Chapter 5
Prokaryotic Selenoprotein Biosynthesis and Function

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Abstract Selenoproteins, i.e., proteins containing selenocysteine (Sec), are found in organisms of all three domains of life, *Eukarya*, *Bacteria* and *Archaea*. Sec is mostly present in the catalytic site of redox-active enzymes with diverse biological functions. The pathway for Sec synthesis and incorporation into proteins during translation differs from that of the 20 canonical amino acids, as Sec is synthesized on its transfer RNA (tRNA) and encoded by UGA, which normally signals termination of translation. The pathway of Sec biosynthesis and incorporation is well understood in Gram-negative bacteria, but considerable gaps of knowledge still exist in the respective systems of Gram-positive bacteria and *Archaea*. This chapter summarizes prokaryotic selenoprotein synthesis and function with a focus on emphasizing the differences between bacterial and archaeal pathways.

Keywords *Archaea* • *Bacteria* • Recoding • Selenocysteine • Selenoprotein • Translation

5.1 Introduction

Selenium was first isolated in lead chambers of a sulphuric acid production factory and named after the Greek goddess of the moon, Selene, by the Swedish chemist Jöns Jacob Berzelius in the early nineteenth century [1]. Initially, selenium was considered to be toxic before its beneficial qualities were recognized in the 1950s. Today, selenium is regarded as an essential trace element for many organisms due to the important roles of selenium-containing cellular macromolecules.

Despite selenium's narrow biologically beneficial "window" for most organisms (deficiency below approximately 0.05 ppm and toxicity above approximately 5 ppm in mammals [2]) and the low environmental concentrations of bioavailable selenium species (typical environmental concentrations of selenate ranging from 0.1 to 20 nM), it is still not clear if organisms requiring selenium possess a dedicated transporter and what selenium species is transported into the cell. For *Escherichia* (*E.*)

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coli, the specific incorporation of selenium into macromolecules is already saturated at 0.1 μM selenite in the medium [3]; still, it is not known how selenium is transported across the cytoplasmic membrane in *E. coli*. It was recently suggested that the putative membrane protein, YedE, which contains two sulfur transport domains and is encoded in an operon with predicted sulfurtransferase, YedF, may serve as a selenium-specific transporter because it occurs only in selenium-utilizing organisms [4]. However, this possibility needs to be experimentally verified. At higher concentrations, *E. coli* metabolizes selenium via the sulfur assimilation pathway [5].

Biologically active selenium occurs in three forms: (1) as a constituent of a base modification (5-[(methylamino)methyl]-2-selenouridine) in certain transfer RNAs (tRNAs, [6]; (2) as a non-covalently bound selenium-containing cofactor [7]; and (3) as the cotranslationally inserted amino acid selenocysteine (Sec). In this chapter, only Sec will be considered.

5.2 Selenocysteine

Sec is by far the best-investigated form of biologically active selenium. It was discovered in 1976 [8] and only 10 years later it was found that Sec is co-translationally inserted into growing polypeptides at the position of an in-frame UGA (opal) nonsense codon in mRNA [9, 10], which led to its designation as the 21st genetically encoded amino acid.

Proteins containing Sec, designated selenoproteins, are found in members of all three domains of life, *Bacteria*, *Archaea*, and *Eukarya*, with Sec almost exclusively located in the catalytic site of redox-active enzymes. Why organisms employ Sec is still not fully understood because: (1) the majority of known organisms do well without Sec; (2) the specific functions of Sec during catalysis are still unknown for most selenoproteins; and (3) homologous proteins with cysteine (Cys) at the respective position exist for all but one selenoprotein, clostridial glycine reductase, in prokaryotes [11]. Still, the use of Sec might be explained by its physico-chemical properties [12]. Its high nucleophilicity and the fact that the selenol group is mostly deprotonated at physiological pH due to its lower pk_a value (5.2 for Sec, 8.3 for Cys) makes Sec more reactive than Cys.

