Published online 11 February 2008 *Nucleic Acids Research, 2008, Vol. 36, No. 6* ***1871–1880***
doi:10.1093/nar/gkn036

Divergence of selenocysteine tRNA recognition by archaeal and eukaryotic *O*-phosphoseryl-tRNA^{Sec} kinase

R. Lynn Sherrer[1], Joanne M. L. Ho[1] and Dieter Söll[1,2,*]

[1]Department of Molecular Biophysics and Biochemistry and [2]Department of Chemistry, Yale University, New Haven, CT 06520-8114, USA

Received December 18, 2007; Revised January 18, 2008; Accepted January 21, 2008

ABSTRACT

Selenocysteine (Sec) biosynthesis in archaea and eukaryotes requires three steps: serylation of tRNA^{Sec} by seryl-tRNA synthetase (SerRS), phosphorylation of Ser-tRNA^{Sec} by *O*-phosphoseryl-tRNA^{Sec} kinase (PSTK), and conversion of *O*-phosphoseryl-tRNA^{Sec} (Sep-tRNA^{Sec}) by Sep-tRNA:Sec-tRNA synthase (SepSecS) to Sec-tRNA^{Sec}. Although SerRS recognizes both tRNA^{Sec} and tRNA^{Ser} species, PSTK must discriminate Ser-tRNA^{Sec} from Ser-tRNA^{Ser}. Based on a comparison of the sequences and secondary structures of archaeal tRNA^{Sec} and tRNA^{Ser}, we introduced mutations into *Methanococcus maripaludis* tRNA^{Sec} to investigate how *Methanocaldococcus jannaschii* PSTK distinguishes tRNA^{Sec} from tRNA^{Ser}. Unlike eukaryotic PSTK, the archaeal enzyme was found to recognize the acceptor stem rather than the length and secondary structure of the D-stem. While the D-arm and T-loop provide minor identity elements, the acceptor stem base pairs G2-C71 and C3-G70 in tRNA^{Sec} were crucial for discrimination from tRNA^{Ser}. Furthermore, the A5-U68 base pair in tRNA^{Ser} has some antideterminant properties for PSTK. Transplantation of these identity elements into the tRNA$^{Ser}_{UGA}$ scaffold resulted in phosphorylation of the chimeric Ser-tRNA. The chimera was able to stimulate the ATPase activity of PSTK albeit at a lower level than tRNA^{Sec}, whereas tRNA^{Ser} did not. Additionally, the seryl moiety of Ser-tRNA^{Sec} is not required for enzyme recognition, as PSTK efficiently phosphorylated Thr-tRNA^{Sec}.

INTRODUCTION

While UGA is typically a stop codon, selenocysteine is co-translationally inserted into proteins in response to in-frame Sec-decoding UGA codons in a limited number of organisms from all three domains of life (1). Sec is formed by a tRNA-dependent transformation of Ser to Sec. In Sec-decoding organisms, tRNA^{Sec} is first aminoacylated with Ser by SerRS (2–4). Bacteria convert Ser to Sec in one step using selenocysteine synthase (SelA) in the presence of the selenium donor selenophosphate (1). Eukaryotes and archaea perform the Ser to Sec conversion using two enzymes: PSTK phosphorylates the serine moiety of Ser-tRNA^{Sec} to Sep-tRNA^{Sec} (5–8), and SepSecS catalyzes the Sep-tRNA^{Sec} to Sec-tRNA^{Sec} conversion (9,10).

About four decades ago the presence of a Sep-tRNA was discovered in rooster (11) and rat liver (12) and later in other eukaryotes and archaea (5,13–15). Also established early (14) was the Sep-tRNA synthesis requirement for two enzymes, SerRS and a 'phosphotransferase activity'. The latter enzyme was purified from bovine liver (7), the human counterpart was characterized with regard to tRNA recognition (8) and finally the protein responsible was identified (5) as PSTK. Detailed characterizations of PSTK activity were subsequently performed on the mouse (5) and *Methanocaldococcus jannaschii* enzyme (15). PSTK transfers the γ-phosphate from ATP to Ser-tRNA^{Sec} yielding Sep-tRNA^{Sec} and ADP (5,15). tRNA^{Sec} binds to PSTK with high affinity and specifically induces its ATPase activity (15). Although SerRS must be able to recognize and aminoacylate both tRNA^{Ser} and tRNA^{Sec}, proper interpretation of the genetic code requires that PSTK differentiate Ser-tRNA^{Sec} from Ser-tRNA^{Ser}. tRNA^{Sec} species from all three domains of life are unusual in both length (>90 nt) and structure. While most tRNAs including tRNA^{Ser} are in a 7/5 cloverleaf form (i.e. 7 bp in the acceptor stem and 5 in the TΨC arm), bacterial tRNA^{Sec} is in an 8/5 form (16), while eukaryal tRNA^{Sec} (17,18) and archaeal tRNA^{Sec} (17,19) likely exist in a 9/4 clover leaf form. Besides the difference in acceptor stem length at 9 bp in archaeal and eukaryotic tRNA^{Sec} versus 7 bp in tRNA^{Ser}, several other features of tRNA^{Sec} are significantly

*To whom correspondence should be addressed. Tel: +1 203 432 6200; Fax: +1 203 432 6202; Email: dieter.soll@yale.edu

different from $tRNA^{Ser}$. Eukaryotic and archaeal $tRNA^{Sec}$ species have 6 or 7 bp D-stems, respectively (17–19); in contrast $tRNA^{Ser}$ has a 3 to 4 bp D-stem. Molecular modeling suggested that a 7 bp D-stem in archaeal $tRNA^{Sec}$ would compensate for the short 4 bp T-stem (5 bp in $tRNA^{Ser}$) thus allowing the normal interaction between the D- and T-loops (19). The sequence, length and orientation of the variable arm of $tRNA^{Sec}$ also vary from those of $tRNA^{Ser}$.

Previous work with HeLa cell extracts (8) demonstrated that the length and secondary structure of the D-stem of human $tRNA^{Sec}$ are the major determinants for serine phosphorylation by a kinase activity (in the following called 'human PSTK'). Here we present the basis of $tRNA^{Sec}$ discrimination from $tRNA^{Ser}$ by an archaeal PSTK from *M. jannaschii*. The G2-C71 and C3-G70 base pairs within the acceptor stem of *Methanococcus maripaludis* $tRNA^{Sec}$ are the major identity elements for tRNA-dependent serine phosphorylation by archaeal PSTK.

MATERIALS AND METHODS

Materials and reagents

All oligonucleotide synthesis and DNA sequencing were carried out by the Keck Foundation Biotechnology Research Laboratory at Yale University. L-[U-^{14}C]Ser (163 mCi/mmol) was from Amersham Biosciences and [α-^{32}P]-ATP (3000 Ci/mmol) was from GE Healthcare. L-[1-^{14}C]Thr (50–60 mCi/mmol) was from American Radiolabeled Chemicals.

Expression and purification of enzymes

Since heterologous overexpression of the *M. maripaludis* PSTK gene in *Escherichia coli* was not successful, *M. jannaschii* PSTK-His_6 (MJ1538) in pET20b (Novagen) was overproduced and purified as described (15). *M. maripaludis* SerRS was overproduced and purified as described (2).

Cloning, purification, transcription and ^{32}P-labeling of tRNAs

All tRNAs were cloned into pUC19, expressed in *E. coli* DH5α, transcribed by T7 RNA polymerase, gel purified and folded as described previously (15). Refolded transcript was ^{32}P-labeled on the 3' terminus using the *E. coli* CCA-adding enzyme and [α-^{32}P]ATP as before (15). After phenol/chloroform extraction the reaction was passed over a Sephadex G25 Microspin column (Amersham Biosciences) to remove excess ATP.

Serylation and phosphorylation of $tRNA^{Sec}$

These assays were carried out in 1× PSTK buffer [50 mM HEPES-KOH (pH 7.5), 10 mM $MgCl_2$, 20 mM KCl, 1 mM DTT] with 1 mM L-Ser (Sigma), 5 mM ATP, 600 nM *M. maripaludis* SerRS, 50 nM *M. jannaschii* PSTK and 1 μM ^{32}P-labeled transcript for 45 min at 37°C. Aliquots (2 μl) of each reaction were quenched on ice with 3 μl of 100 mM sodium citrate (pH 5.0) and 0.66 mg/ml nuclease P1 (Sigma) and incubated at room temperature for 35 min (20,21). Assays that included 100 μM [^{14}C]-Ser were carried out in 1× PSTK buffer with 1 μM $tRNA^{Sec}$ transcript, 5 mM ATP, 1.2 μM SerRS and 200 nM PSTK for 45 min at 37°C. The reactions were stopped by phenol/chloroform extraction and purified over a G25 column to remove unincorporated [^{14}C]-Ser, and aliquots (2 μl) of each reaction were quenched and digested as above. To separate Sep-AMP, Ser-AMP and AMP, 1 μl of quenched, digested sample was spotted on glass polyethyleneimine (PEI) cellulose 20 cm × 20 cm thin layer chromatography (TLC) plates (EMD) and developed for 75 min in 100 mM ammonium acetate and 5% acetic acid. The plates were exposed on an imaging plate (FujiFilms), scanned on a Molecular Dynamics Storm 860 PhosphorImager, and quantified using ImageQuant software.

