Selenocysteine Inserting RNA Elements Modulate GTP Hydrolysis of Elongation Factor SelB†

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ABSTRACT: Elongation factor SelB is required for the incorporation of the amino acid selenocysteine into proteins in *Escherichia coli*. Selenocysteine incorporation is thought to be achieved by simultaneous binding of SelB to selenocysteyl-tRNA^{Sec} and to an mRNA hairpin structure located 3′ adjacent to the UGA selenocysteine codon. SelB was shown previously to bind to GTP or GDP in a molar ratio of 1:1. Here, we demonstrate that SelB, like EF-Tu, exhibits a low intrinsic GTPase activity in the absence of ribosomes. As shown for EF-Tu, GTPase activity of SelB is stimulated by the presence of *E. coli* 70S ribosomes; the apparent K_m for GTP hydrolysis is 55 μM. Interestingly, in the presence of the mRNA hairpin which promotes selenocysteine incorporation, GTPase activity of SelB increases additionally by 3−4-fold; stimulation is due to k_{cat} increasing from 0.05/min in the absence to 0.16/min in the presence of the mRNA hairpin. This mRNA-induced stimulation of SelB GTPase activity depends on the presence of ribosomes. The minimal region of the mRNA hairpin capable to stimulate GTP hydrolysis by SelB locates within the upper half of the hairpin; this part of the mRNA structure was demonstrated previously to be sufficient for binding of the mRNA to SelB. On the basis of these results, we propose that binding of the mRNA hairpin to SelB induces a conformational switch within SelB thereby promoting an increase in ribosome-mediated GTP hydrolysis.

The incorporation of the amino acid selenocysteine into proteins in *Escherichia coli* requires the presence of a UGA codon as well as a specific mRNA hairpin structure located 3′ adjacent to the UGA (*1*, *2*), designated in the following as b-SECIS element (bacterial selenocysteine inserting sequence). In addition, a specific tRNA, selenocysteyl-tRNA^{Sec}, containing a UCA anticodon promotes decoding of the UGA codon. The pathway of selenocysteyl-tRNA^{Sec} synthesis requires acylation with serine by seryl-tRNA synthetase followed by conversion into selenocysteyl-tRNA^{Sec} by selenocysteine synthase (for a review, see ref *3*).

A special elongation factor, SelB, was shown to bind to selenocysteyl-tRNA^{Sec} as well as to the b-SECIS element 3′ to the UGA (*4*−*6*). SelB exhibits extensive sequence homology to elongation factor EF-Tu in its N-terminal domain and also contains a C-terminal extension not found within EF-Tu (*4*, *7*). Gelshift assays demonstrated that the C-terminal extension of SelB was sufficient for binding to the mRNA hairpin, whereas the N-terminal and central part of SelB is required for binding to selenocysteyl-tRNA^{Sec} (*7*). It is thought that selenocysteyl-tRNA^{Sec} is tethered to the UGA codon via simultaneous binding of SelB to the tRNA and to the mRNA hairpin, thereby promoting selenocysteine incorporation into proteins (*3*). Indeed, the existence of such a complex was demonstrated *in vitro* as well as *in vivo* (*5*, *8*).

A sequence alignment of SelB proteins from four different bacterial species and EF-Tu revealed that SelB contains all three protein domains characterized for EF-Tu, in addition to the C-terminal domain only found in SelB (*7*). The similarity includes the GTP binding domains as well as the tRNA binding regions. It was also demonstrated that SelB, like EF-Tu, binds to GTP and GDP in a molar ratio of 1:1 (*4*). In contrast to EF-Tu, however, SelB binds GTP with a 6-fold higher affinity than GDP, which may explain why an EF-Tu like factor is not required. The affinity of SelB for GTP was determined to be 1.7 μM and for GDP as 10 μM (*4*). This deviates significantly from the affinity of EF-Tu for GDP, which is 3 orders of magnitude higher (*9*), while SelB and EF-Tu exhibit similar affinities for GTP.

One of the main questions on the function of SelB is whether and when GTP hydrolysis occurs during the incorporation of selenocysteine into proteins. This analysis is likely to shed some light on the mode of interaction of SelB with the ribosome. To test this, a minimal system was used including ribosomes and the elongation factor, only. By applying this method, it was previously demonstrated that ribosomes stimulate the low intrinsic GTPase activity of elongation factor EF-Tu even in the absence of tRNA or mRNA (*9*, *10*). We therefore assayed the binary complex (SelB-GTP) encountering the ribosome to study GTP hydrolysis by SelB. As the mRNA hairpin structure following the UGA codon is strictly required for selenocysteine incorporation, we also investigated the influence of this element on GTP hydrolysis.