5.3 Selenoprotein Synthesis in Bacteria

5.3.1 Sec Biosynthesis and Incorporation in E. coli

The mechanisms of Sec biosynthesis and its incorporation into selenoproteins (Fig. 5.1a) were first elucidated in *E. coli*. Via genetic screening, four genes were identified, *selA*, *selB*, *selC*, and *selD*, that affected selenium metabolism [13]. *selC* encodes the Sec-specific tRNA ($tRNA^{Sec}$, $tRNA_{UCA}$) [14]. Besides being the longest

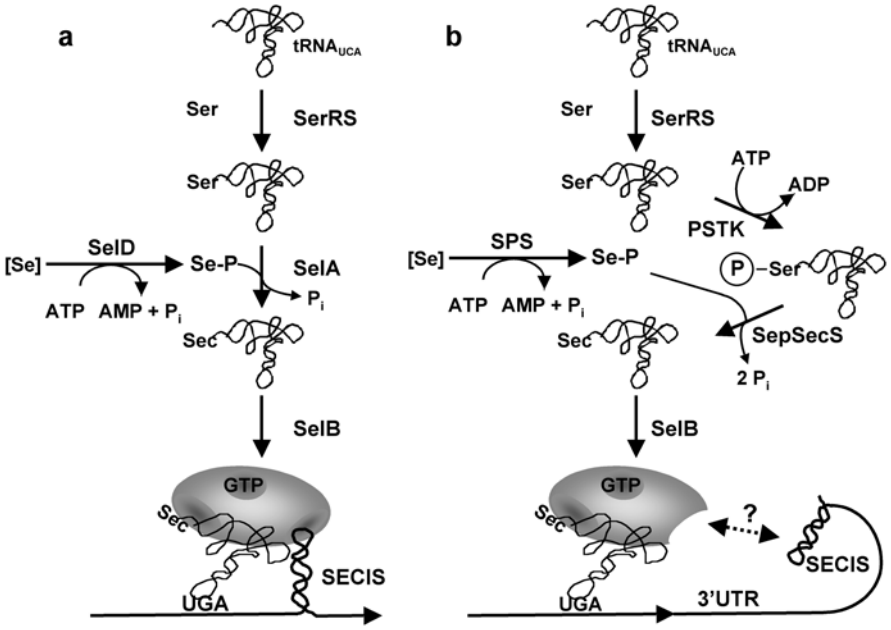

Fig. 5.1 Schematic of selenocysteine biosynthesis and incorporation in *Bacteria* (**a**) and in *Archaea* (**b**). Abbreviations are: *3′ UTR 3′* untranslated region, P_i orthophosphate, *PSTK* seryl-tRNA^{Sec} kinase; *[Se]* reduced selenium-species, *Sec* selenocysteine, *SelA* Sec synthase, *SelB* Sec-specific elongation factor, *SelD* and *SPS* selenophosphate synthetase, *SepSecS O*-phosphoseryl-tRNA^{Sec}:selenocysteine synthase, *Ser* serine, *SerRS* seryl-tRNA synthetase, *Se-P* seleno(mono) phosphate, and the *dashed arrow* indicates an unknown recoding mechanism. The figure is adapted from [77]. See text for details

tRNA in *E. coli*, a number of "invariant" base positions deviate from the consensus sequence of canonical tRNAs (reviewed in [15]). It has an unusually long extra arm and a D stem closed to a six base pair helix minimizing the D loop to four nucleotides, which restricts the types of tertiary interactions within the molecule [16]. However, the most conspicuous difference to canonical elongator tRNAs, is the eight-base-pair aminoacyl-acceptor stem; all other tRNA species have a seven-base-pair stem.

Transfer RNA^{Sec} is aminoacylated with L-serine by canonical seryl-tRNA synthetase (SerRS) (Fig. 5.1a) [14]. The conversion of seryl-tRNA^{Sec} into selenocysteyl-tRNA^{Sec} is catalyzed by Sec synthase (the *selA* gene product), which uses pyridoxal 5′-phosphate (PLP) as a cofactor (reviewed in [15]). The activated selenium donor for this reaction is seleno(mono)phosphate, synthesized from a reduced selenium species by selenophosphate synthetase (SPS), the *selD* gene product [17–19]. Although thiophosphate also serves as substrate in the Sec synthase reaction in vitro leading to cysteyl-tRNA^{Sec}, the catalytic efficiency is much lower [20]. Furthermore, SPS from *E. coli* is highly specific for selenium [20]. Interestingly, mammalian SPS2 (a selenoprotein itself) displays a much higher ambiguity between selenium and sulfur, maybe to "intentionally" generate cysteyl-tRNA^{Sec} in times of selenium starvation [21].

Sec synthase is a ring-shaped homodecamer [22] and binds $tRNA^{Sec}$ in a 1:1 stoichiometry [22, 23]. Furthermore, the $tRNA^{Sec}$•SelA binary complex is bound by SPS resulting in a 1.3 MDa ternary complex, which may serve to channel otherwise toxic selenium species to Sec synthase [24].

