The only difference between our gene-silenced transgenic plants that were resistant to high temperature and the respective wild-type plants was that the chloroplasts of the transgenic plants contained a reduced level of trienoic fatty acids and an elevated level of dienoic fatty acids, which is controlled by chloroplast ω-3 fatty acid desaturase. Of the six different higher plant desaturases whose genes have been cloned, only the expression of the chloroplast FAD8 ω-3 fatty acid desaturase gene changes in response to a change in ambient temperature (*22*).

The ω-3 fatty acid desaturase enzyme, which is expressed in nearly all plant species, may be widely useful in engineering temperature tolerance in plants.

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4. Twenty-nine lines of R_0 plants exhibiting kanamycin resistance were selected. The fatty acid composition of the R_1 plant derived from each line was measured (*15*).
5. The fatty acid composition of a number of individual seeds of the backcrossed T15 line was assayed in order to identify homozygous individuals. This analysis was repeated in one subsequent generation to ensure that the trait was stably inherited.
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9. *Arabidopsis thaliana* mutant lines LK70 (*fad3*) and SH1 (*fad7-1fad8-1*) were obtained from the *Arabidopsis* Biological Resource Center at Ohio State University (Columbus, OH).
10. Sterilized tobacco and *Arabidopsis* seeds were routinely germinated on MS agar [T. Murashige and F. Skoog, *Physiol. Plant.* **15**, 473 (1962)] and cultivated at 25° and 23°C, respectively, under a white fluorescent lamp at an intensity of 70 μmol m^{-2} s^{-1}. The fully expanded fourth leaves of tobacco plants that had been cultivated for 5 days under continuous irradiation from a metal halide lamp at an intensity of 170 μmol m^{-2} s^{-1} were used as experimental samples. In *Arabidopsis*, the fully expanded fifth and sixth leaves were used. The detached leaves were heat-treated on a thermostatic heating block. A sheet of filter paper moistened with sterilized water was placed on the heating block. The leaf was placed on the sheet with the surface facing upward and was covered with a thin layer of polyethylene film to prevent drying. Heat treatment was applied for 5 min at different temperatures as indicated in the text. The leaf samples were then immersed in a transparent acrylic cuvette filled with 10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer saturated with CO_2, and the photosynthetic evolution of O_2 was measured with a Clark-type oxygen electrode at 25°C under irradiation by red light as described [Y. Kobayashi and U. Heber, *Photosynth. Res.* **41**, 419 (1994)].
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23. We thank C. Somerville and M. Nishimura for critical review of the manuscript. We also thank all of the members of our laboratory for technical assistance and discussion.

5 August 1999; accepted 15 November 1999

One Polypeptide with Two Aminoacyl-tRNA Synthetase Activities

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The genome sequences of certain archaea do not contain recognizable cysteinyl–transfer RNA (tRNA) synthetases, which are essential for messenger RNA–encoded protein synthesis. However, a single cysteinyl–tRNA synthetase activity was detected and purified from one such organism, *Methanococcus jannaschii*. The amino-terminal sequence of this protein corresponded to the predicted sequence of prolyl–tRNA synthetase. Biochemical and genetic analyses indicated that this archaeal form of prolyl–tRNA synthetase can synthesize both cysteinyl-$tRNA^{Cys}$ and prolyl-$tRNA^{Pro}$. The ability of one enzyme to provide two aminoacyl-tRNAs for protein synthesis raises questions about concepts of substrate specificity in protein synthesis and may provide insights into the evolutionary origins of this process.

The insertion of cysteine into nascent peptides during protein synthesis is dependent on the interaction of cysteine codons with cysteinyl-tRNA (Cys-tRNA) in the ribosomal A site. Cys-tRNA is synthesized from cysteine and $tRNA^{Cys}$ in an adenosine 5′-triphosphate (ATP)–dependent reaction catalyzed by cysteinyl–tRNA synthetase (CysRS). Genes encoding CysRS, *cysS*, have been detected in over 40 organisms encompassing all the living kingdoms (*1*). The only known exceptions are the thermophilic methanogens *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum*, the complete genome sequences of which contain no open reading frames encoding *cysS* homologs (*2*), raising the question of how these archaea synthesize Cys-tRNA for protein synthesis. It was initially suggested that Cys-$tRNA^{Cys}$ might be synthesized by a pathway involving modification of a mischarged tRNA such as Ser-$tRNA^{Cys}$, using a mechanism reminiscent of those previously described for the synthesis of asparaginyl-tRNA (Asn-tRNA), glutaminyl-tRNA (Gln-tRNA) and, more specifically, selenocysteinyl-tRNA (Sec-tRNA) (*3*). Biochemical analyses revealed no evidence for such a pathway (*4*) but instead showed that Cys-tRNA is synthesized directly from cysteine and tRNA in an ATP-dependent reaction (*5*). The identity of the enzyme responsible for Cys-tRNA synthesis in *M. jannaschii* and *M. thermoautotrophicum* is unknown. The recent finding that some aminoacyl–tRNA synthetase (AARS)–encoding genes may be dispensable for cell viability also raised the possibility that *cysS* genes might be absent altogether from the genomes of *M. jannaschii* and *M. thermoautotrophicum* (*6*).

