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Identification and characterization of phosphoseryl-tRNA[^Ser]Sec kinase

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In 1970, a kinase activity that phosphorylated a minor species of seryl-tRNA to form phosphoseryl-tRNA was found in rooster liver [Maenpaa, P. H. & Bernfield, M. R. (1970) Proc. Natl. Acad. Sci. USA 67, 688–695], and a minor seryl-tRNA that decoded the nonsense UGA was detected in bovine liver. The phosphoseryl-tRNA and the minor UGA-decoding seryl-tRNA were subsequently identified as selenocysteine (Sec) tRNA[^Ser]Sec, but the kinase activity remained elusive. Herein, by using a comparative genomics approach that searched completely sequenced archaeal genomes for a kinase-like protein with a pattern of occurrence similar to that of components of Sec insertion machinery, we detected a candidate gene for mammalian phosphoseryl-tRNA[^Ser]Sec kinase (pstk). Mouse pstk was cloned, and the gene product (PSTK) was expressed and characterized. PSTK specifically phosphorylated the seryl moiety on seryl-tRNA[^Ser]Sec and, in addition, had a requirement for ATP and Mg^2+. Proteins with homology to mammalian PSTK occur in Drosophila, Caenorhabditis elegans, Methanopyrus kandleri, and Methanococcus jannaschii, suggesting a conservation of its function across archaea and eukaryotes that synthesize selenoproteins and the absence of this function in bacteria, plants, and yeast. The fact that PSTK has been highly conserved in evolution suggests that it plays an important role in selenoprotein biosynthesis and/or regulation.

Selenocysteine (Sec) has its own code word, UGA, and its own tRNA, and therefore is viewed as the 21st amino acid in the genetic code (reviewed in refs. 1–4). Although UGA usually codes for the termination of protein synthesis, it also specifies Sec if specific requirements are met. The presence of a stem-loop structure downstream of UGA, called a Sec insertion sequence (SECIS) element, is the critical component in selenoprotein mRNAs that dictates UGA to code for Sec (reviewed in ref. 5). In mammals, the SECIS element occurs in the 3’ untranslated region of selenoprotein mRNAs. A specific elongation factor, EFsec, specifically recognizes selenocysteinyl-tRNA[^Ser]Sec (6, 7), and a SECIS element binding protein, SBP2, binds specifically to the SECIS element (8), directing the insertion of Sec into protein in response to UGA.

It has been known for several years that the biosynthesis of Sec occurs on its tRNA in both bacteria (9) and mammals (10) after the tRNA is initially aminoacylated with serine by seryl-tRNA synthetase. In Escherichia coli, the pathway for the biosynthesis of Sec has been completely established (reviewed in ref. 1). After the aminoacylation of bacterial tRNA[^Ser]Sec with serine, the hydroxyl group is removed from the seryl moiety to yield an aminocarboxylic intermediate, and this step is catalyzed by a pyridoxal phosphate-dependent Sec synthase. The aminocarboxylic intermediate serves as the acceptor for the activated selenium donor, monoselenophosphate, which is synthesized from selenite and ATP in the presence of selenophosphate synthetase (reviewed in ref. 1). Once selenium is donated to the intermediate, the biosynthesis of Sec on tRNA[^Ser]Sec is complete.

In eukaryotes, however, the biosynthesis of Sec has not been established, but several components have been identified over the years that play a role in this process. For example, in 1970, a minor seryl-tRNA was reported to form phosphoryl-tRNA by a kinase activity from rooster liver (11), and a minor seryl-tRNA from bovine, rabbit, and chicken livers was reported to specifically decode the nonsense codon UGA (12). Although it was subsequently shown that both the minor seryl-tRNA that formed phosphoryl-tRNA and the one that decoded UGA were the same tRNA (13), it was not known at the time these components were discovered that they played instrumental roles in the biosynthesis of Sec and its incorporation into protein as the 21st amino acid in the genetic code. The minor serine tRNA was subsequently found to be selenocysteinyl-tRNA[^Ser]Sec (14, 15), but the role of the putative kinase that phosphorylated seryl-tRNA[^Ser]Sec has not been resolved. Higher vertebrates contain two Sec tRNA[^Ser]Sec isoforms that differ from each other by a single methyl group that occurs at the 2’-hydroxyl of ribose in the wobble position of the anticodon. One isoform, containing 5-methylcarboxymethyluridine (mcm^5U), is the precursor of the other, which contains 5-methylcarboxymethyluridine-2’-O-methylriboside (mcm^5Um) (reviewed in ref. 2).