Preparation of seryl-tRNA

$tRNA^{Sec}$, chimera and $tRNA^{Ser}$ ^{32}P-labeled transcripts were each aminoacylated in 1× PSTK buffer with 1 mM L-Ser (Sigma), 5 mM ATP, 3 μM *M. maripaludis* SerRS and 5 μM ^{32}P-labeled transcript as described previously (15). Reactions were incubated at 37°C for 1 h followed by phenol/chloroform extraction, ethanol precipitation and resuspension in water. The samples were passed over Sephadex G25 Microspin columns (Amersham Biosciences) equilibrated with water. To check aminoacylation levels, 2 μl aliquots were removed at the end of the reactions, quenched on ice with 3 μl of 100 mM sodium citrate (pH 5.0) and 0.66 mg/ml nuclease P1 (Sigma), and analyzed as described above.

Phosphorylation of seryl-tRNA

These assays were carried out in 1× PSTK buffer with 1 μM ^{32}P-labeled Ser-tRNA transcript, 5 mM ATP and 50 nM PSTK at 37°C. Reaction mixes were preincubated at 37°C and started by addition of enzyme. At each time point, 2 μl aliquots were taken and treated as described above.

ATPase activity measurement

ATPase activity was determined by measuring the amount of [α-^{32}P]ATP converted to [α-^{32}P]ADP as described before with modifications (15). These assays were carried out in a 12 μl reaction volume including 1× PSTK buffer with 130 nM cold ATP, 100 nM [α-^{32}P]ATP and 1 μM enzyme at 37°C for 30 min. Unless noted otherwise, 1 μM unlabeled tRNA ($tRNA^{Sec}$, G2-C71:C-G $tRNA^{Sec}$, chimera tRNA or $tRNA^{Ser}$) was included. At six time points, 0.75 μl aliquots were taken from each reaction and quenched by the addition of 9.25 μl ice-cold 55 mM EDTA. One microliter of each reaction mixture was spotted on PEI cellulose TLC plates (EMD) and developed in 1 M LiCl for 60 to 75 min. After separation, the [α-^{32}P]ATP and [α-^{32}P]ADP spots were quantified by PhosphorImager using ImageQuant software.

Secondary structure alignment of archaeal tRNAs

Archaeal $tRNA^{Sec}$ and $tRNA^{Ser}$ sequences were acquired from The Institute for Genomic Research, USCS

Archaeal Genome Browser (22) and from the Microbial Genomes at the Joint Genome Institute. The secondary structures were determined by Aragorn 1.1 (23) and aligned manually by secondary structure.

Phosphorylation of threonyl-tRNA^{Sec}

These assays were carried out in 1× PSTK buffer with 5 mM ATP, 2.5 mM threonine (Thr) (Fluka, 99.5% purity), 1 μM ^{32}P-labeled tRNA^{Sec} transcript, 1.2 μM SerRS and with or without 200 nM PSTK for 45 min at 37°C. The reactions were quenched, digested and analyzed by TLC as stated above.

RESULTS

A survey of tRNA identity elements of archaeal PSTK

We decided to undertake a preliminary survey for potential tRNA identity elements for phosphorylation by *M. jannaschii* PSTK. Our assay conditions (see Materials and Methods section and Table 1) guarantee achievement of plateau levels of Ser-tRNA^{Sec} formation, while phosphorylation may or may not reach plateau values for all the different mutants. Such an approach will reveal major elements, but will miss some less important ones. Previous work established that Ser-tRNA^{Sec} but not Ser-tRNA^{Ser} is a substrate for phosphorylation by PSTK (7,8,12–15). Thus, the identity elements for phosphorylation must lie within tRNA^{Sec} (Figure 1A) and be absent from tRNA^{Ser} (Figure 1B), while any antideterminants would be found only in tRNA^{Ser}. Based on a comparison of the sequences and secondary structures of archaeal tRNA^{Sec} and tRNA^{Ser}, we designed transplantation mutants of *M. maripaludis* tRNA^{Sec} by replacing the acceptor stem + T-arm, D-arm, anticodon, anticodon stem–loop, variable arm, T-arm or T-loop of *M. maripaludis* tRNA^{Sec} with those of *M. maripaludis* tRNA$^{Ser}_{UGA}$. Additionally, we made point mutations to tRNA^{Sec} to localize the nucleotides recognized by PSTK. While the mutations did not overlap with previously identified identity elements for the archaeal *Methanosarcina barkeri* SerRS (the long variable arm, G1-C72, the discriminator base G73 and the anticodon stem base-pair G30-C40) (24) some of the mutants did not serylate efficiently (Table 1). Generally, tRNA^{Sec} mutants that did not serylate efficiently would deacylate during purification of Ser-tRNA, leaving little charged tRNA to test for phosphorylation by PSTK. To circumvent this issue we tested the mutants in an activity assay similar to what was described previously (15), in which tRNA was serylated with SerRS and phosphorylated by PSTK in the same reaction. Thus, mutants of tRNA^{Sec} that serylated poorly were phosphorylated by PSTK without a purification of the Ser-tRNA^{Sec} prior to phosphorylation by PSTK. A similar approach for tRNA mutants that serylated inefficiently was implemented in a study of human PSTK in partially purified HeLa cell extracts (8). In our assay, after serylation by purified *M. maripaludis* SerRS and phosphorylation by purified *M. jannaschii* PSTK, the tRNA^{Sec} mutants were digested with nuclease P1, and the Sep-[^{32}P]AMP, [^{32}P]AMP and Ser-[^{32}P]AMP products were separated by TLC. The amounts of aminoacylated (serylated plus phosphorylated) and phosphorylated products were determined by PhosphorImager analysis (Table 1). Additionally, a filter-binding assay (15) was used to determine the affinity of PSTK for each tRNA mutant (Supplementary Table 1). We should note that in this study we used the transcript of the *M. maripaludis* tRNA^{Sec} for our identity studies; SerRS serylated both *M. jannaschii* tRNA^{Sec} and *M. maripaludis* tRNA^{Sec} similarly (Table 1), but the *M. jannaschii* Ser-tRNA^{Sec} deacylated during purification. Additionally, PSTK from *M. jannaschii* was used because we were able to obtain sufficient quantities of purified and active enzyme.

The unusual D-arm provides a minor identity element for archaeal PSTK

The length and structure but not sequence of the D-stem of human tRNA^{Sec} were shown to be the major identity elements for serine phosphorylation by human PSTK (8), but our investigation revealed the D-arm to be a minor identity element for archaeal PSTK. The D-arm of tRNA^{Sec} likely has a 7-bp stem and a 4 nt loop, whereas in the isoacceptors of tRNA^{Ser} the stem and loop are 3 to 4 bp and 9 to 13 nt, respectively. (Figure 1A and B). Replacing the D-arm of tRNA^{Sec} with the D-arm from tRNA^{Ser} resulted in a mutant (D-arm, Table 1) that serylated poorly (1.4%), which made assaying for phosphorylation difficult. The severe effects propagated by the D-arm mutant were possibly due to disruption of tertiary structure. More specific mutations of the D-arm disrupted 1 bp (U16-A20a or C15-G20b) or 2 bp (U16-A20a and C15-G20b) in the 7-bp D-stem: U16A, U16G, C15G, U16A/C15G, A20aU and A20aC. Disrupting 1 bp of the D-stem by mutation of U16 to A or G or A20aU had moderate effects on the relative phosphorylation efficiency of the mutant tRNAs compared to wild-type, while the C15G and A20aC mutations had little effect (Table 1). Compensatory mutations that retained or closed the 7-bp stem retained or restored phosphorylation by PSTK (A20aG, A20aU/U16A, Table 1). The relative phosphorylation efficiency of the U16A/C15G double mutant (disrupted 2 bp in the D-stem) was similar to the single U16 mutants, showing no added effect due to a shorter 5-bp D-stem (Table 1). Although the affinity of PSTK was about 10-fold lower for most of the D-stem mutants, the U16A/C15G double mutant did cause a 28.5-fold decrease over wild-type tRNA^{Sec}, which is comparable to PSTK recognition of tRNA^{Ser} (Supplementary Table 1). Thus, the D-stem of tRNA^{Sec} does provide a minor identity element for PSTK recognition and phosphorylation but is not the signal for phosphorylation by archaeal PSTK.

