The expressions for the correlations are

$$C_{12} = -\frac{1}{2}H_{21}\eta^2_{1\,\mathrm{int}} - \frac{3}{8}H_{21}H^2_{10}\eta^2_{0\,\mathrm{int}} + \eta^2_G\left(1 - \frac{1}{2}H_{21} - \frac{1}{2}H_{10} - \frac{3}{8}H_{21}H^2_{10} + \frac{3}{4}H_{21}H_{10}\right) + \eta^2_N\left(\frac{1}{2} - \frac{3}{8}H_{21}\right)$$

$$C_{13} = \eta^2_G\left(1 - \frac{1}{2}H_{10}\right) + \frac{1}{2}\eta^2_N$$

$$C_{23} = \eta^2_G\left(1 - \frac{1}{2}H_{21} + \frac{1}{4}H_{21}H_{10}\right) + \eta^2_N\left(\frac{1}{2} - \frac{3}{8}H_{21}\right)$$

16. J. Paulsson, *Nature* **427**, 415 (2004).
17. M. A. Savageau, *Biochemical Systems Analysis* (Addison-Wesley, Reading, MA, 1976).
18. The expression for the transmitted noise in η_2 cannot be rewritten as the transmission of the total steady-state noise in gene 1 only (*6*, *15*).
19. J. Paulsson, M. Ehrenberg, *Q. Rev. Biophys.* **34**, 1 (2001).
20. L. H. Hartwell *et al.*, *Nature* **402** (suppl.), C47 (1999).
21. J. Hasty, D. McMillen, J. J. Collins, *Nature* **420**, 224 (2002).
22. D. T. Gillespie, *J. Phys. Chem.* **81**, 2340 (1977).
23. Duplicated measurements are averaged. The error bars reflect the standard deviation of run-to-run differences and the error within each measurement as determined by bootstrapping.

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RNA-Dependent Cysteine Biosynthesis in Archaea

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Several methanogenic archaea lack cysteinyl–transfer RNA (tRNA) synthetase (CysRS), the essential enzyme that provides Cys-tRNA^{Cys} for translation in most organisms. Partial purification of the corresponding activity from *Methanocaldococcus jannaschii* indicated that tRNA^{Cys} becomes acylated with *O*-phosphoserine (Sep) but not with cysteine. Further analyses identified a class II–type *O*-phosphoseryl-tRNA synthetase (SepRS) and Sep-tRNA:Cys-tRNA synthase (SepCysS). SepRS specifically forms Sep-tRNA^{Cys}, which is then converted to Cys-tRNA^{Cys} by SepCysS. Comparative genomic analyses suggest that this pathway, encoded in all organisms lacking CysRS, can also act as the sole route for cysteine biosynthesis. This was proven for *Methanococcus maripaludis*, where deletion of the SepRS-encoding gene resulted in cysteine auxotrophy. As the conversions of Sep-tRNA to Cys-tRNA or to selenocysteinyl-tRNA are chemically analogous, the catalytic activity of SepCysS provides a means by which both cysteine and selenocysteine may have originally been added to the genetic code.

The translation of cysteine codons in mRNA during protein synthesis requires cysteinyl-tRNA (Cys-tRNA^{Cys}). Cys-tRNA^{Cys} is normally synthesized from the amino acid cysteine and the corresponding tRNA isoacceptors (tRNA^{Cys}) in an adenosine triphosphate (ATP)–dependent reaction catalyzed by cysteinyl-tRNA synthetase (CysRS). Genes encoding CysRS, *cysS*, have been detected in hundreds of organisms encompassing all three living domains (*1*). The only exceptions are certain methanogenic archaea, the completed genome sequences of which encode no open reading frames (ORFs) with obvious homology to known *cysS* sequences (*1*). Because of the discovery that the genomes of a number of methanogenic archaea either lack *cysS* (*Methanocaldococcus jannaschii*, *Methanothermobacter thermautotrophicus*, and *Methanopyrus kandleri*) or can dispense with it (*Methanococcus maripaludis*), the formation of Cys-tRNA^{Cys} in these organisms has been a much studied and increasingly contentious topic (*2*, *3*). A noncognate aminoacyl-tRNA synthetase [aaRS (*4*–*6*)] and a previously unassigned ORF (*7*) were variously implicated in Cys-tRNA^{Cys} formation. Recent studies failed to provide conclusive support for either of these routes, leaving the mechanism of Cys-tRNA^{Cys} formation still in doubt (*2*).