In the present study, a computational search of several archaeal and eukaryotic genomes for the presence of a kinase-like gene that occurred in those organisms containing the Sec insertion machinery but not in those organisms lacking this machinery revealed a candidate phosphoryl-tRNA[^Ser]Sec kinase (designated pstk). The pstk gene product (PSTK) was expressed and characterized. It indeed specifically phosphorylates seryl-tRNA[^Ser]Sec. These and other properties of PSTK are reported herein.

**Methods**

**Materials.** Selenium-75 (specific activity 1,000 Ci/mmoll; 1 Ci = 37 GBq) was obtained from the Research Reactor Facility, University of Missouri, Columbia. [γ-32P]ATP (specific activity ~6,000 Ci/mmol), [α-32P]ATP (specific activity ~3,000 Ci/mmol), and [3H]serine (specific activity 29 Ci/mmoll) were obtained from Amersham Biosciences. The following were purchased: Ni-NTA (nitrilotriacetate) agarose from Qiagen (Valencia, CA); Pfu DNA polymerase and pBluescript II from Stratagene; the pET-16b expression vector, the pET-32b vector (Valencia, CA); and puReTaq Ready-To-Go PCR Beads and Production Systems from Amersham Biosciences. The following were purchased: [3H]serine (specific activity 29 Ci/mmoll) from Amersham Biosciences. The following were purchased: Ni-NTA (nitrilotriacetate) agarose from Qiagen (Valencia, CA); Pfu DNA polymerase and pBluescript II from Stratagene; the pET-16b expression vector, the pET-32b vector (encoding 109-aa thioredoxin with a His-tag), and BL21(DE3) competent cells from Novagen; polynucleotide kinase and NuPAGE Bis-Tris gels from Invitrogen; alkaline phosphatase from New England Biolabs; T7 RiboMAX Express Large Scale RNA Production Systems and Wizard Genomic DNA Purification Kit from Promega; and puReTaq Ready-To-Go PCR Beads and Production Systems from Amersham Biosciences.

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Abbreviations: Sec, selenocysteine; mcm^5U, 5-methylcarboxymethyluridine; mcm^5Um, 5-methylcarboxymethyluridine-2’-O-methylriboside; PSTK, phosphoryl-tRNA[^Ser]Sec kinase; SECIS, Sec insertion sequence.

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Hyponurus kandleri proteins was searched against complete, annotated archaeal proteomes with the BLASTP program. A portion amplified by using 
Pfu polymerase (1). The sequence of the cDNA construct was confirmed by sequencing upsteam of, and in-frame with, the Flag-tag and the cloning site in which the vector contained a His-tag immediately downstream of, and in-frame with, the Flag-tag and the cloning site in which the vector contained a His-tag immediately downstream of the termination signal (italicized bases) at the carboxyl terminus. The resulting product was cloned into the pET-16b vector at the NdeI restriction site (italicized bases) at the carboxyl terminus. The resulting product was cloned into the pET-16b vector at the NdeI restriction site (italicized bases) at the carboxyl terminus. The resulting product was cloned into the pET-16b vector at the NdeI restriction site (italicized bases) at the carboxyl terminus.