PSTK recognition of the acceptor stem of archaeal tRNA^{Sec} is essential for phosphorylation

The acceptor stems of archaeal tRNA^{Sec} and tRNA^{Ser} from Sec-decoding archaea differ in both length and sequence (Figure 1A and B and Supplementary Figure 1), which probably affects the secondary and tertiary structures of these tRNAs. The L-form tertiary structure

Table 1. Phosphorylation of $tRNA^{Sec}$ mutants by *M. jannaschii* PSTK

tRNA[a]	Base exchange or domain transplantation	Aminoacylation[b] (%)	Phosphorylation[c] (%)	Relative efficiency[d] (%)
M. maripaludis $tRNA^{Sec}$	Wild-type $tRNA^{Sec}$	80.3 ± 3.8	75.6 ± 3.8	100
M. maripaludis $tRNA^{Ser}$	Wild-type $tRNA^{Ser}_{UGA}$	71.6 ± 8.1	ND[f]	–
Acceptor stem	Acceptor stem + T arm[e]	20.1 ± 1.4	0.4 ± 0.1	2.1
	Δ 5a-67b (G-U)	90.6 ± 0.3	87.0 ± 0.7	102.0
	Δ 5b-67a (G-C)	89.7 ± 0.9	85.8 ± 1.0	101.6
	Δ 5a-67b and 5b-67a	8.9 ± 0.4	4.6 ± 0.4	54.9
	Δ G2-C71	73.4 ± 10.4	0.9 ± 0.3	1.3
	G2-C71 →C-G	63.4 ± 6.0	2.2 ± 0.3	3.7
	→G-U	75.6 ± 0.5	19.6 ± 1.4	27.5
	→A-U	47.2 ± 0.9	5.7 ± 1.6	12.8
	→U-A	35.3 ± 0.6	13.8 ± 2.7	41.5
	C3-G70 →G-C	64.7 ± 1.4	7.6 ± 1.1	12.5
	→A-U	58.3 ± 1.5	41.7 ± 1.7	75.9
	→U-A	70.6 ± 1.6	66.5 ± 1.5	100.0
	→U-G	66.1 ± 3.8	57.9 ± 4.6	93.0
	C5-G68 →G-C	41.6 ± 0.8	36.7 ± 0.9	93.7
	→A-U	65.6 ± 1.3	28.1 ± 0.7	45.5
	→U-A	50.6 ± 1.5	43.8 ± 1.3	91.9
	→U-G	61.3 ± 0.6	49.2 ± 4.7	85.3
D-arm	D-arm[e]	1.4 ± 0.4	ND[f]	–
	U16→A	25.1 ± 0.14	18.4 ± 0.1	77.9
	U16→G	48.7 ± 7.9	32.0 ± 6.0	69.8
	C15→G	79.7 ± 1.0	70.9 ± 3.9	94.5
	U16→A/C15→G	75.8 ± 2.1	57.6 ± 1.5	80.7
	A20a→G	56.6 ± 2.2	50.2 ± 1.3	94.2
	A20a→U	71.3 ± 10.1	43.9 ± 2.1	65.4
	A20a→U/U16→A	57.8 ± 1.2	55.7 ± 1.1	102.3
	A20a→C	69.9 ± 2.7	63.6 ± 2.2	96.6
Anticodon stem	Anticodon stem-loop[e]	46.5 ± 0.2	40.3 ± 1.2	92.1
Anticodon	UCA→UGA[e]	84.9 ± 1.0	80.1 ± 0.4	100.2
Variable arm	Variable arm[e]	52.8 ± 2.6	50.2 ± 2.5	101.0
T-arm	T-arm[e]	37.9 ± 0.8	24.6 ± 1.9	68.9
	G50-C64 →C-G/G51-C63 →C-G	52.6 ± 0.2	40.3 ± 1.2	81.4
	T-loop[e]	44.9 ± 0.9	24.9 ± 6.0	58.9
	G57→A	74.2 ± 1.5	71.7 ± 0.9	102.6
	U59→A	70.2 ± 1.6	58.4 ± 4.3	88.4
M. jannaschii $tRNA^{Sec}$	*M. jannaschii* $tRNA^{Sec}$	75.0 ± 0.5	65.3 ± 6.4	92.5
Heterologous $tRNA^{Sec}$	*M. kandleri* $tRNA^{Sec}$	50.4 ± 1.0	48.2 ± 0.9	101.6
	E. coli $tRNA^{Sec}$	53.3 ± 2.4	7.0 ± 2.1	13.9
	H. sapiens $tRNA^{Sec}$	74.4 ± 3.1	12.8 ± 2.4	18.3
Chimeric tRNA	Transplant 1	4.8 ± 0.3	0.0001 ± 0.0006	0.002
	Transplant 2	9.6 ± 0.7	4.3 ± 0.4	47.6
	Transplant 3	17.7 ± 1.7	5.2 ± 0.7	31.2
	Chimera	18.9 ± 0.1	12.1 ± 1.1	68.0

[a]tRNA transcripts were used.
[b]The percent aminoacylation (serylation plus phosphorylation) refers to the intensity of the Ser-[^{32}P]AMP and Sep-[^{32}P]AMP spots divided by the total intensity of the Ser-[^{32}P]AMP, Sep-[^{32}P]AMP, and [^{32}P]AMP spots and phosphorylation refers to the intensity of the Sep-[^{32}P]AMP spot divided by the total intensity of the Ser-[^{32}P]AMP, Sep-[^{32}P]AMP, and [^{32}P]AMP spots (See 'Materials and Methods' section).
[c]The assay was performed on all tRNAs in triplicate and the standard deviations for each are reported.
[d]The efficiency of phosphorylation was calculated by dividing the percent of tRNA phosphorylated by the percent total aminoacylation. The relative efficiency is a comparison of the efficiency of phosphorylation of each mutant tRNA to that of wild-type *M. maripaludis* $tRNA^{Sec}$ calculated by dividing the percent efficiency of phosphorylation of each mutant by that of wild-type *M. maripaludis* $tRNA^{Sec}$ multiplied by 100%.
[e]Domain from *M. maripaludis* $tRNA^{Ser}_{UGA}$ was transplanted onto the *M. maripaludis* $tRNA^{Sec}$ backbone.
[f]ND, Activity not detectable.

of tRNA has two helical domains; domain I consists of the anticodon stem and the D-stem, and domain II consists of the T-stem and the acceptor stem (25–27). Domain II has 13 bp (9-bp acceptor stem and 4-bp T-stem) in archaeal $tRNA^{Sec}$ and 12 bp (7 bp acceptor stem and 5-bp T-stem) in $tRNA^{Ser}$ (Figure 1A and B). Thus to maintain the domain II structure as that of $tRNA^{Ser}$, we transplanted both the acceptor stem and T-arm from $tRNA^{Ser}_{UGA}$ to $tRNA^{Sec}$, resulting in a 12-bp domain II. The mutant did not serylate efficiently (20.1%), and the relative phosphorylation efficiency was poor (2.1%) (acceptor stem + T arm, Table 1), suggesting that elements within the acceptor stem were required for phosphorylation by archaeal PSTK.

To determine whether the length of the acceptor stem was an important identity element for PSTK recognition of $tRNA^{Sec}$, $tRNA^{Sec}$ mutants with deletions of base pairs within the acceptor stem were analyzed: Δ5a-67b,