Previous investigations of archaeal Cys-tRNA^{Cys} biosynthesis have been hampered by the significant levels of noncognate tRNA routinely cysteinylated and detected by conventional filter binding assays. This problem was circumvented with a more stringent assay of Cys-tRNA^{Cys} formation: gel-electrophoretic separation of uncharged tRNA from aminoacyl-tRNA (aa-tRNA) and subsequent detection of the tRNA moieties by sequence-specific probing (*8*). Given that *M. jannaschii* is a strict anaerobe, and considering that earlier aerobic purification erroneously identified prolyl-tRNA synthetase (*4*, *5*), we used anaerobic conditions for all procedures unless otherwise indicated. When these procedures were used to monitor acylation of total *M. maripaludis* tRNA by an undialyzed *M. jannaschii* cell-free extract (S-100), tRNA^{Cys} was charged with an amino acid that gave rise to the same mobility shift (*9*) exhibited by standard *M. maripaludis* Cys-tRNA^{Cys} generated by *M. maripaludis* CysRS (*1*) (Fig. 1A, lanes 7 and 8). Further optimization of the reaction at this stage showed that Zn^{2+} and ATP were also required for the successful formation of charged tRNA^{Cys}. When the S-

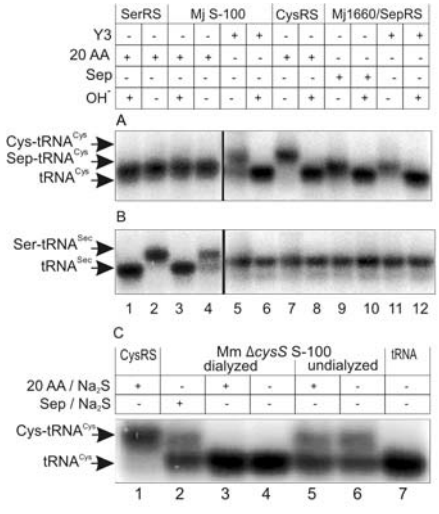

Fig. 1. Acid urea gel electrophoresis and Northern blot analysis of total *M. maripaludis* tRNA charged with *M. maripaludis* SerRS, dialyzed *M. jannaschii* S-100, *M. maripaludis* CysRS, and *M. jannaschii* SepRS in the presence of 20 amino acids (20 AA), phosphoserine, or a *M. jannaschii* S-100 cell-free extract filtrate (Y3). Half of each tRNA sample was deacylated by mild alkaline hydrolysis ($^-$OH). The blots were probed with ^{32}P-labeled oligonucleotides complementary to *M. maripaludis* tRNA^{Cys} (**A**) and *M. maripaludis* tRNA^{Sec} (**B**). Total *M. maripaludis* tRNA charged with dialyzed or undialyzed *M. maripaludis* Δ*cysS* S-100 cell-free extract (*20*) in the presence of 20 amino acids and Na_2S, or Sep and Na_2S (**C**). The blot was analyzed with ^{32}P-labeled oligonucleotides complementary to *M. maripaludis* tRNA^{Cys}.

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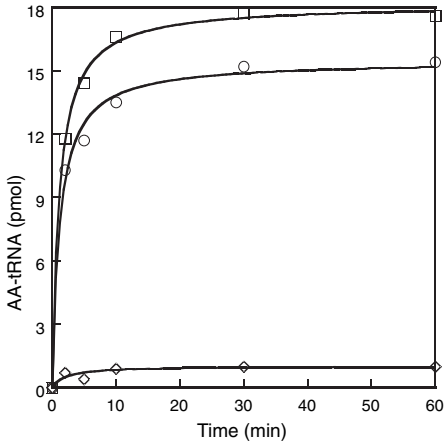

Fig. 2. Amino acid specificity of *M. jannaschii* SepRS. Aminoacylation by the recombinant *M. jannaschii* SepRS was tested with the filter binding assay (as described in SOM). *M. jannaschii* unfractionated tRNA charged with Sep (squares), total *M. maripaludis* tRNA and *M. jannaschii* SepRS incubated with Sep (circles), or with a 20–amino acid mixture (diamonds).

