-Gero,M. and Waller,J.P. (1973) The mechanism of action of methionyl-tRNA synthetase. 3. Ion requirements and kinetic parameters of the ATP–PP$_i$ exchange and methionine-transfer reactions catalyzed by the native and trypsin-modified enzymes. *Eur. J. Biochem.*, **36**, 234–243.
24. Gross,M., Starn,T.K., Rundquist,C., Crow,P., White,J., Olin,A. and Wagner,T. (1992) Purification and initial characterization of peptidyl-tRNA hydrolase from rabbit reticulocytes. *J. Biol. Chem.*, **267**, 2073–2079.
25. Gross,M., Crow,P. and White,J. (1992) The site of hydrolysis by rabbit reticulocyte peptidyl-tRNA hydrolase is the 3′-AMP terminus of susceptible tRNA substrates. *J. Biol. Chem.*, **267**, 2080–2086.
26. Calendar,R. and Berg,P. (1967) D-Tyrosyl RNA: formation, hydrolysis and utilization for protein synthesis. *J. Mol. Biol.*, **26**, 39–54.
27. Soutourina,J., Plateau,P. and Blanquet,S. (2000) Metabolism of D-aminoacyl-tRNAs in *Escherichia coli* and *Saccharomyces cerevisiae* cells. *J. Biol. Chem.*, **275**, 32535–32542.
28. Menninger,J.R. (1978) The accumulation as peptidyl-transfer RNA of isoaccepting transfer RNA families in *Escherichia coli* with temperature-sensitive peptidyl-transfer RNA hydrolase. *J. Biol. Chem.*, **253**, 6808–6813.
29. Menninger,J.R., Mulholland,M.C. and Stirewalt,W.S. (1970) Peptidyl-tRNA hydrolase and protein chain termination. *Biochim. Biophys Acta*, **217**, 496–511.
30. Bult,C.J., White,O., Olsen,G.J., Zhou,L., Fleischmann,R.D., Sutton,G.G., Blake,J.A., FitzGerald,L.M., Clayton,R.A., Gocayne,J.D. *et al.* (1996) Complete genome sequence of the methanogenic archaeon, *Methanococcus jannaschii*. *Science*, **273**, 1058–1073.
31. Fromant,M., Plateau,P. and Blanquet,S. (2000) Function of the extra 5′-phosphate carried by histidine tRNA. *Biochemistry*, **39**, 4062–4067.
32. Schaffer,A.A., Aravind,L., Madden,T.L., Shavirin,S., Spouge,J.L., Wolf,Y.I., Koonin,E.V. and Altschul,S.F. (2001) Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. *Nucleic Acids Res.*, **29**, 2994–3005.
33. Canaday,J., Dirheimer,G. and Martin,R.P. (1980) Yeast mitochondrial methionine initiator tRNA: characterization and nucleotide sequence. *Nucleic Acids Res.*, **8**, 1445–1457.
34. Steinmetz,L.M., Scharfe,C., Deutschbauer,A.M., Mokranjac,D., Herman,Z.S., Jones,T., Chu,A.M., Giaever,G., Prokisch,H., Oefner,P.J. *et al.* (2002) Systematic screen for human disease genes in yeast. *Nature Genet.*, **31**, 400–404.
35. Li,Y., Holmes,W.B., Appling,D.R. and RajBhandary,U.L. (2000) Initiation of protein synthesis in *Saccharomyces cerevisiae* mitochondria without formylation of the initiator tRNA. *J. Bacteriol.*, **182**, 2886–2892.
36. Vial,L., Gomez,P., Panvert,M., Schmitt,E., Blanquet,S. and Mechulam,Y. (2003) Mitochondrial methionyl-tRNA$_f^{Met}$ formyltransferase from *Saccharomyces cerevisiae*: gene disruption and tRNA substrate specificity. *Biochemistry*, **42**, 932–939.
37. Claros,M.G. and Vincens,P. (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur. J. Biochem.*, **241**, 779–786.
38. Emanuelsson,O., Nielsen,H., Brunak,S. and von Heijne,G. (2000) Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *J. Mol. Biol.*, **300**, 1005–1016.
39. Jenkins,B.D. and Barkan,A. (2001) Recruitment of peptidyl-tRNA hydrolase as a facilitator of group II intron splicing in chloroplasts. *EMBO J.*, **20**, 872–879.
40. Miller,H.I. and Friedman,D.I. (1980) An *E.coli* gene product required for lambda site-specific recombination. *Cell*, **20**, 711–719.