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Review

On elongation factor eEFSec, its role and mechanism during selenium incorporation into nascent selenoproteins

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ABSTRACT

Background: Selenium, an essential dietary micronutrient, is incorporated into proteins as the amino acid selenocysteine (Sec) in response to in-frame UGA codons. Complex machinery ensures accurate recoding of Sec codons in higher organisms. A specialized elongation factor eEFSec is central to the process.

Scope of review: Selenoprotein synthesis relies on selenocysteinyl-tRNA^{Sec} (Sec-tRNA^{Sec}), selenocysteine inserting sequence (SECIS) and other selenoprotein mRNA elements, an *in-trans* SECIS binding protein 2 (SBP2) protein factor, and eEFSec. The exact mechanisms of discrete steps of the Sec UGA recoding are not well understood. However, recent studies on mammalian model systems have revealed the first insights into these mechanisms. Herein, we summarize the current knowledge about the structure and role of mammalian eEFSec.

Major conclusions: eEFSec folds into a chalice-like structure resembling that of the archaeal and bacterial orthologues SelB and the initiation protein factor IF2/eIF5B. The three N-terminal domains harbor major functional sites and adopt an EF-Tu-like fold. The C-terminal domain 4 binds to Sec-tRNA^{Sec} and SBP2, senses distinct binding domains, and modulates the GTPase activity. Remarkably, GTP hydrolysis does not induce a canonical conformational change in eEFSec, but instead promotes a slight ratchet of domains 1 and 2 and a lever-like movement of domain 4, which may be critical for the release of Sec-tRNA^{Sec} on the ribosome.

General significance: Based on current findings, a non-canonical mechanism for elongation of selenoprotein synthesis at the Sec UGA codon is proposed. Although incomplete, our understanding of this fundamental biological process is significantly improved, and it is being harnessed for biomedical and synthetic biology initiatives. This article is part of a Special Issue entitled "Selenium research" in celebration of 200 years of selenium discovery, edited by Dr. Elias Arnér and Dr. Regina Brigelius-Flohe.

1. Introduction

Intricate machinery is responsible for Sec synthesis and recoding of the Sec UGA codon. Selenium (Se), an essential dietary micronutrient, is found in two dozen human selenoproteins and selenoenzymes [1] that are pivotal for protecting the cell membrane and DNA from oxidative damage, maintaining redox balance and selenium homeostasis, and aiding protein folding and gene expression [2–4]. The biological function of Se is primarily exerted in a form of the amino acid, selenocysteine (Sec), which is required for function and structure of all selenoproteins and selenoenzymes. An intricate process evolved over time that orchestrates extraction of Se from various metabolites, incorporation of Se into Sec, and precise insertion of Sec into the growing selenoprotein chain.

The remarkable ability of Se to alternate between the oxidized and reduced states with ease [5] provides the basis for catalytic superiority of selenoenzymes over the thiol containing counterparts. Whereas thiol-based enzymes are often inactivated through irreversible oxidation and thus require specific enzymes to revert the abysmal situation, selenium-containing enzymes escape such a trap. This resistance to irreversible oxidation makes selenoenzymes advantageous in redox reactions and explains why they are so crucial for the removal of reactive oxygen species. Failure to incorporate Sec diminishes the catalytic prowess of selenoenzymes [6–14] and compromises the structure of selenoproteins, and introduction of Sec improves function of artificial selenoenzymes and confers resistance to inactivation by oxidation (reviewed in [5]). Consistent with its redox and antioxidant function, levels of many selenoprotein genes and selenoproteins are significantly increased under oxidative stress [15,16]. Disruption or deletion of genes encoding selenocysteine tRNA (tRNA^{Sec}), glutathione peroxidase 4 (GPx4), and thioredoxin reductase 1 (Trx1) and 3 (Trx3) causes embryonically lethal phenotypes in mice, accentuating the significance of