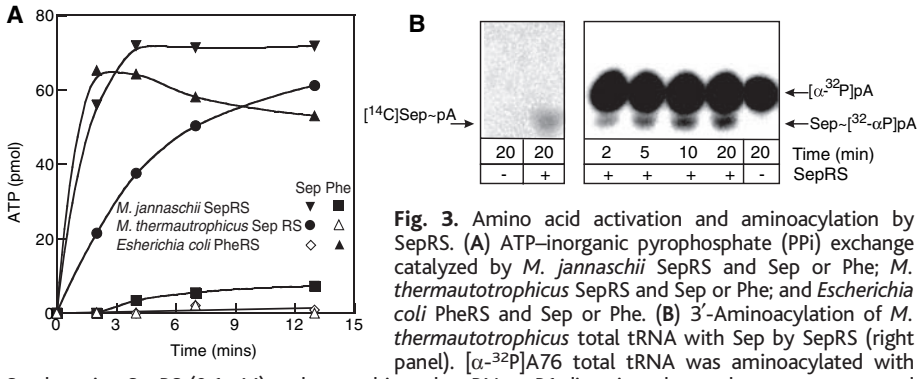

Fig. 3. Amino acid activation and aminoacylation by SepRS. (**A**) ATP–inorganic pyrophosphate (PPi) exchange catalyzed by *M. jannaschii* SepRS and Sep or Phe; *M. thermautotrophicus* SepRS and Sep or Phe; and *Escherichia coli* PheRS and Sep or Phe. (**B**) 3′-Aminoacylation of *M. thermautotrophicus* total tRNA with Sep by SepRS (right panel). [α-^{32}P]A76 total tRNA was aminoacylated with Sep by using SepRS (0.1 μM) and was subjected to RNase P1 digestion; the products were separated by thin-layer chromatography (TLC) and then visualized by phosphor imaging. Quantification of Sep~[α-^{32}P] indicated that about 3% of the total tRNA can be aminoacylated with Sep. The position of migration of Sep~pA was independently confirmed using [^{14}C]Sep (left).

100 fraction was dialyzed, all enzyme activity was lost and could not be recovered by addition of a mixture of the 20 canonical amino acids (Fig. 1A, lanes 3 and 4). These data established that tRNA^{Cys} charging took place in the S-100 extract but not as a result of direct acylation of cysteine to tRNA^{Cys} and not by a Ser-tRNA^{Cys}–dependent conversion mechanism (*10*). In contrast, the dialyzed S-100 extract supplemented with 20 amino acids formed Ser-tRNA^{Sec} (Fig. 1B, lanes 3 and 4), as did *M. maripaludis* seryl-tRNA synthetase (Fig. 1B, lanes 1 and 2). This result is consistent with a tRNA-dependent transformation of serine to selenocysteine (Sec) as seen in bacteria (*11*). On the basis of these results, we reasoned that the Cys-tRNA^{Cys}–forming activity consisted of one or more enzymes and some low-molecular-weight substrates that together participated in a tRNA-dependent amino acid biosynthesis pathway.