Transfer RNA^{Sec} is only recognized by the Sec-specific elongation factor, SelB (the *selB* gene product) [25]. SelB from *E. coli* shares significant homology to the canonical translation elongation factor EF-Tu in its three N-terminal domains [26]; it binds selenocysteyl-$tRNA^{Sec}$ and GTP stoichiometrically and discriminates not only $tRNA^{Sec}$ from the other elongator tRNAs [27], but also distinguishes selenocysteyl-$tRNA^{Sec}$ from uncharged $tRNA^{Sec}$, seryl-$tRNA^{Sec}$ or alanyl-$tRNA^{Sec}$ [28].

A unique property of bacterial SelB is that it interacts with a stem-loop structure of approximately 40 nucleotides, the SECIS (*Sec insertion sequence*) element, immediately 3′ of the UGA codon (the term, SECIS element, was coined for the corresponding eukaryal mRNA element [29]). Binding to the SECIS element is mediated by the L-shaped 17 kDa C-terminal domain IV of SelB, consisting of four winged helix domains [26] not present in EF-Tu (reviewed in [30]). Formation of the quaternary complex between SelB, selenocysteyl-$tRNA^{Sec}$, the SECIS element, and GTP is cooperative, wherein selenocysteyl-$tRNA^{Sec}$ is bound at a much higher affinity in the GTP- than in the GDP-state of SelB [31]. The SECIS element is bound with tenfold higher affinity in the presence of selenocysteyl-$tRNA^{Sec}$ [32]. Furthermore, binding of SECIS not only tethers the tertiary GTP•SelB•tRNA complex to the site of translation, but also serves to activate the molecule. GTP hydrolysis-activity is then stimulated at the ribosome when SECIS is bound [33], which in turn accelerates the release of selenocysteyl-$tRNA^{Sec}$ more than a million-fold [31]. These findings indicate that SECIS-binding induces a conformational switch in the complex, that renders SelB compatible for productive interaction with the ribosome, followed by GTP hydrolysis, and subsequent release of selenocysteyl-$tRNA^{Sec}$ in the proximity of the A site. This causes the affinity of SelB with mRNA to decrease resulting in dissociation of the SelB-SECIS complex. Unlike EF-Tu, SelB has, an approximately 10-fold higher affinity for GTP than for GDP, and a nucleotide exchange factor (EF-Ts) is not required [25].

5.3.2 Selenoprotein Synthesis in Other Bacteria

It is unknown whether all bacteria follow the *E. coli* paradigm during selenoprotein synthesis because no other (non-entero) bacteria have been thoroughly investigated in this respect but presence of the same set of factors involved in Sec biosynthesis and insertion suggests principle conservation of the process. Furthermore, a SECIS consensus, even if weak, can be used to identify selenoprotein genes in bacteria [34]. Still, some bacteria show surprising differences compared to *E. coli*. For example, *Haemophilus influenzae*, a γ-proteobacterium like *E. coli*, synthesizes a Sec-containing selenophosphate synthetase [35].

The likely occurrence of different mechanisms for UGA decoding as Sec among bacteria is illustrated by the fact that selenoprotein genes of Gram-positive bacteria cannot be functionally expressed in *E. coli* [36]. In fact, it is difficult to derive a plausible consensus for a Gram-positive SECIS, even in one organism expressing several selenoprotein genes [37]. On the other hand, the observation that the $tRNA^{Sec}$/SelB pair of *Eubacterium acidaminophilum* effected UGA read-through in *E. coli* of selenoprotein mRNAs from *Desulfomicrobium baculatum*, *Campylobacter jejuni*, and *T. denticola*, respectively, suggests that the SelB/SECIS interaction of Gram-positive bacteria is rather promiscuous [38].

5.4 Selenoprotein Synthesis in *Archaea*

The second prokaryotic domain encompasses the *Archaea*. Due to its accessibility for genetic manipulation and the non-essential nature of its selenoprotein synthesis machinery, *Methanococcus maripaludis* is currently the main model organism for studying selenium metabolism in *Archaea* [39]. It is intriguing to note that six of the eight known archaeal selenoproteins are directly involved in the organisms' primary energy metabolism, methanogenesis [40]. After (draft) sequencing over 500 archaeal genomes, the only *Archaea* containing selenoproteins are still methanogens belonging to the orders *Methanococcales* and *Methanopyrales*.