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MATERIALS AND METHODS

Materials. All reagents were obtained from Sigma (Germany) unless indicated otherwise. T7 polymerase was a generous gift from Thomas Maier (München).

mRNAs. All mRNA constructs were *in vitro* transcribed from DNA templates containing a T7 promotor (*11*). mRNA constructs AH75 and AH90 were obtained by *in vitro* transcription of DNA fragments which were generated by PCR amplification as described previously (*12*), construct *mh* was a generous gift by M. Kromayer (described in ref *7*). *fdhF* and *fdnG* mRNA constructs were *in vitro* transcribed from linearized vectors pAF1 or pAG1, as described previously (*6*). RNAs were purified by PAGE on gels containing 8% acrylamide-7 M urea. RNAs were identified by UV-shadowing, bands were cut out and passively eluted overnight in 0.3 M sodium-acetate (pH 5.2), 0.1% SDS, and 1 mM EDTA. After extraction with phenol and chloroform, RNAs were ethanol precipitated and resuspended in H_2O to the desired concentration. Before use, RNAs were incubated at 55 °C for 2 min and slowly cooled to 30 °C in a waterbath for 15 min.

Preparation of Ribosomes. *E. coli* 600 MRE 0.5 M salt washed 70 S ribosomes were prepared as described in ref *13*.

Preparation of SelB. Preparation of special elongation factor SelB was performed, essentially as described in ref *14*, with the exception that addition of GDP- or GTP-nucleotides was omitted in the SelB storage buffer. SelB was expressed from plasmid pWL 194 (*14*) in *E. coli* strain K38 harboring plasmid pGP1-2. The purification procedure can be summarized as a sedimentation of SelB by a 60 min centrifugation at 150000*g*, solubilization of the protein from the sediment by washing with 1 M NH_4Cl, fractionated ammonium sulfate precipitation between 45 and 63% saturation, and hydroxyl apatite chromatography. The final step, precipitation during dialysis against low salt buffer, takes advantage of the fact that SelB protein aggregates at a low salt concentration, e.g., in the presence of 10 mM potassium phosphate. SelB protein (10 μg) was loaded on a 10% polyacrylamide gel in the presence of sodium dodecyl sulfate. After electrophoresis, the gel was stained with Coomassie Brilliant Blue R-250; a single band corresponding to a molecular mass of approximately 68 kDa was observed.

Assay of GTPase Activity. The hydrolysis of GTP was assayed in a reaction volume of 50 μL containing 50 mM Tris-HCl, pH 7.5/80 mM NH_4Cl/10 mM $MgCl_2$/1 mM dithioerythritol. SelB protein, ribosomes, and mRNA were incubated at 37 °C at the final concentration indicated in the figure legends and tables; if not indicated otherwise, 100 μM [γ-^{32}P]GTP with a specific radioactivity of 100 mCi/μmol was added. Product formation was monitored either by thin-layer chromatography or by liquid-scintillation counting. For analysis by thin-layer chromatography, 10 μL of the assay was drawn from the reaction mixture and the reaction was stopped by addition of 1 μL of 10% sodium dodecyl sulfate containing 20 mM EDTA. After drying in a speed-vaccum centrifuge, samples were redissolved in 3 μL of H_2O, spotted onto polyethyleneimine cellulose plates (Cel 300/PEI/UV_{254} plates; Macherey and Nagel, Germany), and chromatographed in 0.75 M potassium phosphate buffer, pH 3.5, for 1 h. The plates were dried, and the radioactivity was visualized by autoradiography.

Alternatively, 10 μL of the reaction mixture was added to 400 μL of a suspension of activated charcoal (5% in 50 mM KH_2PO_4). After mixing and centrifugation (3 min at 13 000 rpm), 100 μL of the supernatant was transfered into a new tube and γ-$^{32}P_i$ released from GTP was measured in a Kontron MR300 liquid scintillation counter. All experiments were carried out in triplicate.