To identify the components of the Cys-tRNA^{Cys} biosynthetic pathway, the *M. jannaschii* S-100 extract was separated into two fractions: a low-molecular-mass "filtrate" (Y3) derived by a membrane filtration step (cutoff at 3 kD) and a protein fraction. Addition of Y3 to the dialyzed *M. jannaschii* S-100 restored activity (Fig. 1A, lanes 5 and 6). Both the protein and the filtrate fractions were purified individually by various chromatographic procedures; the activity was assayed by reconstitution of purified fractions from both sources [see supporting online material (SOM)]. Chromatographic analysis of the filtrate initially implicated *O*-phosphoserine (Sep) as one of the components in Y3 necessary for formation of Cys-tRNA^{Cys}. This was subsequently verified using the L-enantiomer of this amino acid (see SOM for details). Significant advancement in the protein purification strategy was derived from a proteomic analysis of various partially purified column chromatographic fractions (*12*). Repeated liquid chromatography (LC)–mass spectrometry (MS) analysis in the pattern LC-LC-MS-MS identified 20 proteins in the most active fractions, of which 13 were excluded because of their predicted functions or inconsistent phylogenetic distribution. Of the remaining seven proteins, two of the most abundant (Mj1660 and Mj1678) were consistently observed in genomes lacking *cysS*. Although Mj1660 is a paralog of the α subunit of phenylalanyl-tRNA synthetase (PheRS), it is inactive in Phe-tRNA formation (*13*). Mj1678 has been annotated as a putative pyridoxal phosphate–dependent enzyme. On the basis of its high homology to known class II aaRSs, we speculated that Cys-tRNA^{Cys} biosynthesis could be initiated by Mj1660 with Sep as one of the substrates. His$_6$-Mj1660, produced and purified heterologously from *Escherichia coli*, was found to stably attach Sep to tRNA^{Cys} in an efficient aerobic ATP-dependent reaction, which suggested that it could function as an aaRS (Fig. 1A, compare lanes 9 and 10 with lanes 11 and 12, and Fig. 2). However, tRNA^{Sec} was not a substrate for Mj1660 (Fig. 1B, lanes 9 to 12). Specificity for Sep was further supported by the observation that His$_6$-Mj1660 and its *M. thermautotrophicus* counterpart His$_6$-Mth1501 both catalyzed Sep-dependent and tRNA-independent ATP-[^{32}P]pyrophosphate exchange, a reaction characteristic of aaRSs (Fig. 3A) (*14*). No pyrophosphate exchange activity was detected with either His$_6$-Mj1660 or His$_6$-Mth1501 when Sep was replaced by phenylalanine. Sep was unable to stimulate ATP-[^{32}P]pyrophosphate exchange by *E. coli* PheRS, which indicated that it is a specific substrate for Mj1660-type proteins. Analysis of the position of aminoacylation by using *M. thermautotrophicus* total tRNA labeled with [^{32}P] in the terminal pA residue showed that Sep was attached to the 3′ terminus, the normal site for aminoacylation by aaRSs (Fig. 3B). A similar conclusion came from the protection against periodate oxidation of charged tRNA^{Cys} (*9*). In light of these various enzymatic activities and their specificities, we propose that Mj1660-type proteins are classified as aaRSs and are consequently renamed *O*-phosphoseryl-tRNA synthetase (SepRS, encoded by *sepS*). Like pyrrolysyl-tRNA synthetase (PylRS), which acylates a suppressor tRNA with pyrrolysine, SepRS belongs to an emerging set of synthetases that use modified amino acids but not their canonical counterparts (*15*, *16*). Amino acid sequence similarities indicate that both PylRS and SepRS are subclass IIc aaRSs most closely related to the canonical PheRS. The relative scarcity and narrow phylogenetic distributions of both PylRS and SepRS make it unclear whether these enzymes recently diverged from PheRS or, instead, coevolved with PheRS from a common ancestor.

Attachment of Sep to tRNA^{Cys} by SepRS is a chemically plausible first step in Cys-tRNA^{Cys} synthesis, as Sep-tRNA could feasibly be converted to Cys-tRNA in the presence of a synthase and the appropriate sulfur donor. Analogous pretranslational amino acid modifications have been described for the synthesis of asparaginyl-, formylmethionyl-, glutaminyl-, and selenocysteinyl-tRNAs (*17*). To investigate whether such a transformation accounts for Cys-tRNA^{Cys} formation, preformed Sep-tRNA^{Cys} was incubated with a dialyzed *M. jannaschii* S-100 extract in the presence of Na_2S. Electrophoretic analysis of the resulting aa-tRNA indicated formation of a product whose mobility was consistent with Cys-tRNA^{Cys} (Fig. 4A). On the basis of the above proteomic analysis, we postulated that Mj1678 encoded the enzymatic component responsible for converting Sep-tRNA^{Cys} to Cys-tRNA^{Cys}. His$_6$-Mj1678, produced heterologously in *E. coli*, was found to efficiently convert preformed Sep-tRNA^{Cys} into Cys-tRNA^{Cys} in an anaerobic reaction in the presence of pyridoxal phosphate (PLP) and Na_2S (Fig. 4B,