To investigate how Cys-tRNA is synthesized in *M. jannaschii*, we attempted to purify from cell-free extracts a protein with CysRS activity. Conventional chromatographic procedures (*7*) led to the isolation of a single protein with normal CysRS activity (Fig. 1, A and B). Protein analysis revealed an 18–amino acid peptide sequence matching the predicted NH_2-terminal sequence of *M. jannaschii* prolyl–tRNA synthetase (ProRS)

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(Fig. 1C). A test of this protein with CysRS activity confirmed that it also possessed ProRS activity. This suggested that the determined sequence might arise from a contaminating protein rather than from the bona fide CysRS. To address this question, we cloned the gene encoding *M. jannaschii* ProRS (*proS*) and used it for heterologous expression in *Escherichia coli* and subsequent purification of ProRS (*8*). In vitro aminoacylation assays showed that *M. jannaschii* ProRS could synthesize both Cys-tRNA and Pro-tRNA at comparable rates (Fig. 1, D and E), suggesting dual amino acid specificity for this enzyme during protein synthesis. Prolonged aminoacylation showed that the *M. jannaschii* enzyme could generate 68 pmol of Cys-tRNA per A_{260} (absorbance at 260 nm) unit of unfractionated homologous tRNA (pure tRNA species accept ~1600 pmol per A_{260} unit), a much higher level (4.3%) than observed before (*5*) and consistent with the $tRNA^{Cys}$ content in the tRNA of other organisms. Similarly, the enzyme was efficient in proline charging (78 pmol per A_{260} unit).

The possibility that the observed CysRS-like activity results from the mischarging of $tRNA^{Pro}$ with Cys to yield Cys-$tRNA^{Pro}$ was investigated. A transcript of the *M. jannaschii* $tRNA^{Pro}$ gene was synthesized in vitro and then purified. Attempts to aminoacylate this tRNA transcript with *M. jannaschii* ProRS showed that it can readily be charged with Pro but not with Cys (*9*). Additionally, unfractionated *M. jannaschii* tRNA was charged with Pro and subsequently treated with sodium metaperiodate which oxidized, and thus inactivated, all uncharged tRNAs (*10*). After deacylating the Pro-tRNA, we attempted to recharge the tRNA preparation; although Pro charging activity was still detectable, Cys charging had now been abolished, indicating that Pro was exclusively attached to $tRNA^{Pro}$ in the initial reaction (*11*). *Methanococcus jannaschii* total tRNA was also partially fractionated into its various isoacceptors by reversed-phase liquid chromatography, and these fractions were subsequently tested for their ability to be charged with Cys and Pro by *M. jannaschii* ProRS (*12*). A single fraction solely chargeable with Cys and two discrete fractions solely chargeable with Pro were detected, in agreement with the prediction from the genome sequence that *M. jannaschii* contains one $tRNA^{Cys}$ and two $tRNA^{Pro}$ isoacceptors. These data indicate that *M. jannaschii* ProRS is able to synthesize both Cys-$tRNA^{Cys}$ and Pro-$tRNA^{Pro}$, but not Cys-$tRNA^{Pro}$ or Pro-$tRNA^{Cys}$.