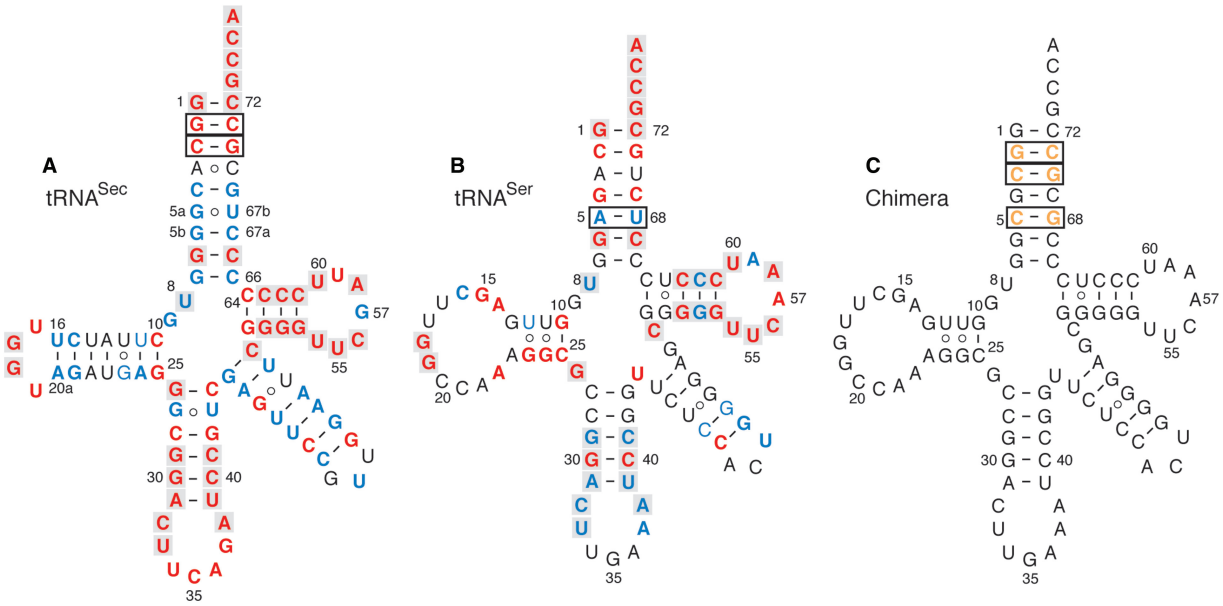

Figure 1. Conservation of archaeal $tRNA^{Sec}$ and $tRNA^{Ser}$ sequences and transplantation of $tRNA^{Sec}$ identity elements into $tRNA^{Ser}$. The cloverleaf structures of *M. maripaludis* $tRNA^{Sec}$ (**A**) and $tRNA^{Ser}_{UGA}$ (**B**) are shown. The secondary structures of presently available archaeal $tRNA^{Sec}$ (seven sequences) and $tRNA^{Ser}$ from Sec-decoding archaea (18 sequences) were compared (See Supplementary Figure 1). Bold and red nucleotides are invariant within each $tRNA^{Sec}$ or $tRNA^{Ser}$. Bold and blue nucleotides are at least 70% conserved within each $tRNA^{Sec}$ or $tRNA^{Ser}$. Blue nucleotides are at least 70% conserved within each $tRNA^{Sec}$ or $tRNA^{Ser}$ but not in the pictured *M. maripaludis* tRNAs. Gray shading shows residues conserved at >70% between $tRNA^{Sec}$ and $tRNA^{Ser}$. Black boxes on G2-C71 and C3-G70 of $tRNA^{Sec}$ in (A) indicate identity elements transplanted into the $tRNA^{Ser}$ scaffold to produce the tRNA chimera pictured in (**C**). The black box on A5-U68 of $tRNA^{Ser}$ in (B) indicates an antideterminant that was mutated to C5-G68 in the tRNA chimera (C). The mutated nucleotides are boxed, bold and orange in the tRNA chimera. The numbering of $tRNA^{Sec}$ is according to Sturchler *et al.* (18), and the numbering of $tRNA^{Ser}$ is according to Sprinzl *et al.* (39).

Δ5b-67a, Δ5a-67b/Δ5b-67a and ΔG2-C71. Deletion of 5a-67b or 5b-67a did not influence phosphorylation efficiency (Table 1). Deletion of both 5a-67b and 5b-67a resulted in mutant $tRNA^{Sec}$ that serylated poorly, similarly to the acceptor stem + T arm mutant (8.9% and 20.1%, respectively). Unlike the acceptor stem + T arm mutant, the relative phosphorylation efficiency was higher at 54.9% of the wild-type $tRNA^{Sec}$ level (Table 1). This may suggest that the length of the acceptor stem plays a role in phosphorylation. Additionally, mutant $tRNA^{Sec}$ with a G2-C71 deletion serylated efficiently (73.4%) but its relative phosphorylation efficiency was only 1.3% (Table 1), signifying a possible sequence-specific interaction with PSTK.

Archaeal $tRNA^{Sec}$ has invariant G2-C71 and C3-G70 base pairs and a well-conserved C5-G68 base pair in the acceptor stem (Figure 1A) (C5-A68 in *Methanococcus voltae* and G5-C68 in *Methanopyrus kandleri*, Supplementary Figure 1). We mutated each of these base pairs to determine their importance for PSTK recognition. Mutation of the invariant $tRNA^{Sec}$ G2-C71 bp was detrimental to phosphorylation with a C2-G71 mutation producing the most severe decrease in relative phosphorylation efficiency at 96.3% below wild-type level (Table 1); interestingly, C2-G71 is highly conserved in all archaeal $tRNA^{Ser}$ but invariant in $tRNA^{Ser}$ from Sec-decoding archaea (Supplementary Figure 1). Mutation of the invariant C3-G70 base pair of archaeal $tRNA^{Sec}$ to G3-C70 had a severe effect on phosphorylation with an 87.5% decrease in the relative efficiency, while mutation to A3-U70 had a moderate decrease (24.1%) (Table 1). G3-C70 and A3-C70 are the most common base pairs found at this position in $tRNA^{Ser}$ from Sec-decoding archaea although the isoacceptors of *M. kandleri* $tRNA^{Ser}$ have a conserved C3-G70 bp (Supplementary Figure 1). It should be noted that the genome of *M. kandleri* has an accelerated rate of evolution among the archaea (28). Mutation of C3-G70 to U3-A70 or U3-G70 had little to no influence on the relative phosphorylation efficiency (Table 1). Mutation of the well-conserved C5-G68 base pair of $tRNA^{Sec}$ to G5-C68, U5-A68, or U5-G68 had a minimal effect on phosphorylation while mutation to A5-U68, the most common base pair at that position in $tRNA^{Ser}$ from Sec-decoding archaea, caused a 54.5% decrease in phosphorylation over wild-type $tRNA^{Sec}$ (Table 1). Additionally, Ser-$tRNA^{Sec}$ from *M. kandleri*, which has a G5-C68 base pair, phosphorylates as efficiently as *M. maripaludis* Ser-$tRNA^{Sec}$ (Table 1). These data demonstrate that A5-U68 in $tRNA^{Ser}$ has some antideterminant value for PSTK recognition.

The anticodon stem–loop and long variable arm are not identity elements for phosphorylation by PSTK

The anticodon stem–loop is highly conserved in archaeal $tRNA^{Sec}$ compared to the anticodon stem–loops of $tRNA^{Ser}$ isoacceptors (with the anticodons UGA, GGA and GCU) found in Sec-decoding archaea (Figure 1 and Supplementary Figure 1). Neither a mutation of the anticodon of $tRNA^{Sec}$ from UCA to one of a $tRNA^{Ser}$ isoacceptor, UGA (anticodon UCA to UGA), nor

replacing the entire anticodon stem–loop of $tRNA^{Sec}$ with that of $tRNA^{Ser}_{UGA}$ (anticodon stem–loop) had a negative effect on the relative phosphorylation efficiency by PSTK (Table 1).

The variable arms of $tRNA^{Sec}$ and $tRNA^{Ser}$ differ in sequence, length and orientation (Figure 1A and B and Supplementary Figure 1). Replacement of the variable arm of $tRNA^{Sec}$ with that of $tRNA^{Ser}_{UGA}$ did not influence the relative phosphorylation efficiency (variable arm, Table 1).

The T-loop of $tRNA^{Sec}$ is a minor identity element for phosphorylation

The structure of the T-arm of archaeal $tRNA^{Sec}$ is quite unusual in that the T-stem has 4 bp while most tRNAs, including $tRNA^{Ser}$, have 5-bp T-stems (Figure 1A and B and Supplementary Figure 1). Unlike the T-stems of archaeal $tRNA^{Ser}$ isoacceptors, the sequences of the T-stems of archaeal $tRNA^{Sec}$ found to date are invariant (Figure 1A and B and Supplementary Figure 1). Replacement of the T-arm of $tRNA^{Sec}$ with that of $tRNA^{Ser}_{UGA}$ resulted in a 31.1% decrease in relative phosphorylation efficiency compared to wild-type $tRNA^{Sec}$ (T-arm, Table 1) and a 29.1-fold decrease in the affinity of PSTK for the mutant tRNA (Supplementary Table 1). The T-arm mutation also had a significant effect on serylation, serylating at 37.9% as compared to 80.3% for wild-type $tRNA^{Sec}$ (Table 1), possibly due to disruption of the tertiary structure. Mutation of the first 2 bp of the T-stem from G50-C64 to C-G and G51-C63 to C-G had little effect on the relative phosphorylation efficiency (G50-C64 to C-G/G51-C63 to C-G, Table 1).