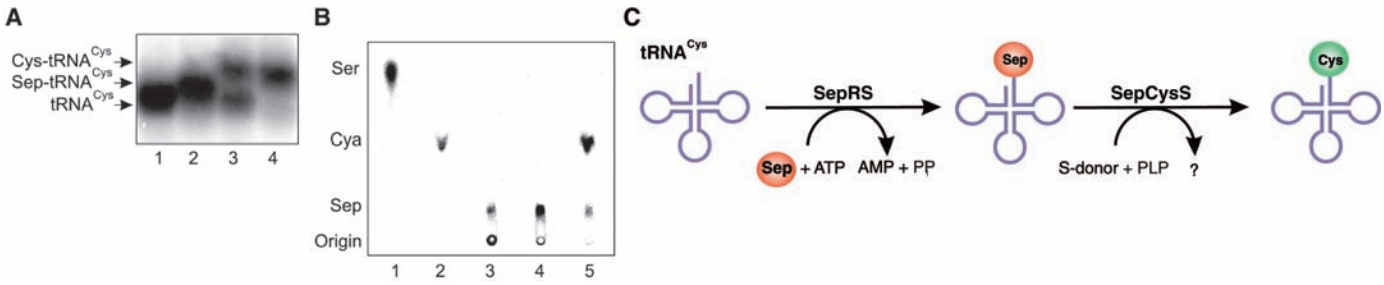

Fig. 4. Conversion of in vitro synthesized Sep-tRNA^{Cys} to Cys-tRNA^{Cys}. (**A**) Aminoacylation of tRNA^{Cys} monitored by acid urea gel electrophoresis and Northern blotting. Lane 1, total *M. maripaludis* tRNA; lane 2, tRNA^{Cys} charged with Sep by recombinant *M. jannaschii* SepRS; lane 3, Sep-tRNA^{Cys} incubated with dialyzed *M. jannaschii* cell-free S-100 extract in the presence of dithiothreitol (DTT) and Na_2S; lane 4, tRNA^{Cys} charged with cysteine by *M. maripaludis* CysRS. (**B**) Phosphorimages of TLC separation of [^{14}C]Sep and [^{14}C]Cys recovered from the aa-tRNAs of the SepCysS activity assay (see SOM). Cysteine was analyzed in its oxidized form as cysteic acid (Cya). Lane 1, Ser marker; lane 2, cysteine from Cys-tRNA^{Cys} generated with *M. maripaludis* CysRS; lane 3, Sep from Sep-tRNA^{Cys} made with *M. jannaschii* SepRS; lane 4, Sep-tRNA^{Cys} incubated with *E. coli* S-100 cell-free extract in the presence of DTT and Na_2S (see SepCysS assay in SOM); lane 5, Sep-tRNA^{Cys} converted to Cys-tRNA^{Cys} with recombinant MJ1678 protein in the presence of DTT and Na_2S (see SepCysS assay in SOM). (**C**) Scheme of Cys-tRNA^{Cys} formation in methanogenic archaea.

lane 5). The natural sulfur donor of the reaction remains uncharacterized. On the basis of the conversion activity, we suggest that Mj1678 is a Sep-tRNA:Cys-tRNA synthase (SepCysS; encoded by *pscS*). SepRS and SepCysS, both of which are encoded in all archaea lacking *cysS*, together provide a facile two-step pathway for the synthesis of Cys-tRNA^{Cys} by means of Sep-tRNA^{Cys} (Fig. 4C). This route is consistent with the earlier observation that Sep is a precursor of cysteine in *M. jannaschii* (*18*). As in other organisms (*19*), the proposed route of Sep formation involves D-3-phosphoglycerate dehydrogenase (MJ1018) and an as yet unidentified phosphoserine aminotransferase.