To examine the ability of *M. jannaschii* ProRS to synthesize Cys-tRNA in vivo, we attempted to rescue growth at a restrictive temperature of an *E. coli cysS* temperature-sensitive mutant strain using the archaeal *proS* gene (*13*). Coexpression of the genes encoding *M. jannaschii* $tRNA^{Cys}$ and various methanogen ProRS proteins restored growth of *E. coli* UQ818 at 42°C, indicating that ProRS can synthesize Cys-$tRNA^{Cys}$ in vivo (Fig. 2). The slow growth of the rescued transformants was attributed to the high number of rare codons (with respect to normal *E. coli* usage; e.g., for arginine) in the archaeal genes, and perhaps also to an unfavorable cellular Cys:Pro ratio. In addition, the apparent need for modification of $tRNA^{Cys}$ to render it active (evidenced by the inactivity of a gene transcript) suggests that the tRNA substrate may be less than optimal when expressed in *E. coli*. In vivo complementation was strictly dependent on the presence of the *M. jannaschii* $tRNA^{Cys}$ gene, indicating the archaeal *proS* gene products could not charge *E. coli* $tRNA^{Cys}$ sufficiently to sustain growth. The ability of *M. jannaschii* ProRS to synthesize Cys-tRNA both in vitro and in vivo, together with the lack of a *cysS* gene in the genome of *M. jannaschii*, indicates that this enzyme can specify two amino acids during protein synthesis.

The observation that *M. jannaschii* ProRS can recognize both Cys and Pro raised the question of how such recognition is achieved in the context of a single protein. Synthesis of [^{35}S]Cys-tRNA and [^{3}H]Pro-tRNA were both inhibited by the addition of excess unlabeled Cys or Pro (Fig. 3, A and B) and by the ProRS inhibitor thiaproline (*14*) (Fig. 3C). Thus, either the active center of ProRS contains both Cys and Pro binding sites in close proximity, or the protein contains two functionally linked active sites. Another pos-

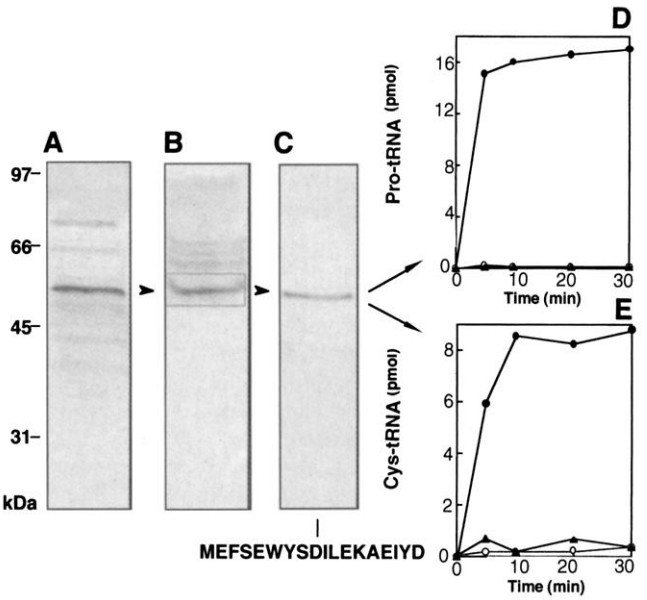

Fig. 1. Purification and NH_2-terminal sequencing of a protein with CysRS activity from *M. jannaschii*. Protein purification was monitored by SDS-PAGE (molecular size standards are in kD). (**A**) Active eluate from the final chromatographic step (Sepharose 4B-CNBr activated with 100 mg of total *E. coli* tRNA as a coupling ligand). (**B**) Samples (20 μl) of active fractions from the previous step were loaded on a 10 to 20% gradient tris-glycine native gel and subjected to PAGE. The bands were located by staining of part of the gel with Coomassie Blue, then were excised from the gels. After overnight elution in reaction buffer, CysRS activity was tested. The boxed band corresponds to the only sample that contained CysRS activity. (**C**) The active band from (B) was further analyzed by SDS-PAGE, blotted onto polyvinylidene difluoride membrane, and subjected to NH_2-terminal sequencing. The 18–amino acid sequence derived (shown) corresponds to ProRS from *M. jannaschii*. This enzyme was then heterologously produced in *E. coli* and purified and tested for both CysRS and ProRS activities. *M. jannaschii* ProRS was found to catalyze the direct attachment of both cysteine (**D**) and proline (**E**) to a fraction of *M. jannaschii* total tRNA. Aminoacylation reactions (20-μl samples) were performed as described in the presence of the following amino acids: (D) 20 μM [^{3}H]proline (●); 20 μM [^{3}H]proline and 800 μM nonradiolabeled cysteine (▲); and 20 μM [^{3}H]proline and 800 μM nonradiolabeled proline (○). (E) Same as (D), but with 20 μM [^{35}S]cysteine instead of 20 μM [^{3}H]proline. Abbreviations for the amino acid residues are as follows: A, Ala; D, Asp; E, Glu; F, Phe; I, Ile; K, Lys; L, Leu; M, Met; S, Ser; W, Trp; and Y, Tyr.