Isolation, Aminoacylation, and Fractionation of Natural and Synthetic tRNA. Total tRNA was isolated from bovine liver and then aminoacylated with 20 unlabeled amino acids or 19 unlabeled amino acids and \( {^3}H \)serine in the presence of a fresh preparation of rabbit reticulocyte aminocyt-tRNA synthetases containing all 20 active synthetases (14). The resulting aminoacyl-tRNAs were fractionated on an RPC-5 column (15) and tRNA was precipitated from individual fractions and collected as described in ref. 14. Both Sec tRNA\([Ser]_{Sec}\) isoforms and serine tRNA\([Ser]_{Ser}\) were purified to homogeneity, as described in ref. 16. The 3'-labeled probe used in detecting seryl-tRNA\([Ser]_{Ser}\) or tRNA\([Ser]_{Sec}\) was described in ref. 17. Synthetic tRNA\([Ser]_{Sec}\) was prepared from the pBluescript II expression vector that encoded the mouse Sec tRNA\([Ser]_{Sec}\) gene. The gene had been amplified by using a PCR and then cloned into the vector. The 3' end of the Sec tRNA\([Ser]_{Sec}\) gene was cut with HindIII and blunt-ended with mung bean nuclease. Sec tRNA\([Ser]_{Sec}\) was transcribed by using the T7 RibomAX Express Large Scale RNA System, and the resulting product was purified according to the manufacturer's instructions. The 5' triphosphate that was present on synthetic tRNA\([Ser]_{Sec}\) was removed by incubation of the freshly synthesized RNA with alkaline phosphatase and isolation of tRNA\([Ser]_{Sec}\) after extraction with phenol and precipitation in ethanol (16).

PSTK Assay. Total aminocyt-tRNA, fractionated aminoacyl-tRNAs, purified seryl-tRNA\([Ser]_{Sec}\), purified tRNA\([Ser]_{Sec}\), synthetic seryl-tRNA\([Ser]_{Sec}\), or purified seryl-tRNA\([Ser]_{Sec}\) was used as a substrate for PSTK in 50 \( \mu \)l of reaction mixture [20 mM Tris-HCl (pH 7.4)/0.01 mM EDTA/1 mM DTT/10 mM MgCl2/50 \( \mu \)M ATP/1 \( \mu \)l of \( \gamma-{^3}P\)ATP (final specific activity \( \sim 600 \text{ Ci} / \text{mmol})/0.5 \mu \text{g of pure PSTK)]. Coupled aminocytlation-phosphorylation reactions involved the same components plus rabbit reticulocyte aminocyt-tRNA synthetases (14). Reactions were stopped for 20 min or at time intervals designated in figures at 30°C and were spotted on P81 phosphocellulose filter paper. The filters were washed four times with 0.75% phosphoric acid (\( \sim 50 \mu \text{m} \) per wash) and dried, and radioactivity was measured in a liquid scintillation counter.

\( \gamma-{^3}P\)phospho[\( {^3}H \)]seryl-tRNA was deacylated, and the products were identified as described in ref. 13. The phosphorylated adenosine product of the kinase reaction was identified by using [\( \alpha-{^3}P\)]ATP and analyzing the \( {^3}P \)-labeled product by TLC. The reversibility of PSTK was assessed by assaying aliquots as described above in reactions with and without ADP. The level of \( \gamma-{^3}P\)-labeled ATP generated from ADP and O-[\( {^{32}P} \)]phospho-lysyl-tRNA\([Ser]_{Ser}\) at the end of the incubation period and the amount of remaining O-[\( {^{32}P} \)]phosphoserine (after deacylation of O-[\( {^{32}P} \)]phospho-lysyl-tRNA\([Ser]_{Ser}\) were determined by analyzing the \( {^{32}P} \) counts in the products (\( \gamma-{^3}P\)ATP and O-[\( {^{32}P} \)]phosphoserine) by cutting out filter strips after TLC (18).