From available genome sequences, the organismal distributions of SepRS and SepCysS are apparently coupled. To date, *sepS* and *pscS* have only been detected in the genomes of the methanogenic archaea *M. jannaschii*, *M. maripaludis*, *M. thermautotrophicus*, *M. kandleri*, *Methanococcoides burtonii*, the *Methanosarcinaceae*, and in *Archaeoglobus fulgidus*. Although some of these organisms lack *cysS*, others, such as *M. maripaludis*, also encode a canonical CysRS and thus contain two potentially functional pathways for Cys-tRNA^{Cys} synthesis (*20*). Comparable redundancy is seen for Asn-tRNA^{Asn} synthesis in many bacteria, where the tRNA-dependent route is the sole pathway for asparagine biosynthesis (*21*). Present knowledge of the genes required for archaeal amino acid biosynthesis suggests that the SepRS/SepCysS pathway may provide the only means for de novo production of cysteine in a number of organisms (e.g., *M. jannaschii, M. maripaludis*), whereas other organisms (e.g., *Methanosarcinaceae*) have both tRNA-dependent and tRNA-independent routes to cysteine. In contrast, most nonmethanogenic archaea with known genomes (e.g., *Aeropyrum, Sulfolobus, Pyrococcus, Pyrobaculum, Thermoplasma, Picrophilus*, *Halobacteria*) encode *O*-acetylserine sulfhydrylase (*22*) or cysteine synthase, which suggests that cysteine biosynthesis is tRNA-independent in these organisms.

To investigate whether the SepRS/SepCysS pathway can act as the sole route for cysteine biosynthesis we used *M. maripaludis*, which has a facile genetic system. This organism has both a dispensable CysRS (*20*) and the *sepS* and *pscS* genes but no known pathway for de novo biosynthesis of free cysteine. Biochemical evidence of a functional SepRS/SepCysS pathway in *M. maripaludis* extracts is presented in Fig. 1C. In dialyzed extracts of a *cysS* deletion mutant, Cys-tRNA^{Cys} biosynthesis is dependent on the addition of Sep and Na_2S (Fig. 1C, lane 2). To test if the SepRS/SepCysS pathway is necessary for cysteine biosynthesis, the *sepS* gene was deleted from the chromosome of the wild type of *M. maripaludis*. The resulting *ΔsepS* strain was a cysteine auxotroph (Fig. 5). Although it grew at a rate comparable to that of wild type on complete medium, it was unable to grow in the absence of exogenous cysteine. These findings indicate that under certain conditions the SepRS/SepCysS pathway can provide the sole source of cysteine for the cell via Cys-tRNA^{Cys}. Reliance on such a route clearly satisfies the requirements for cysteine during protein synthesis, but how cysteine is made available for other metabolic processes is less clear. One possibility is that hydrolysis of Cys-tRNA^{Cys} directly provides free cysteine, as previously proposed for free Asn synthesis via Asn-tRNA^{Asn} in certain bacteria (*21*). In addition, protein turnover in the cell would be expected to contribute more significantly to the cellular cysteine pool when CysRS is absent, as the free amino acid is not itself a substrate for protein synthesis in such cases. Finally, most of the organisms harboring the SepRS/SepCysS pathway are methanogens, which, even in the absence of glutathione, may not require a large pool of free cysteine for redox buffering in the cytoplasm. Methanogens contain high levels of the essential coenzyme 2-mercaptoethanesulfonate (*23*), which may

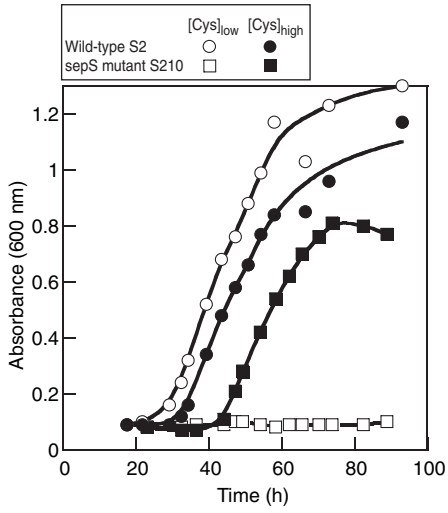

Fig. 5. Growth response of the *ΔsepS* mutant and wild-type *M. maripaludis* strain S2 to the presence and absence of cysteine in mineral media containing acetate. About 2 × 10^3 cells were inoculated into prewarmed McAV medium containing 3 mM coenzyme M for a final cysteine concentration of <0.16 μM ([Cys]$_{low}$) or into the same medium with 3 mM cysteine ([Cys]$_{high}$). Wild-type S2 (circles), and *sepS* mutant S210 (squares).

fulfill the redox buffering function of free cysteine. For thermophilic organisms, replacement of the heat-labile cysteine with the thermostable 2-mercaptoethanesulfonate may be an additional benefit.