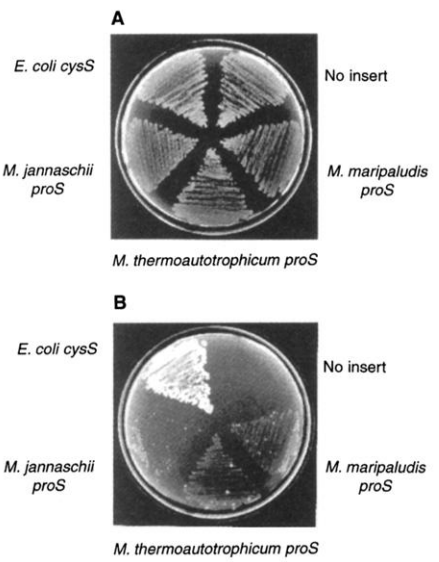

Fig. 2. Complementation of a temperature-sensitive *cysS* mutation in *E. coli* strain UQ818 with *proS* genes of *M. maripaludis*, *M. jannaschii*, *M. thermoautotrophicum*, and a *cysS* gene from *E. coli*. The experiment was performed as described (*11*). An additional plasmid (pTech-Mj-$tRNA^{Cys}$) containing the *M. jannaschii* $tRNA^{Cys}$ gene was necessary in strain UQ818 (see text). The plates were incubated for 4 days at the permissive temperature (30°C) (**A**) or at the nonpermissive temperature (42°C) (**B**).

sible explanation is that ProRS displays a broad range of amino acid specificity under the in vitro experimental conditions used, as recently described for *E. coli* lysyl–tRNA synthetase (*15*). However, the inability of any of the other 18 canonical amino acids to inhibit aminoacylation by ProRS (Fig. 4, A and B) indicates that binding is specific for Cys and Pro. In addition, Cys (but not Pro) activation was found to require the presence of tRNA (Fig. 4C), indicating that in vivo there are effectively two separate entities, a free ProRS that recognizes proline and a ProRS:$tRNA^{Cys}$ complex that recognizes cysteine.

The finding that *M. jannaschii* ProRS also functions as a CysRS is unexpected. Normally, individual aminoacyl-tRNAs are synthesized by a particular AARS specific for the appropriate amino acid and tRNA, with errors in substrate recognition being corrected by proofreading (*16*). The only known exceptions are Asn-tRNA, Gln-tRNA, and Sec-tRNA, which can be synthesized by reaction schemes dependent on the initial recognition of apparently noncognate tRNAs by AARSs, although amino acid recognition by the appropriate enzyme remains specific (*3*). The CysRS activity of *M. jannaschii* ProRS differs in that it is dependent on the enzyme using both Cys and $tRNA^{Cys}$ as cognate substrates.

The amino acid sequences of the *M. jannaschii* and *M. thermoautotrophicum* ProRSs show a high degree of similarity to the sequences of other ProRS proteins (*17*), indicating that any differences associated with their CysRS function cannot be detected by phylogenetic methods alone (*18*). Furthermore, CysRS activity may exist in ProRS enzymes from organisms with a conventional *cysS* gene, in which case the *M. jannaschii* and *M. thermoautotrophicum* ProRSs would not be expected to be distinctive in amino acid sequence. This is supported by the observation that *Methanococcus maripaludis* contains both a ProRS with CysRS activity (Fig. 2) and a CysRS (*1*). It raises the question of whether the CysRS synthetic activity is an ancestral feature or has been more recently acquired by ProRS to compensate for the loss of a conventional *cysS* gene. The detection of vestigial thiol-binding sites in other class II aminoacyl–tRNA synthetases (*19*) suggests that Cys-tRNA synthetic activity could have evolved in such enzymes. A sampling of other ProRS proteins to delineate the distribution of CysRS activity is now needed to address the evolutionary timing of such an event and how it might relate to the distribution of *cysS* genes.