Binding of tRNA to PSTK. PSTK or thioredoxin containing a His-tag, which was used as a control protein, was added in a total volume of 100 \( \mu \)l containing reaction mixture (see above) with or without 50 \( \mu \)M unlabeled ATP and 500 \( \mu \)g of a highly purified sample of a mixture of both tRNA\([Ser]_{Ser}\) isoforms or highly purified tRNA\([Sec]_{Sec}\), tRNA\([Sec]_{Sec}\), or synthetic tRNA\([Ser]_{Sec}\) for 30 min at room temperature. Ni-NTA agarose (20 \( \mu \)l) was added to pull down PSTK or the control protein. After washing three times with 1 ml of 1X TBS/0.1% Tween, the beads were suspended in 40 \( \mu \)l of TBE-urea loading buffer (90 mM Tris/64.6 mM boric acid/2.5 mM EDTA/3.5 M urea, pH 8.3), and 5 \( \mu \)l of each sample was loaded onto a 15% TBE-urea gel. After electrophoresis and transfer of the RNA to a nylon membrane, RNA was detected by Northern blotting with the appropriate probe (17).
Results

An amino-terminal peptide of mouse PSTK (Fig. 2B, lane 1) or exposed to antibodies generated against an amino-terminal peptide of mouse PSTK (Fig. 2B, lane 2).

To test this hypothesis, we attempted to identify a putative kinase-like protein that is encoded in Methanopyrus kandleri and Methanococcus jannaschii genomes, the two archaea that encode the Sec insertion machinery (19, 20), but is absent in 12 other completely sequenced archaeal genomes that do not code for the Sec insertion machinery. The search, performed as described in Methods, yielded only 27 predicted ORFs common to Methanococcus jannaschii, Methanopyrus kandleri, and C. elegans, with the exception of seven bases at the amino terminus. As described in Methods, the missing bases were inserted at the 5′ end (Fig. 2A). The pstk coding sequence is 1,080 nucleotides in length and encodes a 359-aa protein.

Expression and Isolation of PSTK. PSTK, after its expression and purification (see Methods), was run on a denaturing gel, and, after electrophoresis, the gel was either stained with Coomassie blue (Fig. 2B, lane 1) or exposed to antibodies generated against an amino-terminal peptide of mouse PSTK (Fig. 2B, lane 2). This highly purified sample of PSTK was used for subsequent characterization.

PSTK Antibodies. Polyclonal antibodies against PSTK were generated in rabbits by Spring Valley Laboratories (Woodbine, MD) by using a peptide encoding a conjugated predicted amino-terminal epitope region (GATRRDGQPKLGLC-OH) as antigen.

Results

Computational Search for a Sec Kinase. The requirements for Sec biosynthesis appear to be similar in eukaryotes and archaea in regard to the location of SECIS elements in the 3′ untranslated region and the separation of functions for SECIS binding and Sec insertion in separate proteins in these organisms (2–4). In contrast, in bacteria, the SECIS elements are located immediately downstream of the UGA Sec codon, and SECIS binding and Sec insertion functions are carried out by a single protein, designated SelB (1). We therefore hypothesized that the formation of phosphoserine on Sec tRNA might also be conserved between eukaryotes and archaea.

To test this hypothesis, we attempted to identify a putative kinase-like protein that is encoded in Methanococcus jannaschii and Methanopyrus kandleri genomes, the two archaea that encode the Sec insertion machinery (19, 20), but is absent in 12 other completely sequenced archaeal genomes that do not code for the Sec insertion machinery. The search, performed as described in Methods, yielded only 27 predicted ORFs common to Methanococcus jannaschii, Methanopyrus kandleri, and C. elegans, with the exception of seven bases at the amino terminus. As described in Methods, the missing bases were inserted at the 5′ end (Fig. 2A). The pstk coding sequence is 1,080 nucleotides in length and encodes a 359-aa protein.

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more slowly over the course of the reaction period (Fig. 3B). The data demonstrated that the candidate PSTK phosphorylated seryl-tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\).