Archaeal $tRNA^{Sec}$ is more similar to the eukaryal than to the bacterial counterparts and possesses all structural features differentiating it from other elongator tRNAs [15]. No homolog of SelA was found in *Archaea*, and instead, a kinase was detected, which occurs only in selenoprotein-containing archaea and eukaryotes [41]. Biochemical analysis revealed that the protein transfers a phosphate group from ATP to seryl-$tRNA^{Sec}$ generating *O*-phosphoseryl-$tRNA^{Sec}$, which led to its designation as *O*-phosphoseryl-$tRNA^{Sec}$ kinase (PSTK) [41, 42]. The physiological significance of this reaction in vivo was unclear until a PLP-containing protein from *Methanococcus maripaludis*, which is homologous to the soluble liver antigen/liver pancreas protein (SLA/LP) [43, 44], was shown to catalyze the selenophosphate-dependent conversion of *O*-phosphoseryl-$tRNA^{Sec}$ to selenocysteyl-$tRNA^{Sec}$. As a result of these findings, the enzyme was named *O*-phosphoseryl-$tRNA^{Sec}$:selenocysteine synthase (SepSecS) [45]. An elaborate biochemical study, conducted simultaneously, showed that the pathway in mammals is identical, and therein, SepSecS was designated SecS [46]. Although catalyzing very similar reactions, the tetrameric SepSecS and the decameric SelA seem to have arisen via convergent evolution [22, 47].

As the function of bacterial Sec synthase in converting seryl-$tRNA^{Sec}$ to selenocysteyl-$tRNA^{Sec}$ is expanded during evolution and was separated into two reactions, PSTK and SepSecS, in *Archaea* and *Eukarya*, the question arose as to what evolutionary advantage is conferred by employing an "extra" enzyme, PSTK, that was so stably retained in evolution. Currently, there are three plausible explanations: since (1) *O*-phosphoseryl- would provide a better leaving group (phosphate) than seryl- (water) for replacement with selenium, the overall kinetic efficiency may

be improved in the case of PSTK/SepSecS as compared to SelA [45]; since (2) seryl-tRNA^{Sec} is more sensitive to deacylation than other aminoacyl-tRNAs [48], phosphorylation by PSTK could stabilize the aminoacyl-tRNA [41]; since (3) cysteyl-tRNA^{Sec} is formed in vitro with thiophosphate more efficiently from *O*-phosphoseryl-tRNA^{Sec} [21] than from seryl-tRNA^{Sec} [20], the replacement of Sec with Cys could be achieved in the event selenium is scarce. Indeed, severely selenium starved rats incorporate Cys at the position of Sec in thioredoxin reductase, probably in order to salvage at least some enzymatic activity [49]. However, Sec-utilizing archaea like *Methanococcus voltae* or *M. maripaludis* synthesize whole sets of separately encoded isoenzymes containing Cys at the position of Sec, which substitute for the selenoproteins under selenium-deprived conditions [40]. Furthermore, *M. maripaludis* is able to insert Cys or tryptophan at Sec-encoding UGA via a mechanism not involving the Sec machinery [50]. Thus, it appears not necessary for *M. maripaludis* to render Sec codons prone to Cys insertion via cysteyl-tRNA^{Sec}.

As observed in *Bacteria* and *Eukarya*, Sec insertion in *Archaea* is directed by UGA on the mRNA. However, *Archaea* do not contain SECIS elements within the coding region of the selenoprotein mRNAs, but in the 3′ untranslated regions (3′ UTRs) [51, 52]. Notably, the SECIS elements employed in the three domains of life appear to be completely unrelated.

Since SelB is a key component of the bacterial selenoprotein synthesis machinery, it was assumed that the situation would be the same in *Archaea*. Indeed, the archaeal SelB homolog (aSelB) binds guanosine nucleotides and aminoacyl-tRNA^{Sec} as expected [53] and a *M. maripaludis* strain lacking aSelB could not produce selenoproteins [54]. The C-terminal extension of aSelB is rather short and completely unrelated to that of bacterial SelB, where it is responsible for SECIS-binding. Although analysis of the crystal structure suggested that the C-terminal extension of aSelB may bind RNA [5], all our efforts to demonstrate direct aSelB-SECIS interaction in the *M. maripaludis* system were thus far unsuccessful ([53] and C. Sattler, S. Goetz, M. Rother, unpublished). These observations, and the fact that the situation is similar in the eukaryal system, suggest that in *Archaea*, communication between the SECIS element in the 3′ UTR effecting recoding and the site of that recoding (on the ribosome at UGA) is established by one or more as of yet unknown factor(s).