The existence of AARSs able to catalyze the synthesis of more than one aminoacyl-tRNA is assumed to have been an important step in the evolution of these enzymes (*20*). This is at odds with the narrow substrate ranges seen in contemporary AARSs, a characteristic critical for their role in translation. However, the ability of ProRS to synthesize two aminoacyl-tRNAs suggests that the AARSs could have evolved via ancestors characterized by wide substrate specificities. The fact that most organisms now contain separate AARSs for the synthesis of each aminoacyl-tRNA, rather than a limited number of enzymes with multiple activities, suggests that functional isolation of these pathways offers a selective advantage. Such an advantage may be related to the known ability of individual aminoacyl-tRNA and AARS levels to fine tune the expression of AARS-encoding genes, thus providing a means to more precisely regulate individual components of the translational machinery (*21*).

Numerous schemes have been proposed for the evolution of translation (*22*), many of which suggest that early protein synthesis was a relatively unspecific process giving rise to mixed populations of polypeptides [e.g., (*23*)]. Within such schemes, amino acid activation is assumed to have been achieved by ancestral AARS-like enzymes able to recognize a broad range of amino acids (*24*). The evolution of the extant AARS proteins from such precursors would require intermediates with multiple substrate specificities, an activity now shown to exist also in a contemporary AARS, *M. jannaschii* prolyl–tRNA synthetase.

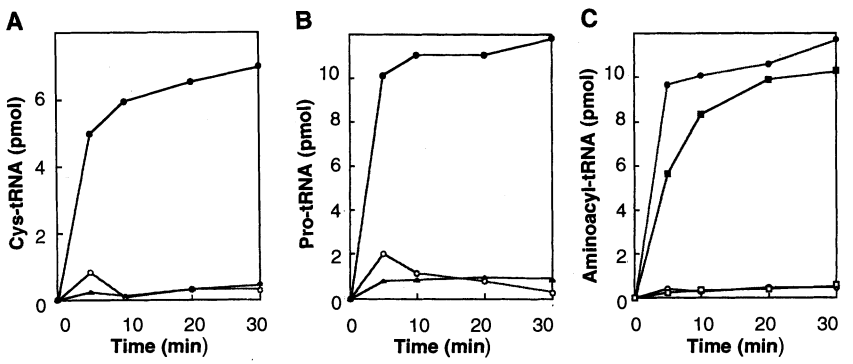

Fig. 3. Aminoacylation of *M. jannaschii* tRNA by purified *M. jannaschii* His_6-ProRS. Aminoacylation reactions (20-μl samples) were performed as described in the presence of the following amino acids: (**A**) 20 μM [^{35}S]cysteine (●); 20 μM [^{35}S]cysteine and 800 μM nonradiolabeled cysteine (▲) and 20 μM [^{35}S]cysteine and 800 μM nonradiolabeled proline (○). (**B**) Same as (A), but with 20 μM [^{3}H]proline. (**C**) Inactivation of the formation of Cys-$tRNA^{Cys}$ (●) and Pro-$tRNA^{Pro}$ (■) by 800 μM thiaproline (open squares and open circles, respectively).