Several divalent cations were examined to assess their requirement for kinase activity (Fig. 3C). Although there was no activity at 0.01 mM with any of the three divalent cations examined, a small amount of activity occurred at 0.1 mM. At higher levels, Mg\(^{2+}\) was found to be essential for PSTK activity, and the maximal activity level occurred at 1.0 mM. Mn\(^{2+}\) could replace Mg\(^{2+}\) as a divalent metal cation, but it was not as efficient as Mg\(^{2+}\).

To examine the reversibility of the kinase reaction, O-\(^{32P}\)phosphoseryl-tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\) was incubated in a mixture with Mg\(^{2+}\) and PSTK and with or without 50 μM ADP, and the loss of \(^{32P}\) was monitored over a 20-min period (Fig. 3D). In the absence of ADP, ~18% of the total O-phosphoseryl-tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\) was lost, whereas ~45% was lost in the presence of ADP. These data suggest that ~30% of O-phosphoseryl-tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\) was converted to seryl-tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\) and ATP during the course of the reaction. This result was confirmed by determining the \(^{32P}\) radioactivity present in these two components at the end of the incubation period (Fig. 3D). Comparison of the reversibility of the aminocyclation and phosphorylation reactions suggests that attachment of the phosphate moiety to serine enhances the stability of this aminoacyl group on tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\).

**PSTK Reaction Products.** As shown above, the starting components in the PSTK reaction were tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\), ATP, and Mg\(^{2+}\). However, it was important to also determine the products in the reaction. Clearly, the γ-phosphate group on ATP was transferred to seryl-tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\) to yield O-phosphoseryl-tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\). To verify that the adenosine phosphate product is ADP, we used [α-\(^{32P}\)]ATP as a substrate in place of [γ-\(^{32P}\)]ATP and identified the adenosine phosphate product as ADP by TLC (see Methods; data not shown).

Furthermore, deacylation of [\(^{32P}\)phospho\(^{3H}\)]seryl-tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\) and chromatography of the resulting amino acid before and after treatment with alkaline phosphatase demonstrated that the deacylated product migrated with O-phosphoserine, whereas the alkaline phosphatase-treated products migrated with inorganic phosphate (\(^{32P}\)) and serine (\(^{3H}\)) (data not shown).

Because tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\) is the only known tRNA to begin transcription within the coding sequence (2) and therefore has a 5′ triphosphate, we examined whether the presence or absence of the 5′ triphosphate had any effect on PSTK activity and whether PSTK could possibly replace a phosphate group at the 5′ end. The presence or absence of the 5′ triphosphate had no effect on PSTK activity, and PSTK did not replace the 5′ phosphate group (data not shown).

**PSTK Specificity.** To determine the specificity of PSTK, total aminoacyl-tRNA was fractionated on an RPC-5 column as shown in Fig. 4. The two major Sec tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\)isoforms are known to be hydrophobic and elute late from the column (see ref. 17 and references therein). Their elution in the latter portion of the column run was confirmed by assaying individual fractions with a \(^{32P}\)-labeled probe that specifically recognized Sec tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\). Both isoforms eluted within column fractions 50 and 72. Assay of the column fractions with PSTK and [γ-\(^{32P}\)]ATP demonstrated that only the two Sec tRNA\(^{\text{Ser}}\)\(^{\text{Sec}}\)
isoforms became labeled, providing strong evidence that the kinase is specific for these tRNAs. In addition, seryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] did not serve as a substrate for PSTK.