The discovery of the SepRS/SepCysS pathway raises the question as to whether this mechanism predates direct charging by CysRS and tRNA-independent cysteine biosynthesis. Similar scenarios have been suggested for Asn-tRNA and Gln-tRNA biosynthesis, where the tRNA-dependent pathways have been proposed as the original routes for synthesis of both the aa-tRNAs and the corresponding amino acids (*24–26*). If SepRS/SepCysS was indeed the ancestral pathway for cysteine synthesis via Cys-tRNA, a lack of alternative cysteine bio-

synthetic capacity may explain why certain organisms have retained this route. This would be consistent with earlier proposals that CysRS (*27*, *28*) and cysteine itself (*22*, *29*, *30*) were the last—or very late—canonical additions to the genetic code. The recent demonstration in mammalian cells (*31*) of the Ser-tRNA^{Sec} to Sep-tRNA^{Sec} conversion by a special kinase [present also in archaea (*31*)] implicates the Sep moiety as an intermediate in Sec synthesis. As the conversions of Sep-tRNA to Cys-tRNA or Sec-tRNA are chemically analogous (using suitable sulfur or selenium donors, respectively), the addition of selenocysteine to the genetic code may have been patterned on an accepted route for cysteine formation, the SepRS/SepCysS pathway.

Note added in proof: A recently published bioinformatics analysis has suggested that Mj1660 is a class II CysRS (*32*).

References and Notes

1. T. Li *et al.*, *FEBS Lett.* **462**, 302 (1999).
2. B. Ruan *et al.*, *J. Bacteriol.* **186**, 8 (2004).
3. A. Ambrogelly *et al.*, *Cell. Mol. Life Sci.* **61**, 2437 (2004).
4. C. Stathopoulos *et al.*, *Science* **287**, 479 (2000).
5. R. S. Lipman, K. R. Sowers, Y. M. Hou, *Biochemistry* **39**, 7792 (2000).
6. C. M. Zhang, Y. M. Hou, *RNA Biol.* **1**, 35 (2004).
7. C. Fabrega *et al.*, *Nature* **411**, 110 (2001).
8. U. Varshney, C. P. Lee, U. L. RajBhandary, *J. Biol. Chem.* **266**, 24712 (1991).
9. A. Sauerwald, unpublished observations.
10. H. S. Kim, U. C. Vothknecht, R. Hedderich, I. Celic, D. Söll, *J. Bacteriol.* **180**, 6446 (1998).
11. A. Böck, M. Thanbichler, M. Rother, A. Resch, in *Aminoacyl-tRNA Synthetases*, M. Ibba, C. S. Francklyn, S. Cusack, Eds. (Landes Bioscience, 2004), pp. 320–327.
12. C. S. Giometti *et al.*, *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **782**, 227 (2002).
13. R. Das, U. C. Vothknecht, *Biochimie* **81**, 1037 (1999).
14. R. Calendar, P. Berg, *Prog. Nucl. Acid Res. Mol. Biol.* **1**, 375 (1966).
15. C. Polycarpo *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12450 (2004).
16. S. K. Blight *et al.*, *Nature* **431**, 333 (2004).
17. M. Ibba, H. D. Becker, C. Stathopoulos, D. L. Tumbula, D. Söll, *Trends Biochem. Sci.* **25**, 311 (2000).
18. R. H. White, *Biochim. Biophys. Acta* **1624**, 46 (2003).
19. M.-J. Basurko *et al.*, *IUBMB Life* **48**, 525 (1999).
20. C. Stathopoulos *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 14292 (2001).
21. B. Min, J. T. Pelaschier, D. E. Graham, D. Tumbula-Hansen, D. Söll, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 2678 (2002).
22. K. Mino, K. Ishikawa, *FEBS Lett.* **551**, 133 (2003).
23. W. E. Balch, R. S. Wolfe, *J. Bacteriol.* **137**, 264 (1979).
24. J. T. Wong, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1909 (1975).
25. D. C. Jeffares, A. M. Poole, D. Penny, *J. Mol. Evol.* **46**, 18 (1998).
26. M. Di Giulio, *J. Mol. Evol.* **55**, 616 (2002).
27. J. Avalos, L. M. Corrochano, S. Brenner, *FEBS Lett.* **286**, 176 (1991).
28. K. Farahi, G. D. Pusch, R. Overbeek, W. B. Whitman, *J. Mol. Evol.* **58**, 615 (2004).
29. M. Di Giulio, M. Medugno, *J. Mol. Evol.* **49**, 1 (1999).
30. D. J. Brooks, J. R. Fresco, *Mol. Cell. Proteomics* **1**, 125 (2002).
31. B. A. Carlson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 12848 (2004).
32. A. Sethi, P. O'Donoghue, Z. Luthey-Schulten, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 4045 (2005).