The T-loop size is conserved between archaeal $tRNA^{Sec}$ and $tRNA^{Ser}$ but the sequences are variant in two positions, 57 and 59 (Figure 1A and B and Supplementary Figure 1). A57 is invariant in the isoacceptors of $tRNA^{Ser}$ from Sec-decoding archaea, whereas G57 is found in most archaeal $tRNA^{Sec}$. U59 is invariant in archaeal $tRNA^{Sec}$, while A59 is highly conserved in archaeal $tRNA^{Ser}$. Transplanting the T-loop from $tRNA^{Ser}_{UGA}$ onto $tRNA^{Sec}$ which mutates G57A and U59A, causes a 41.1% decrease in the relative phosphorylation efficiency and a decrease in serylation to 44.9% compared to wild-type $tRNA^{Sec}$ (T-loop, Table 1); these results are similar to replacement of the entire T-arm with that of $tRNA^{Ser}_{UGA}$, suggesting that G57 and U59 might be responsible. Nevertheless, the single mutations G57A or U59A serylated efficiently and had minimal effects on the relative phosphorylation efficiency (Table 1). Consequently, although neither single mutation had a significant effect, the collective mutation of G57 and U59 in the T-loop mutant had a moderate effect on serylation, phosphorylation and binding (T-loop, Table 1 and Supplementary Table 1).

Transplantation of acceptor stem base pairs G2-C71, C3-G70 and C5-G68 into $tRNA^{Ser}$ allows robust phosphorylation by PSTK

Although mutation of G2-C71 in $tRNA^{Sec}$ resulted in a mutant that only phosphorylated at 3.7% of the wild-type level, transplantation of G2-C71 alone into the $tRNA^{Ser}_{UGA}$ backbone was not sufficient to confer phosphorylation onto this mutant (transplant 1, Table 1). The archaeal $tRNA^{Sec}$ identity elements found at base pairs G2-C71 and C3-G70 were transplanted into the $tRNA^{Ser}_{UGA}$ backbone, and the negative determinant A5-U68 was mutated to G5-C68 as found in $tRNA^{Sec}$ (Figure 1C). PSTK phosphorylated the chimera with a relative phosphorylation efficiency of 68% compared to wild-type $tRNA^{Sec}$ (chimera, Table 1). Furthermore, the affinity of PSTK for the chimeric tRNA was higher than that for $tRNA^{Ser}_{UGA}$ (Supplementary Table 1). Phosphorylation of the chimeric Ser-$tRNA^{Ser}$ by PSTK demonstrated the admirable substrate qualities of the transplanted $tRNA^{Ser}$ species (Figure 2). Neither mutation of the D-arm in the tRNA chimera to add base pairs to the D-stem (transplant 2, Table 1), thus making a smaller D-loop as seen for $tRNA^{Sec}$, nor addition of an additional base pair to the acceptor stem of the chimera to lengthen it from 7 to 8 bp improved phosphorylation (transplant 3, Table 1). Thus, the major identity elements for phosphorylation by archaeal PSTK are found in the acceptor stem at base pairs G2-C71 and C3-G70.

Chimeric tRNA induces the ATPase activity of PSTK

The ATPase activity of PSTK is specifically induced by $tRNA^{Sec}$ and there was minimal ATPase activity of the enzyme in the presence of $tRNA^{Ser}$ or in the absence of tRNA (15). When the ATPase activity of PSTK was tested in the presence of the G2-C71 to C-G $tRNA^{Sec}$ mutant, which was poorly phosphorylated (Table 1), the little ATPase activity detectable was similar to the ATPase activity in the presence of $tRNA^{Ser}$ (Figure 3). Yet, the chimeric tRNA (G2-C71, C3-G70, and C5-G68 of $tRNA^{Sec}$ transplanted into $tRNA^{Ser}_{UGA}$) (Figure 1C) significantly induced the ATPase activity of PSTK (Figure 3), further demonstrating the importance of these acceptor stem base pairs in phosphorylation by PSTK.

Divergence in $tRNA^{Sec}$ acceptor stems from *Archaea*, *Eukarya* and *Bacteria*

Alignment of archaeal and eukaryal $tRNA^{Sec}$ by secondary structure (Figure 4A) revealed insightful differences in the acceptor stems in light of the described identity elements for serine phosphorylation of *M. maripaludis* $tRNA^{Sec}$ by *M. jannaschii* PSTK and human $tRNA^{Sec}$ by human PSTK (8). As established by the phosphotransferase assay with *M. jannaschii* PSTK and *M. maripaludis* $tRNA^{Sec}$, G2-C71 is a major identity element for phosphorylation (Table 1) and is invariant in archaeal $tRNA^{Sec}$ (Figures 1A and 4A). Eukaryal $tRNA^{Sec}$, however, has a highly conserved C2-G71 bp at this position (Figure 4A and Supplementary Figure 2); only 2 out of 57 eukaryal $tRNA^{Sec}$ genes analyzed were variant at this position; $tRNA^{Sec}$ from the diatom *Thalassiosira pseudonana* has a U2-A71 bp (29). A BLAST search against the genome of the marsupial *Monodelphus domestica* identified a probable $tRNA^{Sec}$ (by secondary structure and sequence conservation), which has a U2-G71 bp at this position (Supplementary Figure 2). Archaeal $tRNA^{Sec}$ is invariant

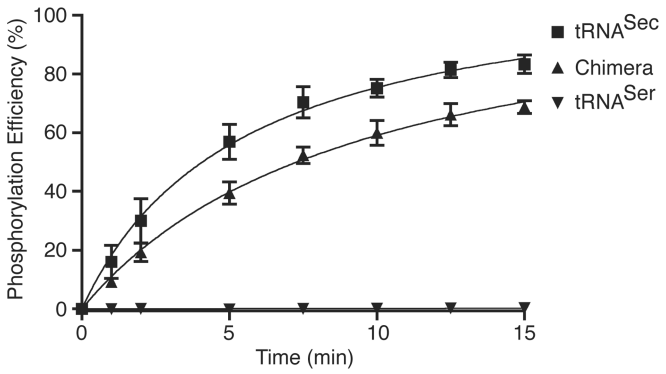

Figure 2. *In vitro* conversion of Ser-tRNA to Sep-tRNA by *M. jannaschii* PSTK. One micromolar ^{32}P-labeled Ser-tRNA^{Sec} (70.8% serylated), chimera Ser-tRNA (64.5% serylated) or Ser-tRNA^{Ser} (63.4% serylated) was incubated with 50 nM PSTK at 37°C for 15 min. Aliquots of the reactions were quenched with 100 mM sodium citrate, pH 5.0 and digested with 0.66 mg/ml nuclease P1 for 35 min at room temperature. Samples were then spotted onto a PEI-cellulose TLC plate and developed in 100 mM ammonium acetate, 5% acetic acid for 75 min. Following quantification of the intensities of Ser-[^{32}P]AMP, [^{32}P]AMP and Sep-[^{32}P]AMP using ImageQuant, the fraction (%) of aminoacyl-tRNA formed at each time point was calculated by dividing the intensity of the Sep-[^{32}P]AMP and Ser-[^{32}P]AMP spots by the total intensity and the fraction (%) of Sep-tRNA^{Sec} formed at each time point was calculated by dividing the intensity of the Sep-[^{32}P]AMP spot by the total intensity. The phosphorylation efficiency was determined by dividing the percent Sep-tRNA by the percent aminoacyl-tRNA. Error bars represent the standard deviation of three separate experiments.

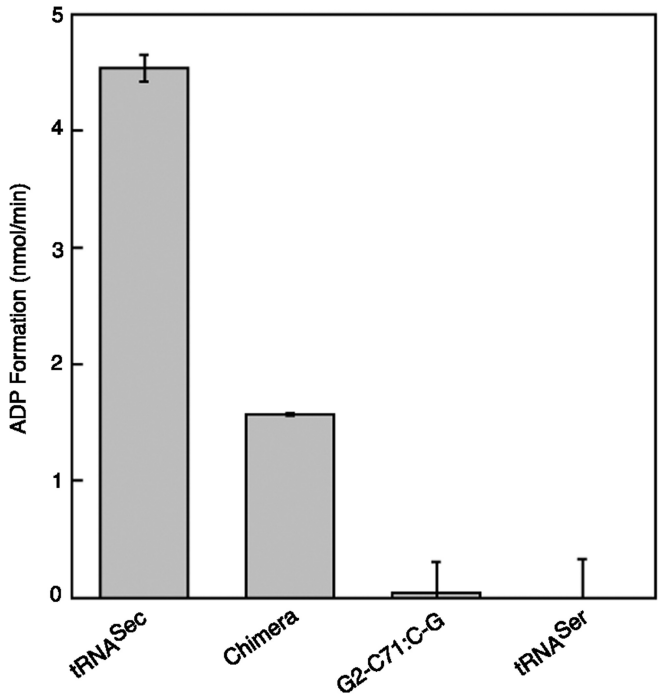

Figure 3. tRNA^{Ser} chimera with transplanted (tRNA^{Sec}) identity elements induces the ATPase activity of PSTK. A graph is shown of the ratio of [α-^{32}P]ATP converted to [α-^{32}P]ADP by PSTK (1 μM) in the presence of 1 μM tRNA^{Sec}, chimera tRNA, G2-C71:C-G tRNA^{Sec} mutant or tRNA^{Ser}. The minimal ATPase activity in the absence of tRNA was subtracted. Error bars represent the standard deviation of three separate experiments.