**Discussion**

PSTK was originally discovered >30 years ago when a kinase activity was detected in rooster liver that catalyzed the formation of phosphoseryl-tRNA on a minor isoform of the total seryl-tRNA population (11). Since this original report, the identity, isolation, and cellular role of PSTK, as well as phosphoseryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\], have remained largely obscure. The minor phosphoseryl-tRNA and another minor seryl-tRNA identified in bovine, rabbit, and chicken livers that decoded the stop codon UGA (12) were shown to be Sec tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] (13). It was subsequently reported that phosphoseryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] as the intermediate in the biosynthesis of Sec in mammals (21, 22) and in bacteria (23). However, after the identification of aminoacyl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] as the correct intermediate in bacteria (reviewed in ref. 1), it was reported that phosphoseryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] was not an intermediate in Sec biosynthesis in mammals and that Sec biosynthesis proceeded by the same pathway as that in bacteria (24, 25). The intermediate in the latter studies, however, was not identified, nor was phosphoseryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] ruled out as a possible intermediate. Thus, the biosynthesis of Sec in mammalian cells and the role of phosphoseryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] have not been resolved. It should be noted that the identity elements in Sec tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] for PSTK have been identified and found to reside primarily in the secondary structure and length of the D-stem (26).

In the present study, PSTK was identified by using an algorithm that searched for a kinase-like protein gene in the genomes of archaea that are known to encode the Sec insertion machinery vs. those that do not and then comparing the purported kinase gene sequences to genes manifesting homology in eukaryotes that do and do not contain the Sec insertion machinery. The single candidate gene that was found in the genomes of two archaea and in eukaryotes containing the ability to insert Sec into proteins, but not in those that do not, was further characterized. After its expression in bacteria, it was isolated as a pure protein, and *in vitro* assays confirmed that this purported kinase was indeed PSTK. PSTK was found to be specific for seryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\], whereas no activity with other tRNAs was detected.

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**Fig. 4.** Specificity of PSTK. Total calf liver tRNA that had been aminoacylated with all 20 unlabeled amino acids in the presence of rabbit reticulocyte synthetases was fractionated on an RPC-5 column (total of 150 A\textsubscript{260} units). The aminoacyl-tRNAs in individual fractions were precipitated, the precipitants were collected and redissolved, and an aliquot was spotted on a nitrocellulose filter and hybridized with a specific Sec tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] probe or added to a reaction containing PSTK and the components for measuring kinase activity. Arbitrary units represent either the \(^{32}\)P determined in the kinase reaction by measuring radioactivity of samples in a scintillation counter (e.g., 10,000 cpm = 10,000 arbitrary units) or the PhosphorImager units measured with \(^{32}\)P-labeled probe in a PhosphorImager (Molecular Dynamics) (e.g., 10,000 PhosphorImager units = 400 arbitrary units).

**Fig. 5.** Interaction of tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] and PSTK. (A) Binding of tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] to PSTK. Synthetic seryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] was used in lanes 1–3, synthetic tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] in lanes 3–6, a mixture of both naturally occurring seryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] isoforms in lanes 7–9, a mixture of both naturally occurring tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] isoforms in lane 10, tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] in lane 11, and tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] in lane 12. The control protein was thioredoxin. Reaction mixtures were incubated, the proteins were pulled down with Ni-NTA agarose, and Northern blots were prepared as described in Methods. Each reaction was carried out at least three times, yielding similar results, and representative samples are shown. (B) Effect of tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] on phosphorylation. Synthetic \[^{3}\]H]seryl-tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] (2 \(\mu\)g) was added to PSTK reactions with increasing concentrations of synthetic tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] as indicated. The percent aminoacylation used in reactions was 62%, and this amount of unacylated tRNA\[^{\text{Ser}}\]\[^{\text{Sec}}\] (0.76 \(\mu\)g) was taken into account, as indicated in the initial reaction.
PSTK catalyzed the formation of phosphoseryl-tRNA\[Ser\]Sec from seryl-tRNA\[Ser\]Sec and ATP in the presence of Mg\(^{2+}\). Although Mn\(^{2+}\) can also serve as a divalent metal cation for the reaction, it was not as effective as Mg\(^{2+}\). The products were identified as O-phosphoseryl-tRNA\[Ser\]Sec and ADP, and the reaction was found to be reversible. Therefore, the reaction can be written as

\[
\text{seryl-tRNA}[\text{Ser}],[\text{Sec}] + \text{ATP} \xrightarrow{\text{PSTK}, \text{Mg}^{2+}} O\text{-phosphoseryl-tRNA}[\text{Ser}],[\text{Sec}] + \text{ADP}
\]