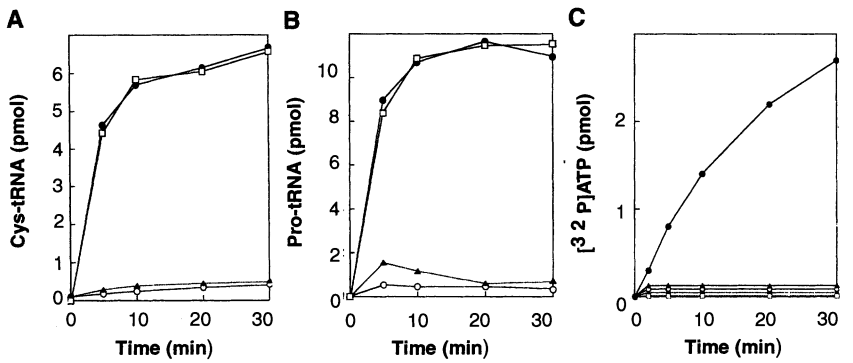

Fig. 4. Specific activation of Cys and Pro by *M. jannaschii* His_6-ProRS. Reactions were performed as described (**A**) in the presence of 20 μM [^{35}S]cysteine (●), 20 μM [^{35}S]cysteine and the 18 nonradiolabeled amino acids (800 μM), with the exception of cysteine and proline (□), and 20 μM [^{35}S]cysteine and 800 μM nonradiolabeled cysteine (▲) or proline (○). (**B**) The same as in (A) but with 20 μM [^{3}H]proline instead of 20 μM [^{35}S]cysteine. (**C**) Cysteine-dependent pyrophosphate exchange. Activation of cysteine by *M. jannaschii* ProRS was observed only in the presence of total *M. jannaschii* tRNA (1 μg/μl) (●). In the conditions used for the reaction {2 mM cysteine, 1 mM [^{32}P]PP_i (NEN DuPont, 4.6 Ci/mmol)}, no amino acid activation was observed in the presence of in vitro–transcribed $tRNA^{Pro}$ (▼), in vitro–transcribed $tRNA^{Cys}$ (○), with fractionated *M. jannaschii* tRNA lacking $tRNA^{Cys}$ (▲), or in the presence of the enzyme alone (□).

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7. Frozen *M. jannaschii* cells (200 g) were resuspended in two volumes of buffer A [50 mM tris-HCl (pH 8), 10 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 2 mM benzamidine, and 10% glycerol], sonicated, and centrifuged at 120,000*g* for 1.5 hours. The sample was dialyzed overnight against the same buffer and loaded on a DE52 DEAE-cellulose column (Whatman). A linear gradient of KCl (0 to 500 mM) was applied and the active fractions were pooled and dialyzed against 20 mM phosphate buffer (pH 6.8) containing 10 mM 2-mercaptoethanol and 10% glycerol. After dialysis the sample was applied to a P11 phosphocellulose column (Whatman), and active fractions were eluted with a linear gradient of 20 to 500 mM phosphate buffer. After dialysis in phosphate buffer the active fractions were applied to a Bio-Gel HT hydroxyapatite column (Bio-Rad) that was eluted with a linear gradient of 0.02 to 1 M phosphate buffer (pH 6.8). Active fractions were concentrated with solid polyethyleneglycol 20000 in dialysis bags, dialyzed against buffer A, and fractionated again by anion-exchange chromatography with a Uno-Q column (Bio-Rad). Active samples were then loaded on a gel filtration column (Superdex 200, Pharmacia), and fractions from this separation with CysRS activity were then loaded on a tRNA affinity column (Sepharose 4B–CNBr activated, Pharmacia) prepared according to the manufacturer's instructions, with 100 mg of total *E. coli* tRNA (Boehringer Mannheim) as coupling ligand. Samples (20 μl) from the most active fractions were then analyzed on tris-glycine native gels (10 to 20% polyacrylamide, Bio-Rad). After visualization of proteins either by Coomassie Blue or silver staining, samples were excised from the gels, eluted overnight in reaction buffer B [50 mM Hepes (pH 7), 50 mM KCl, 15 mM $MgCl_2$, 5 mM dithiothreitol (DTT)] containing 1 mM benzamidine and 10% glycerol, and tested for CysRS activity. Cys-tRNA and Pro-tRNA synthesis was assayed at 70°C in reaction buffer B in the presence of total *M. jannaschii* tRNA (1 mg/ml, prepared by standard methods) and 20 μM radioactive amino acid {[^{35}S]cysteine (1075 Ci/mmol; NEN DuPont) or [^{14}C]cysteine (303 mCi/mmol, NEN DuPont; reduced by DTT)]} or proline (103 Ci/mmo, Amersham). Samples taken at various time points were spotted on Whatman 3MM filter disks, presoaked in 10% trichloroacetic acid. The disks were washed and radioactivity was measured by liquid scintillation counting.
8. The *proS* gene was cloned from *M. jannaschii* genomic DNA by polymerase chain reaction (PCR) with the primers G**CATATG**TTGGAATTTTCAGAATGGTATTCAGATATA and **GGATCC**TTAGTAGGTTTTAGCTATTGCTATATATTTATTAC containing Nde I and Bam HI restriction sites, respectively (indicated in bold). For expression in *E. coli*, *M. jannaschii proS* was subcloned into pET 15b (Invitrogen) and used to transform the strain BL21 (DE3). The derived strain was then used for the production of His_6-ProRS, which was subsequently purified by nickel-affinity chromatography (Qiagen) followed by cation exchange chromatography with a Mono-S column (Pharmacia). After these purification steps, the His_6-ProRS was judged to be at least 99% pure by Coomassie Blue staining after SDS–polyacrylamide gel electrophoresis (PAGE).
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12. Total *M. jannaschii* tRNA (20 mg) was fractionated by reversed-phase chromatography with an RPC-5 column (70 cm by 1 cm) pre-equilibrated with buffer C [10 mM magnesium acetate, 10 mM sodium acetate, 1 mM EDTA (pH 4.5), and 450 mM NaCl] and developed in a gradient of 0.45 to 1.2 M NaCl in buffer C [R. L. Pearson, J. F. Weiss, A. Kelmers, *Biochim. Biophys. Acta* **228**, 770 (1971)]. Fractions were assayed for charging with both Pro and Cys by His_6-ProRS as described (*7*).
13. *Escherichia coli cysS* and *proS* genes from *M. thermoautotrophicum* and *M. jannaschii* were cloned into plasmid pCBS1 (*19*) to yield pCBS-Ec-cysS, pCBS-Mt-proS, and pCBS-Mj-proS. After obtaining some partial sequence from the University of Washington Genome Center (http://kandinsky.genome.washington.edu/Maripaludis/html/top.html), the *M. maripaludis proS* gene was cloned from genomic DNA by PCR. The complete gene was sequenced (GenBank accession number AF163997) and cloned into pCBS1 to generate pCBS-Mm-proS. The *E. coli* strain UQ818 [*cysS*^{ts}; K. Bohman and L. A. Isaksson, *Mol. Gen. Genet.* **176**, 53 (1979)] was transformed at 30°C with these plasmids and the resulting transformants tested for growth on Luria-Bertani agar supplemented with ampicillin (100 mg/liter), chloramphenicol (34 mg/liter), and Cys (0.5 mM) at 30° and 42°C.
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25. We thank C. A. Hutchison III for sharing unpublished results, and A. Ambrogelly, C. Becker, G. Raczniak, and D. Tumbula for assistance and advice. H.D.B. is a European Molecular Biology Organization postdoctoral fellow. M.I. is supported by an Investigator Fellowship from the Alfred Benzon Foundation and D.S. by grants from the National Institute of General Medical Sciences, NASA, and the Department of Energy.