5.5 Selenoproteins of Prokaryotes

Despite the fact that the majority of prokaryotes do not contain selenoproteins, nearly all phylogenetic clades harbor members which do. Their unifying feature appears to be an (facultative) anaerobic life style, which possibly reflects the primordial nature of the Sec-utilizing trait. Unambiguous identification of selenoproteins still relies on experimentally verifying the presence of Sec in the protein, either via mass spectrometry or via incorporation of radioactive selenium. However, bioinformatic approaches proved to be very helpful in identifying potential selenoprotein

genes and, thus, the selenoprotein-containing organisms [55, 56]. Such predictions are based on searching sequenced genomes for UGA codons, either in conjunction with potential SECIS elements [34], or through analyses of sequences adjacent to in-frame UGA codons, because most selenoproteins contain homologs in which Sec is replaced with Cys [57]. Based on bioinformatic analyses, selenoproteins have been classified into more than 50 families [58, 59], 25 of which occur in prokaryotes [60]. Another surprising insight revealed by analyzing large data sets is the potential size of prokaryotic selenoproteomes. While *Mycobacterium* species only encode a single selenoprotein, formate dehydrogenase, *Alkaliphilus oremlandii* OhILA encodes 13, and *Syntrophobacter fumaroxidans* encodes 39 [56, 57, 61].

5.5.1 Predicted Selenoproteins

Numerous "new" prokaryotic selenoproteins were identified by bioinformatic approaches (reviewed in [59]), most of which are oxidoreductases potentially involved in signaling, detoxification, e.g., of reactive oxygen species, and maintaining cellular redox homeostasis (e.g., alkylhydroperoxidase-like protein, arsenate reductase, disulfide-bond oxidoreductase-like protein, glutaredoxin, glutathione peroxidase, glutathione S-transferase, peroxiredoxin, thiol-disulfide isomerase-like protein, and thioredoxin). Intriguing is the presence of homologs of mammalian deiodinases and selenoprotein W (encoded in, for example, *Gemmata obscuriglobus*). The function of these "eukaryal" selenoproteins in these organisms is not known.

5.5.2 Formate Dehydrogenase

Formate dehydrogenase (FDH) is the most widely distributed selenoprotein found in nature and it has been suggested that the genes encoding FDH, together with the genes encoding the system for Sec biosynthesis and incorporation into protein, were extensively transferred laterally [60]. FDH catalyzes the reversible reduction of CO_2 to formate and can be involved in energy metabolism, carbon fixation or pH homeostasis. The diverse cellular roles of FDH are reflected by the considerable differences found in subunit composition, kinetic properties and types of electron acceptors utilized [62]. The natural electron acceptor for Sec-containing FDH can be cytochrome (cyt_b for the *E. coli* FdhN), $NADP^+$ (for the *Moorella thermoacetica* enzyme), ferredoxin (for the *Clostridium pasteurianum* enzyme) or F_{420} (a 2-deazaflavin derivative functionally analogous to NAD^+; in the FDH of methanogenic archaea), where FDH functions in formate oxidation rather than CO_2 reduction. FDH contains Fe/S clusters and a pterin cofactor coordinating either molybdenum or tungsten [63].

5.5.3 Hydrogenase

Sec-containing Ni-/Fe-hydrogenase is the second most common selenoprotein in prokaryotes. Hydrogenases catalyze the reversible reduction of protons and are widely distributed among prokaryotes and lower eukaryotes. The electron carriers used vary depending on the organism. In the active site of Ni/Fe hydrogenase either three Cys plus a Sec (or four Cys) residues coordinate a Ni atom, with two of these Cys also binding a Fe atom [64]. The Sec-containing hydrogenases of methanogenic archaea are either coenzyme F_{420}-reducing or F_{420}-non-reducing. The latter enzyme is tightly associated with the heterodisulfide reductase and electrons derived from H_2 oxidation are transferred via Fe/S clusters and FAD from the hydrogenase to both the heterodisulfide and ferredoxin by flavin-based electron bifurcation [65]. Central to this tight interaction and to electron transfer is the hydrogenase delta subunit VhuD, which contains two Sec residues [66].

5.5.4 Glycine Reductase

The glycine reductase system is almost exclusively found in amino acid fermenting clostridia [67]. It consists of three proteins, P_A, P_B, and P_c. While P_c does not contain Sec, P_B is a substrate-specific selenoprotein. Although the corresponding subunits of all characterized substrate-specific P_B proteins of glycine, betaine, sarcosine and proline reductase, respectively (see below), contain Sec, [67], homologous sequences in which the Sec UGA codon is replaced by a Cys UGU or UGC codon were identified in databases [11]. P_A is the only selenoprotein for which no Cys-containing homolog is known [57]. Sarcosine reductase and betaine reductase share their P_A and P_C components with glycine reductase but contain different substrate-specific, Sec-containing P_B proteins [68].