In a binding assay, either tRNA\[Ser\]Sec or seryl-tRNA\[Ser\]Sec bound to PSTK, but inclusion of ATP with the two tRNA substrates had different effects. ATP appeared to enhance the binding of tRNA\[Ser\]Sec but decrease the binding of seryl-tRNA\[Ser\]Sec. The decreased binding of seryl-tRNA\[Ser\]Sec was likely due to its conversion to phosphoseryl-tRNA\[Ser\]Sec, which was found to have low affinity for PSTK. Interestingly, tRNA\[Ser\]Sec was a potent inhibitor of the phosphorylation reaction, and although the significance of this inhibition is unclear, PSTK will likely be inhibited significantly when selenoprotein biosynthesis is down-regulated and the unacylated Sec isoforms are enriched. PSTK has been highly conserved in evolution in genomes that encode the machinery for Sec insertion into protein, because it was found in archaea and numerous lower and higher animals containing this machinery. What, then, is the possible role of PSTK and phosphoseryl-tRNA\[Ser\]Sec in cellular metabolism? It has been speculated that phosphoseryl-tRNA\[Ser\]Sec may serve as an active storage form which can, after its dephosphorylation, be re-generated as seryl-tRNA\[Ser\]Sec for the biosynthesis of Sec (27). Several lines of evidence argue against this proposal. For example, the poor rate of dephosphorylation of phosphoseryl-tRNA\[Ser\]Sec by PSTK observed in the present study suggests that the phosphate group cannot readily be removed, as has similarly been found for other kinases, unless the unlikely possibility of a specific phosphatase might exist. Furthermore, seryl-tRNA\[Ser\]Sec is an authentic suppressor tRNA that decodes UGA stop codons in vivo (28), and phosphoseryl-tRNA\[Ser\]Sec is capable of decoding UGA in vitro (29), suggesting that these forms of tRNA\[Ser\]Sec are likely maintained intracellularly only transiently as substrates for other reactions. Seryl-tRNA\[Ser\]Sec is a substrate for PSTK, and if the product of this reaction is not an intermediate in the biosynthesis of Sec, then seryl-tRNA\[Ser\]Sec would also be used in Sec biosynthesis by another route. In any case, it is easy to visualize that seryl-tRNA\[Ser\]Sec is, and must be, ephemeral.

The fact that PSTK has been conserved in evolution as one of the components of the Sec insertion machinery also strongly argues that it must play an important role in Sec incorporation into protein and that it has not been conserved solely for converting seryl-tRNA\[Ser\]Sec to a storage form. If phosphoseryl-tRNA\[Ser\]Sec is used ephemerally, as proposed above for seryl-tRNA\[Ser\]Sec, then it would most likely be used as an intermediate in the biosynthesis of Sec (reviewed in ref. 2). Indeed, the phosphate moiety of phosphoseryl-tRNA\[Ser\]Sec would make an excellent leaving group for replacement with selenium. If it is an intermediate in the biosynthesis of Sec, then it, too, would likely exist only ephemerally intracellularly. In the event phosphoseryl-tRNA\[Ser\]Sec is not an intermediate in the biosynthesis of Sec, and if it does not exist as a storage form for Sec-tRNA\[Ser\]Sec, additional hypotheses must be advanced to address its function. Like the serylated form of tRNA\[Ser\]Sec (6), phosphoseryl-tRNA\[Ser\]Sec does not bind to EF\(\text{Sec}\) (Z. Stoytcheva and M.J.B., unpublished work), and therefore, it cannot be used directly in incorporating phosphoseryl into protein through EF\(\text{Sec}\) and UGA. Although several important steps in the identity and characterization of \(\text{pstk}\) and PSTK have been carried out in the present study, the function of this kinase and its biosynthetic product, phosphoseryl-tRNA\[Ser\]Sec, must await future studies.

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