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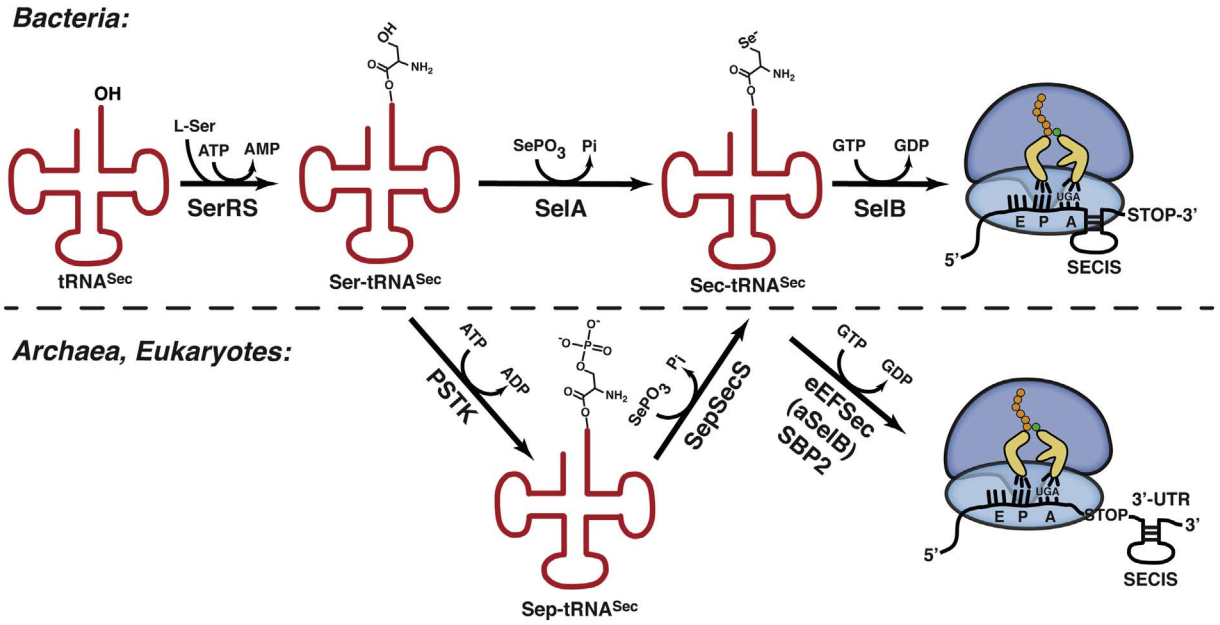

Fig. 1. Synthesis and co-translational incorporation of Sec across kingdoms of life. (Top) The bacterial process is initiated with serylation of $tRNA^{Sec}$. SelA then directly catalyzes Ser-to-Sec conversion. In the end, SelB delivers Sec-$tRNA^{Sec}$ to the 70S ribosome in response to an in-frame Sec UGA. The bacterial SECIS, which is in ORF and immediately downstream of the Sec codon, coordinates recoding. (Bottom) As in bacteria, the process begins with $tRNA^{Sec}$ serylation. However, here the Ser-to-Sec conversion proceeds in two steps: PSTK phosphorylates the Ser group and SepSecS then promotes conversion of Sep-$tRNA^{Sec}$ to Sec-$tRNA^{Sec}$. eEFSec and aSelB promote recoding in eukaryotes and archaea, respectively. In both instances, the 3'-UTR SECIS element coordinates the recoding step. The distinction is that only eukaryotes rely on SBP2 protein factor.

maintaining selenoproteome integrity [17–20]. Furthermore, mutations in enzymes facilitating Sec and selenoprotein synthesis cause systemic disorders [3,4,21–27], including severe early childhood degeneration of the human brain [28–30]. Recently, a mutation in $tRNA^{Sec}$ has been shown to cause deficit in stress-related, but not housekeeping, selenoproteins resulting in a complex clinical profile [31]. Lastly, Se deficiency, low levels of selenoproteins, and mutations in selenoprotein genes cause pathologies of cardiac, muscular, nervous, endocrine, immune, and reproductive systems (reviewed in [2,32–39]). Thus, faithful insertion of Se and Sec into proteins is an essential biological process. Surprisingly, this process is still poorly understood [4,40] and is largely being modeled using the general mechanism of bacterial protein synthesis (reviewed in [41]). While these models permit extrapolations to be drawn, they are often insufficient in their capacity to explain observations and, even worse, may be misleading. The absence of detailed models of selenoprotein synthesis is a consequence of only recent focus on the system and the sheer complexity of the Sec system which presents a number of technical and intellectual challenges.