5.5.5 Proline Reductase

The reduction of D-proline to 5-aminovalerate in clostridia seems to proceed by a different mechanism compared to glycine reduction. Still, D-proline reductase is similar to P_B of glycine reductase and composed of three different subunits, PrdB which contains Sec, and two proteins resulting from processing of proprotein PrdA [69].

5.5.6 Methionine Sulfoxide Reductase

Methionine sulfoxide reductase (Msr) reduces oxidized methionine residues in proteins, which arise by action of reactive oxygen species [70]. MsrA is specific for the *S*-form of methionine sulfoxide, whereas MsrB is specific for the *R*-form. Both

MsrA and MsrB can either be selenoproteins or non-selenoproteins, depending on the organism. Sec-containing MsrA was characterized in *Alkaliphilus oremlandii* OhILA [61]. Biochemical analysis showed that Sec-containing Msr has a vastly higher activity, but also that it is more difficult to reductively regenerate the selenoproteins, than the Cys-containing isoforms [71, 72].

5.5.7 Seleno(mono)phosphate Synthetase

Sec-containing SPS has been found in all three domains of life. The enzyme provides the activated selenium donor during Sec synthesis. What exact role the Sec residue plays during catalysis in these enzymes has not been shown, but it could account for the enzyme's promiscuity towards sulfide in generating thiophosphate. Also, requirement of a Sec-containing enzyme for Sec synthesis may allow for direct integration of environmental signals, like the availability of selenium, into regulating selenoprotein synthesis.

5.5.8 Formyl-Methanofuran Dehydrogenase

Formyl-methanofuran dehydrogenase (FMD) catalyzes the reduction of CO_2 and methanofuran to formyl-methanofuran, which is the initial step in methanogenesis from CO_2 in all methanogenic archaea. FMD contains either Mo or W, and Fe/S clusters and is composed of five subunits, wherein two share considerable similarity with FDH [73].

5.5.9 Heterodisulfide Reductase

Obligate hydrogenotrophic methanogens reduce the heterodisulfide of coenzyme M and coenzyme B by a cytoplasmic multienzyme complex composed of the F_{420}-nonreducing hydrogenase (see above) and a soluble heterodisulfide reductase, an iron-sulfur flavoprotein, which in *Methanococcus* and *Methanopyrus* species also contains Sec. However, the role of Sec in this enzyme is not known.

5.5.10 HesB-Like Selenoprotein

M. maripaludis synthesizes a small, approximately 11 kDa selenoprotein, similar to HesB from *Synechococcus*, that was originally identified through genome analyses [57]. However, it is not an essential protein [74]. Beside its distant relation to IscA, which is involved in Fe/S-cluster assembly, nothing is known about the function of the HesB-like selenoprotein.

5.5.11 Benzoyl-CoA Reductase

Strictly anaerobic sulfate-reducing bacteria capable of aromatic degradation (e.g., *Geobacter* and *Desulfococcus*) synthesize three selenoproteins [75]. As deduced from genomic sequences, one of them, BamF, which is homologous to F_{420}-non-reducing hydrogenase, is potentially a Sec-containing subunit of benzoyl-CoA reductase, BamBCDEFGHI [76]. However, the role of Sec in this complex remains to be elucidated.

5.6 Concluding Remarks

Despite the remarkable depth of knowledge gained about physiological, mechanistic, structural, and evolutionary aspects of selenoprotein synthesis in prokaryotes, considerable gaps in our understanding remain to be closed to fully appreciate selenium biology in these organisms. For example, it is still not known how selenium is specifically transported across the cytoplasmic membrane. Furthermore, some microorganisms, like *M. maripaludis*, regulate gene expression in a selenium-responsive fashion, but the underlying mechanism and how the availability of selenium in the environment is sensed is unknown. In *Archaea*, the recoding of UGA from a nonsense to a Sec codon by a SECIS element in the 3′ UTR appears similar to the mechanism present in eukaryotes. However, factors mediating this recoding in *Archaea* have not yet been identified. Lastly, the cellular roles of the many newly identified selenoproteins will have to be elucidated. Thus, research dedicated to selenium biology in prokaryotes promises exiting new insights into the complex metabolic and regulatory networks within such simple organisms.

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