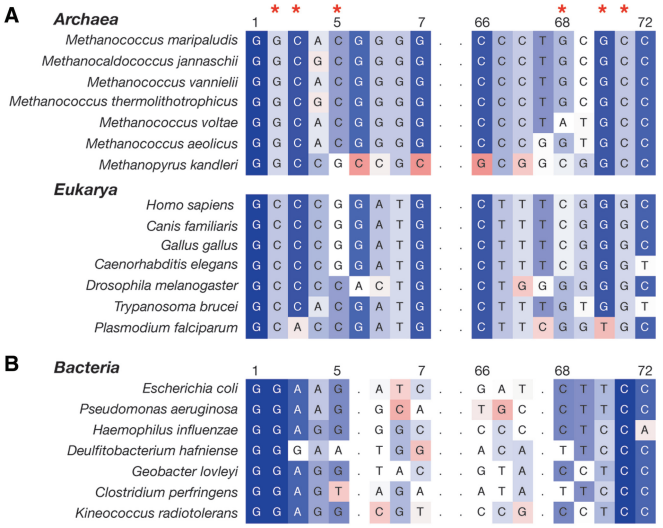

Figure 4. Comparative alignment of archaeal, eukaryotic and bacterial tRNA^{Sec} acceptor stems. The secondary structures of archaeal (seven sequences, Supplementary Figure 1), eukaryotic (57 sequences, Supplementary Figure 2) and bacterial tRNA^{Sec} (50 sequences) were aligned. (**A**) Alignment of the acceptor stems of all presently known archaeal tRNA^{Sec} and representative eukaryotic tRNA^{Sec} are shown. Nucleotides are colored according to sequence similarity (BLOSUM 50) between the archaeal and eukaryotic tRNA^{Sec}. Red asterisks indicate key nucleotides in the acceptor stem of archaeal tRNA^{Sec} for phosphorylation by PSTK. (**B**) Alignment of the acceptor stems of representative bacterial tRNA^{Sec} to those of the archaeal and eukaryotic tRNA^{Sec}. Nucleotides are colored according to sequence similarity (BLOSUM 50) among the bacterial tRNA^{Sec}.

at bp C3-G70, and 84% of eukaryal tRNA^{Sec} genes analyzed also have a C3-G70 bp (Figure 4A and Supplementary Figure 2). Of the remaining 16% of eukaryal tRNA^{Sec}, only 3.5% have a base pair (G3-C70) that caused poor phosphorylation in the context of archaeal tRNA^{Sec} by archaeal PSTK (Table 1). The A5-U68 with negative determinant properties in archaeal tRNA^{Ser} is found in 4% of eukaryal tRNA^{Sec} genes (Supplementary Figure 2). Although human tRNA^{Sec} has a C2-G71 bp rather than the major identity element G2-C71 found in archaeal tRNA^{Sec}, it retains the C3-G70 bp in the acceptor stem (Figure 4A), as well as other similar structural features such as the unusual D-arm, and archaeal PSTK is able to weakly phosphorylate human tRNA^{Sec} (Table 1). Thus, eukaryal tRNA^{Sec} has diverged at a critical identity element, G2-C71, for serine phosphorylation by archaeal PSTK.

The acceptor stem of bacterial tRNA^{Sec} consists of 8 bp (16) rather than 9 bp as found in archaeal and eukaryal tRNA^{Sec}. Acceptor stems from 50 divergent Sec-decoding bacterial species were analyzed, and the G2-C71 bp is highly conserved if not invariant (Figure 4B). The third base pair in the acceptor stem, however, was 75% conserved as an A3-U70, 21% as a G3-C70 bp and 4% as a U3-A70 or C3-G70 bp. Most bacterial tRNA^{Sec} analyzed did not have the antideterminant for archaeal PSTK, A5-U68. *E. coli* tRNA^{Sec} has G2-C71, A3-U70 and G5-C68 bp in the acceptor stem (Figure 4B) and is phosphorylated weakly at a relative phosphorylation efficiency of 13.9% compared to wild-type *M. maripaludis*

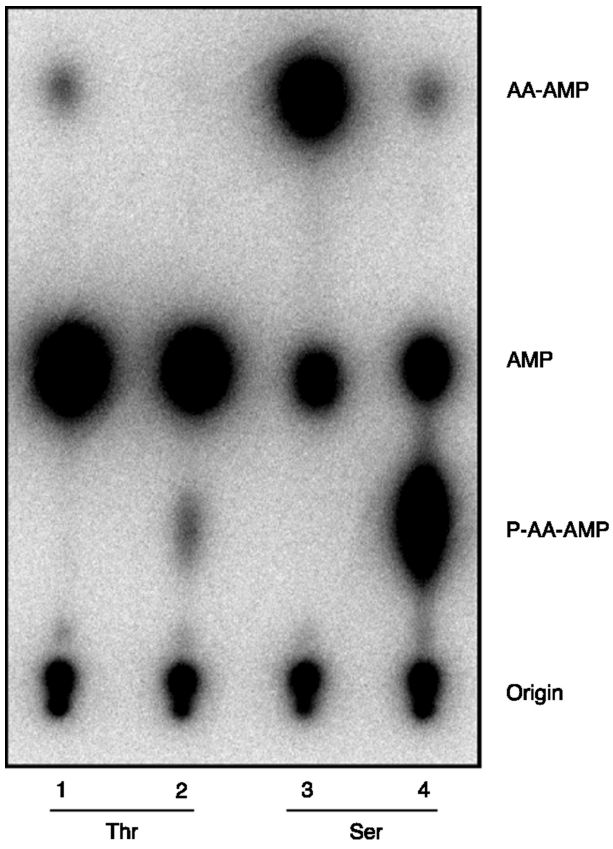

Figure 5. *In vitro* conversion of threonyl-tRNA^{Sec} to phosphothreonyl-tRNA^{Sec} by PSTK. One micromolar ^{32}P-labeled tRNA^{Sec} transcript was incubated with 600 nM SerRS and Thr (lane 1), 600 nM SerRS, 100 nM PSTK and Thr (lane 2), 600 nM SerRS and Ser (lane 3), or 600 nM SerRS, 100 nM PSTK and Ser (lane 4) at 37°C for 45 min. Aliquots of the reactions were quenched with 100 mM sodium citrate, pH 5.0 and digested with 0.66 mg/ml nuclease P1 for 35 min at room temperature. Samples were then spotted onto a PEI-cellulose TLC plate and developed in 100 mM ammonium acetate, 5% acetic acid for 75 min.

tRNA^{Sec} (Table 1). Although *E. coli* tRNA^{Sec} has the major identity element for phosphorylation by archaeal PSTK, G2-C71, other features such as the A3-U70 bp, the C59 in the T-loop, the 8/5 cloverleaf arrangement (8-bp acceptor stem and 5-bp T-stem), and other possible differences in tertiary structure could contribute to the poor phosphorylation and binding by archaeal PSTK (Table 1 and Supplementary Table 1).

Amino acid recognition by PSTK

It was observed earlier that *M. barkeri* SerRS misactivates threonine (30) and inefficiently forms Thr-tRNA^{Ser} (I. Weygand-Durasevic, personal communication). Given that *M. jannaschii* PSTK has a similar affinity for tRNA^{Sec} as Ser-tRNA^{Sec} and thus does not seem to recognize the serine moiety on the tRNA (15), we considered whether PSTK could phosphorylate Thr-tRNA^{Sec}. *M. maripaludis* [^{32}P]tRNA^{Sec} was mischarged with Thr by *M. maripaludis* SerRS, producing Thr-tRNA^{Sec} (Figure 5, lane 1). Thr-tRNA^{Sec} was subsequently phosphorylated with *M. jannaschii* PSTK (Figure 5, lane 2) and digested with nuclease P1. The products, [^{32}P]AMP, Thr-[^{32}P]AMP and phosphothreonyl-[^{32}P]AMP were separated by TLC on PEI cellulose. While aminoacylation with Thr was poor (only 3.7%), PSTK converted 94.4% of the Thr to phosphothreonine, which migrated similarly to phosphoserine (Figure 5, compare lanes 2 and 4). This corresponds well to the 96.3% conversion of Ser to Sep by PSTK (Figure 5, lane 4). The experiment was performed with [^{14}C]Thr to confirm the results (Supplementary Figure 3). Again, [^{14}C]Thr-tRNA^{Sec} was phosphorylated by PSTK (Supplementary Figure 3, compare lanes 2 and 4). The results provide further evidence that PSTK primarily recognizes the tRNA and not the amino acid, if any, that is attached to the 3' end of the tRNA.