The Sec UGA recoding process is intimately interlocked with the elaborate cycle of Sec synthesis (Fig. 1). This anabolic cycle provides the obligate substrate for selenoprotein synthesis, but it is so embroidered with particularities that it is prudent to provide a brief mention. In a classical case, proteinogenic amino acids are coupled to cognate tRNAs via specific aminoacyl-tRNA synthetases (aaRSs). However, in case of Sec, indirect aminoacylation is the only pathway to its synthesis and subsequent incorporation into protein. This is because the putative SecRS that would attach Sec onto $tRNA^{Sec}$ is the only aaRS that never arose during evolution. Also, a cellular pool of free Sec necessary for the direct coupling does not exist due to its high reactivity. Hence, each organism that relies on Sec had to devise a different mechanism for Sec synthesis. The solution involves a multistep process during which Sec is synthesized from a serine (Ser) precursor on the cognate tRNA while utilizing a Se donor molecule [42]. In all organisms, the process begins with $tRNA^{Sec}$ serylation (Fig. 1), which is catalyzed by a promiscuous SerRS (acts primarily on $tRNA^{Ser}$). Subsequently, a single bacterial Sec synthase (SelA) directly converts the seryl moiety to Sec in a reaction mechanism that requires a pyridoxal phosphate (PLP) co-factor and selenophosphate [43] (top panel, Fig. 1). Conversely, in archaea and eukaryotes, the conversion occurs in two steps (bottom panel, Fig. 1). First, a specialized kinase (PSTK) phosphorylates Ser [44,45], and then, the Sec synthase (SepSecS) replaces the phosphoryl group with a selenol moiety using the mechanism analogous to that of the bacterial SelA [44,46,47]. To ensure process fidelity, Sec synthetic enzymes recognize and 'read' the distinct fold and structure of $tRNA^{Sec}$ [43,48–51], the largest and truly remarkable molecule among elongator tRNAs. But where does Se come from and in what form for reactions catalyzed by SelA and SepSecS? Another PLP enzyme, Sec lyase, extracts Se from Sec [52] and releases selenide, which is then used by selenophosphate synthetase (SPS or SPS2) to form selenophosphate [53,54], the major Se donor. In the end, the process achieves its goals: Sec is 'attached' to its tRNA while circumventing both the need for free Sec and the absent SecRS.

Once formed, Sec-$tRNA^{Sec}$ is delivered to the ribosome in response to a context-dependent in-frame UGA codon (Fig. 1). The delivery and recoding is delegated to a specialized elongation factor, SelB in prokaryotes [55] and eEFSec in eukaryotes [56], which evolved to bind only Sec-$tRNA^{Sec}$ and no other aa-tRNA. Initially, the name of the bacterial *selB* gene was born out of a set of pleiotropic, and at the time unmapped, mutations in *E. coli* that had caused inactivation of formate dehydrogenase [57]. One of these later turned out to be the Sec elongation factor and the name SelB was then used irrespective of the organism. It is now more widely accepted that SelB is used when bacterial and archaeal Sec systems are discussed, and that eEFSec is used in conjunction with the eukaryotic process. Hence, to simplify matters, in the next few sections we shall use SelB, aSelB, and eEFSec for bacterial, archaeal, and eukaryotic Sec elongation factors, respectively.

The deletion of *EEFSEC* obliterates selenoprotein synthesis in fruit flies [58], further emphasizing the significance of this specialized elongation factor. Intriguingly, eEFSec and SelB cannot recode the Sec UGA on its own. Instead, they require an accessory RNA and/or protein factors. The first such element identified was designated as the SElenoCysteine Insertion Sequence (SECIS). This *in-cis* element forms a hairpin structure and is present in selenoprotein mRNAs across kingdoms [59–62]. In bacteria, it resides in an open reading frame (ORF)