On the road to selenocysteine

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Decades ago, a mammalian tRNA that exclusively bound the UGA stop codon was identified by using the triplet binding assay that was instrumental in deciphering the genetic code (1). The possibility of a naturally occurring suppressor tRNA presented the dilemma of explaining the biological function of such a molecule. In the same year, an equally perplexing report indicated the existence of tRNA from either rooster or rat liver that was aminoacylated with phosphoserine (2). That observation was verified several years later when phosphoseryl tRNA was also identified from lactating bovine mammary glands (3). Phosphoserine was well established to be present in proteins at that time, but the synthesis of this amino acid was known to occur posttranslationally, subsequent to the insertion of serine in response to one of the six serine codons. Not until several years later was it discovered that these two tRNAs, that which recognized UGA and that which formed phosphoseryl tRNA, were one and the same (4), and that this tRNA was atypical with respect to structure, size, and modified base content (5). The mystery as to the biological role this molecule played was solved with the determination that this tRNA could support the synthesis of the selenium-containing amino acid selenocysteine (Sec) (6), which addressed yet another perplexing observation, the existence of Sec-containing proteins in which that amino acid was encoded by the UGA codon, the first mammalian example being glutathione peroxidase (7). As the pieces of the puzzle of selenoprotein biosynthesis fell into place, the role of phosphoseryl-tRNA remained obscure, its low relative abundance raising the issue of whether it was an anomaly without physiological significance. In a recent issue of PNAS, Carlson *et al*. (8) employed a clever comparative genomics approach to identify a kinase that is most likely dedicated to Sec synthesis, providing the missing link in the route from serine to Sec in eukaryotes and archaea, as well as adding a new player to the translation "team" whose sole purpose is to produce selenoproteins.

Carlson *et al*. (8) provide data indicating that PSTK is the kinase that phosphorylates seryl-tRNA$^{[Ser]Sec}$ *in vivo*. Studies using bacterially synthesized, purified PSTK established the substrate specificity of this enzyme exclusively for both seryl-tRNA$^{[Ser]Sec}$ isoforms, which differ by only a single modified base (5). It is highly significant that the initial method of detection, a computer-assisted analysis of genomes that were known either to contain or to be missing the selenoprotein synthesis machinery, was successful at all. The presence of PSTK in archaeal and eukaryote genomes that were known to translationally synthesize selenoproteins, but its absence in the genomes of organisms that do not, is consistent with the role of PSTK indicated by Carlson *et al*. (8). This correlation also argues for a dedicated role of this kinase in selenoprotein synthesis and the absence of closely related, yet distinct, kinases. With the characterization of PSTK, the remarkable list of cellular components whose biological role is apparently restricted to the synthesis of mammalian selenoproteins is expanded.

Biosynthesis of selenocysteine from serine in mammalian cells is atypical among eukaryotic tRNAs.

Sec typically, but not exclusively, is present at the active site of selenium-containing enzymes across evolution. There are several proteins in bacteria that contain UGA-encoded Sec, and these have been well characterized. The list includes glycine and proline reductases, as well as formate and formylmethanofuran dehydrogenases (for a review, see ref. 9). In fact, genetic analyses of bacterial mutants unable to synthesize Sec-containing formate dehydrogenases resulted in the identification of the components of the prokaryotic selenoprotein biosynthesis machinery (10).

In contrast to the relatively few prokaryotic selenoproteins, there are several dozen selenoproteins in archaea and eukaryotes, with a comprehensive computational analysis of the human genome identifying 25 selenoproteins (11). These include a diverse collection of proteins, with families of related enzymes with antioxidant capabilities (glutathione peroxidases) and those involved with the maintenance of the reduced cellular environment (thioredoxin reductases) and the maturation of thyroxin (thyroid hormone deiodinases). Other mammalian selenoproteins of known function include SelP, which is involved with selenium transport; SPS2, which is required for Sec synthesis; and SelR, a methionine sulfoxide reductase (reviewed in ref. 12). Although the biochemical properties (i.e., cellular location and binding partners) are known for several other selenoproteins, their biological roles await further investigation. Genetic data have also implicated at least three selenoproteins in human disease: SelN1 in muscular dystrophy (13) and GPX1 (14–16) and Sep15 in cancer etiology (17, 18). It is also likely that multiple biological roles for individual selenoproteins will be identified: one of the most interesting examples to date is the dual role of GPX4 as a membrane-located antioxidant enzyme and as a major sperm structural protein (19).

Just as there are clear evolutionary differences among the functions of selenoproteins, the results presented by Carlson *et al*. (8) highlight the evolutionary differences in the process of selenoprotein synthesis between prokaryotes and eukaryotes. Although commonalities exist, such as the use of UGA to encode Sec, the use of dedicated tRNA$^{[Ser]Sec}$, and translational elongation factors, differences, such as the location of the SECIS element 3′ and adjacent to the Sec-encoding UGA triplet in prokaryotes and in the 3′ UTR in eukaryotes and the need for an additional protein, SBP2, in eukaryotes, also are apparent (reviewed in ref. 12). In prokaryotes, an aminoacrylyl intermediate between the conversion from serine to Sec was identified (20). Now, with the identification of PSTK and the all-but-certain assignment of a phosphoserine intermediate in eukaryotic Sec synthesis, an additional difference can be catalogued.

The resolution of the biochemical pathway by which serine is converted to Sec in mammals is also a reminder

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of the highly unusual dual role played by $tRNA^{[Ser]Sec}$. The biosynthesis of Sec from serine via phosphoserine in mammalian cells is certainly atypical among eukaryotic tRNAs in that $tRNA^{[Ser]Sec}$ serves as a scaffold for the synthesis of an amino acid that ultimately and specifically finds its way into protein (this is in addition to the role of $tRNA^{[Ser]Sec}$ as the UGA-recognizing adaptor).

As pointed out by Carlson *et al*. (8), the functional implications of a phosphoseryl-$tRNA^{[Ser]Sec}$ intermediate remain to be determined. Did this pathway evolve just to take advantage of the chemistry of phosphorylation and serine activation, or are there other cellular benefits? Because of the challenge created by UGA's functioning as both a stop and Sec codon, as well as the benefits of the selenoprotein biosynthesis machinery's responsiveness to selenium availability, selenoprotein synthesis is highly regulated, at the levels of both transcription and translation. It will be particularly interesting to determine whether PSTK activity is expressed in a tissue-specific manner and whether it is influenced by cellular or environmental signals.

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