DISCUSSION

The pathway for selenocysteine formation differs in bacteria from that present in eukaryotes and archaea; however, tRNA^{Sec} remains a common factor for selenocysteine formation among the three. The tRNA-dependent conversion of Ser to Sec in eukaryotes and archaea requires that the unusual tRNA^{Sec} be recognized initially by enzymes that interact with many tRNAs such as processing and modifying enzymes and the CCA-adding enzyme prior to 'mischarging' with Ser by SerRS, phosphorylation of the resulting Ser-tRNA^{Sec} by PSTK and conversion of Sep-tRNA^{Sec} to Sec-tRNA^{Sec} by SepSecS. Subsequently, the tRNA^{Sec}-specific elongation factor SelB must recognize Sec-tRNA^{Sec} specifically for selenoprotein synthesis (31–33). While we have speculated that a complex of the enzymes involved in selenocysteine biosynthesis might exist (15) as has been suggested and shown for the tRNA-dependent amidotransferases (34,35), it is not known which components of the pathway might exist in a complex or whether the enzymes might compete for the tRNA^{Sec} substrate. Yet, PSTK provides the first line of defense in maintaining the fidelity of genetic code translation by binding with high affinity to Ser-tRNA^{Sec} (7,15). This sequesters mischarged tRNA from use in translation and discriminates Ser-tRNA^{Sec} from Ser-tRNA^{Ser}.

A previous study detailed how a human PSTK discriminates human Ser-tRNA^{Sec} from Ser-tRNA^{Ser} by virtue of the atypical D-stem structure of tRNA^{Sec} (8). While archaeal tRNA^{Sec} retains a D-stem structure similar to that of eukaryal tRNA^{Sec}, mutations that open the last two base pairs in the D-stem of *M. maripaludis* tRNA^{Sec}—leading to the formation of a 5-bp rather than a 7-bp stem—did not result in a complete loss of serine phosphorylation by *M. jannaschii* PSTK as occurred when similar mutations were made to human tRNA^{Sec} (phosphorylation by human PSTK) (8). Some mutations to the D-stem of *M. maripaludis* tRNA^{Sec} (U16A, U16G, U16A/C15G and A20aU) caused a moderate decrease in phosphorylation but others (C15G and A20aC) did not, which brings into question whether a 7-bp stem has an effect on phosphorylation by archaeal PSTK. The intact 7-bp D-stem *per se* may not be recognized for phosphorylation; these data might suggest a sequence-specific interaction of archaeal PSTK with U16, but the

compensatory mutation A20aU/U16A that keeps the D-stem intact phosphorylated efficiently even with a mutation of U16. Perhaps mutations of A20aU or of U16 perturb the tertiary structure of $tRNA^{Sec}$, causing the decrease in phosphorylation efficiency by PSTK.

The T-loop of $tRNA^{Sec}$ also provides a minor identity element for archaeal PSTK phosphorylation. The combined mutation of G57A and U59A in the T-loop, which resulted in a T-loop identical to that found in $tRNA^{Ser}$ from Sec-decoding archaea, caused a moderate decrease in phosphorylation that was not completely attributable to a single mutation of either G57 or U59. Conceivably these mutations, specifically that of U59, could disrupt a tertiary interaction of the D- and T-loops between U17 and U59. A novel tertiary interaction was reported for *E. coli* $tRNA^{Sec}$ between C16 (located at the same position as U17 in archaeal $tRNA^{Sec}$) in the D-loop and C59 (U59 in archaeal $tRNA^{Sec}$) in the T-loop (16) although a similar interaction was not found between U16 (located at the same position as U17 in archaeal $tRNA^{Sec}$) and U59 in a study of eukaryotic-type *Xenopus laevis* $tRNA^{Sec}$ (18). If archaeal $tRNA^{Sec}$ is able to form interactions common to all known tRNA crystal structures between the D-loop and T-loop (25–27) as was supported by molecular modeling of the archaeal-type *M. jannaschii* $tRNA^{Sec}$ (19), an alternative explanation is possible; nucleotide 57 stacks with other purines in an intercalation of bases from both the D- and T-loops, and nucleotide 59 stacks to the tertiary base pairs 15–48, playing a crucial role in fixing the juxtaposition of domains I (anticodon stem and D-stem) and II (T-stem and acceptor stem) (19). Although nucleotide 57 is typically conserved as a purine and nucleotide 59 can be any nucleotide, the invariant nature of U57 and highly conserved G59 may be essential for tertiary interactions in archaeal $tRNA^{Sec}$. A future structural study of archaeal $tRNA^{Sec}$ would be helpful in making these determinations.

Despite the fact that at present we cannot conclusively state that the length of the acceptor stem is involved in phosphorylation of Ser-$tRNA^{Sec}$ by archaeal PSTK, our data definitively revealed that critical identity elements are found within the acceptor stem. This finding is quite unlike what was shown for eukaryotic-type PSTK and $tRNA^{Sec}$ where neither the sequence nor length of the acceptor stem was important for phosphorylation (8). Mutagenesis of *M. maripaludis* $tRNA^{Sec}$ at the G2-C71 and C3-G70 bp demonstrated their importance for phosphorylation, and transplantation of these base pairs into $tRNA^{Ser}_{UGA}$ along with mutating the A5-U68 antideterminant endowed efficient phosphorylation to the chimeric tRNA. Additionally, we previously demonstrated that $tRNA^{Sec}$ stimulated the ATPase activity of PSTK by a potential induced fit mechanism (15); the fact that our chimeric tRNA unlike $tRNA^{Ser}$ stimulated the ATPase activity of PSTK is further confirmation of the essentiality of the G2-C71 and C3-G70 bp.

A fascinating aspect revealed by this study is the divergence in recognition of archaeal and eukaryal $tRNA^{Sec}$ by archaeal and eukaryal PSTKs; there is precedence for this as aminoacyl-tRNA synthetases from the three domains of life sometimes utilize variant tRNA identity elements (36), and additionally, the tRNA-dependent amidotransferase GatCAB from bacteria and archaea evolved to recognize different elements within $tRNA^{Asn}$ (37,38). While the length and structure of the D-stem of human $tRNA^{Sec}$ were essential for phosphorylation by human PSTK (8), the unusual D-stem of archaeal $tRNA^{Sec}$ only moderately affected phosphorylation by archaeal PSTK, possibly by means of maintenance of proper tertiary structure. On the contrary, invariant base pairs within the acceptor stem of archaeal $tRNA^{Sec}$ were vital for phosphorylation by archaeal PSTK. Moreover, the invariant G2-C71 of archaeal $tRNA^{Sec}$ was found to be highly conserved as C2-G71 in eukaryotic $tRNA^{Sec}$, which resulted in minimal phosphorylation by archaeal PSTK in the context of archaeal $tRNA^{Sec}$. This distinction between archaeal and eukaryotic $tRNA^{Sec}$ corresponds to a deep evolutionary divide between archaeal-type and eukaryotic-type PSTKs (15). While $tRNA^{Sec}$ transverses interactions with a number of enzymes in the selenocysteine biosynthesis pathway, that distinction may indicate co-evolution of archaeal and eukaryotic $tRNA^{Sec}$ with their respective PSTKs.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

We thank Sotiria Palioura, Lennart Randau, Kelly Sheppard and Jing Yuan for helpful discussions and critical reading of the paper. We appreciate the assistance of William B. Whitman in providing us with archaeal $tRNA^{Sec}$ sequences and Ivana Weygand-Durasevic for helpful advice. R.L.S. is the recipient of a Ruth L. Kirschstein National Research Service Award (F32 GM075602) from the National Institute of General Medical Sciences. J.H. holds an Edward A. Bouchet Undergraduate Fellowship from Yale University. This work was supported by grants from the Department of Energy and the National Institute of General Medical Sciences (to D.S.). Funding to pay the Open Access publication charges for this article was provided by National Institute of General Medical Sciences grant GM22854 (to D.S.).

Conflict of interest statement. None declared.

REFERENCES

1. Böck,A., Thanbichler,M., Rother,M. and Resch,A. (2005) In Ibba,M., Francklyn,C. and Cusack,S. (eds), *Aminoacyl-tRNA Synthetases*. Landes Bioscience, Georgetown, TX, pp. 320–327.
2. Bilokapic,S., Korencic,D., Söll,D. and Weygand-Durasevic,I. (2004) The unusual methanogenic seryl-tRNA synthetase recognizes $tRNA^{Ser}$ species from all three kingdoms of life. *Eur. J. Biochem.*, **271**, 694–702.
3. Leinfelder,W., Zehelein,E., Mandrand-Berthelot,M.A. and Böck,A. (1988) Gene for a novel tRNA species that accepts L-serine and cotranslationally inserts selenocysteine. *Nature*, **331**, 723–725.
4. Wu,X.Q. and Gross,H.J. (1993) The long extra arms of human $tRNA^{((Ser)Sec)}$ and $tRNA^{(Ser)}$ function as major identify elements for serylation in an orientation-dependent, but not sequence-specific manner. *Nucleic Acids Res.*, **21**, 5589–5594.