25 October 1999; accepted 16 December 1999

A H^+-Gated Urea Channel: The Link Between *Helicobacter pylori* Urease and Gastric Colonization

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Acidic media trigger cytoplasmic urease activity of the unique human gastric pathogen *Helicobacter pylori*. Deletion of *ureI* prevents this activation of cytoplasmic urease that is essential for bacterial acid resistance. UreI is an inner membrane protein with six transmembrane segments as shown by in vitro transcription/translation and membrane separation. Expression of UreI in *Xenopus* oocytes results in acid-stimulated urea uptake, with a pH profile similar to activation of cytoplasmic urease. Mutation of periplasmic histidine 123 abolishes stimulation. UreI-mediated transport is urea specific, passive, nonsaturable, nonelectrogenic, and temperature independent. UreI functions as a H^+-gated urea channel regulating cytoplasmic urease that is essential for gastric survival and colonization.

The Gram-negative pathogen *H. pylori* is unique in its ability to colonize the human stomach. *H. pylori* infection is acquired during childhood, persists lifelong if not eradicated, and is associated with chronic gastritis and an increased risk of peptic ulcer disease and gastric cancer (*1*). An acid-tolerant neutralophile, *H. pylori* expresses a neutral pH–optimum urease to maintain proton motive force (PMF) and to enable gastric colonization (*2*).

Most urease is found in the bacterial cytoplasm, although up to 10% appears on the surface, owing to cell lysis during culture (*3*). Surface or free urease has a pH optimum between pH 7.5 and 8.0 but is irreversibly inactivated below pH 4.0 (*4*, *5*). The activity of cytoplasmic urease is low at neutral pH but increases 10- to 20-fold as the external pH falls between 6.5 and 5.5, and its activity remains high down to pH ~2.5 (*5*). Thus, cytoplasmic, not surface, urease is required for acid resistance. The unmodified urea permeability of the inner membrane is insufficient to supply enough urea to intrabacterial urease for urease activity to buffer the bacterial periplasm in the face of gastric acidity (the median diurnal acidity of the human stomach is pH 1.4). The data here show that *H. pylori* expresses a urea transport protein with unique acid-dependent properties that activates the rate of urea entry into the cytoplasm.

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