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Structural Insights into the Activity of Enhancer-Binding Proteins

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Activators of bacterial σ^{54}–RNA polymerase holoenzyme are mechanochemical proteins that use adenosine triphosphate (ATP) hydrolysis to activate transcription. We have determined by cryogenic electron microscopy (cryo-EM) a 20 angstrom resolution structure of an activator, phage shock protein F [PspF$_{(1\text{-}275)}$], which is bound to an ATP transition state analog in complex with its basal factor, σ^{54}. By fitting the crystal structure of PspF$_{(1\text{-}275)}$ at 1.75 angstroms into the EM map, we identified two loops involved in binding σ^{54}. Comparing enhancer-binding structures in different nucleotide states and mutational analysis led us to propose nucleotide-dependent conformational changes that free the loops for association with σ^{54}.

Gene expression is regulated at the level of RNA polymerase (RNAP) activity. Bacterial RNAP containing the σ^{54} factor requires specialized activator proteins, referred to as bacterial enhancer-binding proteins (EBPs), that interact with the basal transcription complex from remote DNA sites by DNA looping (*1*–*4*). EBPs bind upstream activating sequences via their C-terminal DNA binding domains and form higher order oligomers that use adenosine triphosphate (ATP) hydrolysis to activate transcription (*5*, *6*). The central σ^{54}-RNAP–interacting domain of EBPs is responsible for adenosine triphosphatase (ATPase) activity and transcription activation (*7*–*9*) and belongs to the larger AAA+ (ATPase associated with various cellular activities) family of proteins (*10*–*12*). Well-studied EBPs include phage shock protein F (PspF), nitrogen-fixation protein A (NifA), nitrogen-regulation protein C (NtrC), and C_4-dicarboxylic acid transport protein D (DctD) (*1*–*3*, *7*, *13*).

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PspF from *Escherichia coli* forms a stable oligomeric complex with σ^{54} at the point of ATP hydrolysis (*14*). PspF-ADP.AlF$_x$ (a complex of adenosine diphosphate and aluminum fluoride, where x is the number of fluorine atoms equal to 3 or 4) alters the interaction between σ^{54} and promoter DNA similarly to PspF hydrolyzing ATP (*15*) and was thus deemed a functional hydrolysis intermediate. Activator nucleotide hydrolysis–dependent events couple the chemical energy of hydrolysis to transcriptional activation. The highly conserved and EBP-specific GAFTGA amino acid motif (fig. S1) (*16*) is a crucial mechanical determinant for the successful transfer of energy from ATP hydrolysis in EBPs to the RNAP holoenzyme via the small N-terminal EBP-interacting domain of σ^{54} (called region I, ~56 residues and sufficient for PspF interaction) (*1*, *14*, *17*–*19*).

The lack of structural information has hindered progress toward understanding the basis of this energy transfer process required for transcriptional activation. We now present a structure-function analysis of one such system using the following: (i) a cryo-EM reconstruction of PspF's AAA+ domain [residues 1 to 275, PspF$_{(1\text{-}275)}$] in complex with σ^{54} at the point of ATP hydrolysis (mimicked by in situ–formed ADP.AlF$_x$), (ii) the crystal structure of nucleotide-free (apo) PspF$_{(1\text{-}275)}$ at 1.75 Å resolution, and (iii) mutational analysis.

Nanoelectrospray mass spectroscopy of a PspF$_{(1\text{-}275)}$–σ^{54} complex with ADP.AlF$_x$ established that six monomers of PspF$_{(1\text{-}275)}$ are in complex with a monomeric σ^{54}, consistent with AAA+ proteins functioning as hexamers (*10*, *12*). The three-dimensional (3D) recon-

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