5. Carlson,B.A., Xu,X.M., Kryukov,G.V., Rao,M., Berry,M.J., Gladyshev,V.N. and Hatfield,D.L. (2004) Identification and characterization of phosphoseryl-tRNA$^{[Ser]Sec}$ kinase. *Proc. Natl Acad. Sci. USA*, **101**, 12848–12853.
6. Kaiser,J.T., Gromadski,K., Rother,M., Engelhardt,H., Rodnina,M.V. and Wahl,M.C. (2005) Structural and functional investigation of a putative archaeal selenocysteine synthase. *Biochemistry*, **44**, 13315–13327.
7. Mizutani,T. and Hashimoto,A. (1984) Purification and properties of suppressor seryl-tRNA: ATP phosphotransferase from bovine liver. *FEBS Lett.*, **169**, 319–322.
8. Wu,X.Q. and Gross,H.J. (1994) The length and the secondary structure of the D-stem of human selenocysteine tRNA are the major identity determinants for serine phosphorylation. *EMBO J.*, **13**, 241–248.
9. Xu,X.M., Carlson,B.A., Mix,H., Zhang,Y., Saira,K., Glass,R.S., Berry,M.J., Gladyshev,V.N. and Hatfield,D.L. (2007) Biosynthesis of selenocysteine on its tRNA in eukaryotes. *PLoS Biol.*, **5**, e4.
10. Yuan,J., Palioura,S., Salazar,J.C., Su,D., O'Donoghue,P., Hohn,M.J., Cardoso,A.M., Whitman,W.B. and Söll,D. (2006) RNA-dependent conversion of phosphoserine forms selenocysteine in eukaryotes and archaea. *Proc. Natl Acad. Sci. USA*, **103**, 18923–18927.
11. Carlsen,E.N., Trelle,G.J. and Schjeide,O.A. (1964) Transfer ribonucleic acids. *Nature*, **202**, 984–986.
12. Mäenpää,P.H. and Bernfield,M.R. (1970) A specific hepatic transfer RNA for phosphoserine. *Proc. Natl Acad. Sci. USA*, **67**, 688–695.
13. Hatfield,D., Diamond,A. and Dudock,B. (1982) Opal suppressor serine tRNAs from bovine liver form phosphoseryl-tRNA. *Proc. Natl Acad. Sci. USA*, **79**, 6215–6219.
14. Sharp,S.J. and Stewart,T.S. (1977) The characterization of phosphoseryl tRNA from lactating bovine mammary gland. *Nucleic Acids Res.*, **4**, 2123–2136.
15. Sherrer,R.L., O'Donoghue,P. and Söll,D. (2008) Characterization and evolutionary history of an archaeal kinase involved in selenocysteinyl-tRNA^{Sec} formation. *Nucleic Acids Res.*, doi:10.1093/nar/gkm1134 (Advance access published 3 January 2008).
16. Baron,C., Westhof,E., Böck,A. and Giege,R. (1993) Solution structure of selenocysteine-inserting tRNA^{Sec} from *Escherichia coli*. Comparison with canonical tRNA^{Ser}. *J. Mol. Biol.*, **231**, 274–292.
17. Hubert,N., Sturchler,C., Westhof,E., Carbon,P. and Krol,A. (1998) The 9/4 secondary structure of eukaryotic selenocysteine tRNA: more pieces of evidence. *RNA*, **4**, 1029–1033.
18. Sturchler,C., Westhof,E., Carbon,P. and Krol,A. (1993) Unique secondary and tertiary structural features of the eucaryotic selenocysteine tRNA^{Sec}. *Nucleic Acids Res.*, **21**, 1073–1079.
19. Ioudovitch,A. and Steinberg,S.V. (1999) Structural compensation in an archaeal selenocysteine transfer RNA. *J. Mol. Biol.*, **290**, 365–371.
20. Bullock,T.L., Uter,N., Nissan,T.A. and Perona,J.J. (2003) Amino acid discrimination by a class I aminoacyl-tRNA synthetase specified by negative determinants. *J. Mol. Biol.*, **328**, 395–408.
21. Wolfson,A.D. and Uhlenbeck,O.C. (2002) Modulation of tRNA^{Ala} identity by inorganic pyrophosphatase. *Proc. Natl Acad. Sci. USA*, **99**, 5965–5970.
22. Schneider,K.L., Pollard,K.S., Baertsch,R., Pohl,A. and Lowe,T.M. (2006) The UCSC Archaeal Genome Browser. *Nucleic Acids Res.*, **34**, D407–D410.
23. Laslett,D. and Canback,B. (2004) ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. *Nucleic Acids Res.*, **32**, 11–16.
24. Korencic,D., Polycarpo,C., Weygand-Durasevic,I. and Söll,D. (2004) Differential modes of transfer RNA^{Ser} recognition in *Methanosarcina barkeri*. *J. Biol. Chem.*, **279**, 48780–48786.
25. Ladner,J.E., Jack,A., Robertus,J.D., Brown,R.S., Rhodes,D., Clark,B.F. and Klug,A. (1975) Structure of yeast phenylalanine transfer RNA at 2.5 Å resolution. *Proc. Natl Acad. Sci. USA*, **72**, 4414–4418.
26. Moras,D., Comarmond,M.B., Fischer,J., Weiss,R., Thierry,J.C., Ebel,J.P. and Giege,R. (1980) Crystal structure of yeast tRNA^{Asp}. *Nature*, **288**, 669–674.
27. Quigley,G.J., Seeman,N.C., Wang,A.H., Suddath,F.L. and Rich,A. (1975) Yeast phenylalanine transfer RNA: atomic coordinates and torsion angles. *Nucleic Acids Res.*, **2**, 2329–2341.
28. Brochier,C., Forterre,P. and Gribaldo,S. (2004) Archaeal phylogeny based on proteins of the transcription and translation machineries: tackling the *Methanopyrus kandleri* paradox. *Genome Biol.*, **5**, R17.
29. Hatfield,D.L., Lee,B.J., Price,N.M. and Stadtman,T.C. (1991) Selenocysteyl-tRNA occurs in the diatom *Thalassiosira* and in the ciliate *Tetrahymena*. *Mol. Microbiol.*, **5**, 1183–1186.
30. Bilokapic,S., Maier,T., Ahel,D., Gruic-Sovulj,I., Söll,D., Weygand-Durasevic,I. and Ban,N. (2006) Structure of the unusual seryl-tRNA synthetase reveals a distinct zinc-dependent mode of substrate recognition. *EMBO J.*, **25**, 2498–2509.
31. Fagegaltier,D., Hubert,N., Yamada,K., Mizutani,T., Carbon,P. and Krol,A. (2000) Characterization of mSelB, a novel mammalian elongation factor for selenoprotein translation. *EMBO J.*, **19**, 4796–4805.
32. Forchhammer,K., Leinfelder,W. and Böck,A. (1989) Identification of a novel translation factor necessary for the incorporation of selenocysteine into protein. *Nature*, **342**, 453–456.
33. Rother,M., Wilting,R., Commans,S. and Böck,A. (2000) Identification and characterisation of the selenocysteine-specific translation factor SelB from the archaeon *Methanococcus jannaschii*. *J. Mol. Biol.*, **299**, 351–358.
34. Oshikane,H., Sheppard,K., Fukai,S., Nakamura,Y., Ishitani,R., Numata,T., Sherrer,R.L., Feng,L., Schmitt,E., Panvert,M. *et al.* (2006) Structural basis of RNA-dependent recruitment of glutamine to the genetic code. *Science*, **312**, 1950–1954.
35. Bailly,M., Blaise,M., Lorber,B., Becker,H.D. and Kern,D. (2007) The transamidosome: a dynamic ribonucleoprotein particle dedicated to prokaryotic tRNA-dependent asparagine biosynthesis. *Mol. Cell*, **28**, 228–239.
36. Giege,R., Sissler,M. and Florentz,C. (1998) Universal rules and idiosyncratic features in tRNA identity. *Nucleic Acids Res.*, **26**, 5017–5035.
37. Bailly,M., Giannouli,S., Blaise,M., Stathopoulos,C., Kern,D. and Becker,H.D. (2006) A single tRNA base pair mediates bacterial tRNA-dependent biosynthesis of asparagine. *Nucleic Acids Res.*, **34**, 6083–6094.
38. Namgoong,S., Sheppard,K., Sherrer,R.L. and Söll,D. (2007) Co-evolution of the archaeal tRNA-dependent amidotransferase GatCAB with tRNA^{Asn}. *FEBS Lett.*, **581**, 309–314.
39. Sprinzl,M., Dank,N., Nock,S. and Schon,A. (1991) Compilation of tRNA sequences and sequences of tRNA genes. *Nucleic Acids Res.*, **19**, 2127–2171.