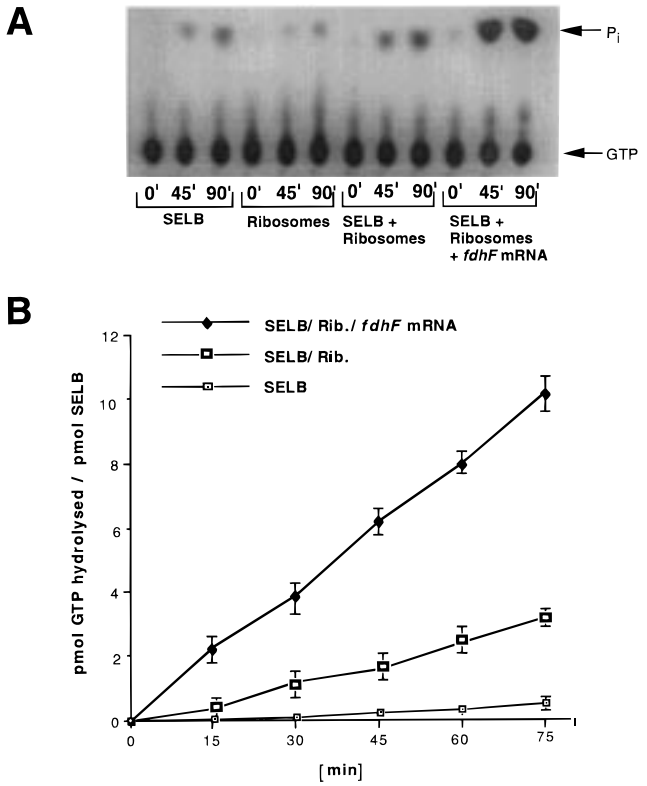

FIGURE 1: (A) Autoradiograph of a representative experiment of GTP hydrolysis by SelB in the presence or absence of ribosomes or the *fdhF* mRNA analysed by thin-layer chromatography. Samples were taken after 0, 45, and 90 min. As a control, free ribosomes were also analysed for GTP hydrolyses; as observed by other groups (*9*) ribosomes show a low background level of GTP hydrolysis due to contaminating GTPases; accordingly, the level of background activity was determined and subtracted from all samples containing ribosomes (see Materials and Methods). (B) Quantitative analysis of the rate of GTP hydrolysis by elongation factor SelB in the absence and presence of ribosomes (Rib.) and the *fdhF* mRNA. SelB was present at a concentration of 2.5 μM, the *fdhF* mRNA at a concentration of 5 μM and 70 S ribosomes at a concentration of 0.12 μM.

RESULTS

GTPase Activity of SelB Is Stimulated by Ribosomes and the b-SECIS Element. Analysis of the SelB amino acid sequence revealed a potential GTP binding motif and GTP binding to SelB was demonstrated by equilibrium dialysis (*4*). Therefore, we first investigated GTP hydrolysis in the absence of ligands such as ribosomes, tRNA, or mRNA. [γ-^{32}P]GTP was incubated with purified SelB, and the reaction products were analyzed by thin-layer chromatography. Indeed, a low intrinsic GTPase activity of SelB was observed (Figure 1). It was demonstrated previously that

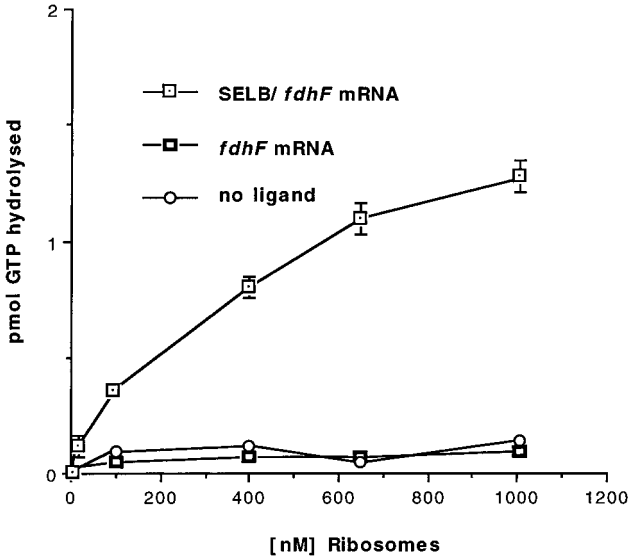

FIGURE 2: Dependence of GTP hydrolysis by SelB (2.5 μM) and the *fdhF* mRNA b-SECIS element (5 μM) on the presence of ribosomes (0.12 μM). Samples were incubated at 37 °C for 10 min. As a control, GTP hydrolysis by ribosomes without SelB was analysed in the presence (*fdhF* mRNA) or absence (no ligand) of the the b-SECIS element.

the low intrinsic GTPase activity of elongation factor EF-Tu is stimulated by the presence of ribosomes even in the absence of aminoacyl-tRNA and mRNA (*10*). Thus, we analyzed whether this is also the case for special elongation factor SelB. In fact, a significant increase in GTP hydrolysis of SelB is observed in the presence of ribosomes (Figure 1).

As the selenocysteine mRNA hairpin 3′ adjacent to the UGA selenocysteine codon is strictly required for selenocysteine incorporation, we next wanted to determine whether the presence of the b-SECIS element influences the ribosome-induced GTP hydrolysis of SelB. Interestingly, addition of the mRNA increased GTP hydrolysis by 3−4-fold (Figure 1). GTPase activity of SelB was not stimulated in the presence of the *fdhF* mRNA but in the absence of ribosomes, demonstrating that the stimulation is a strictly ribosome-dependent event (data not shown). Addition of other RNAs, like poly U mRNA or bulk tRNA did not increase GTP hydrolysis of SelB in the presence of ribosomes, even when added in a 50-fold excess compared to the *fdhF* mRNA (Figure 3). Ribosome-induced GTP hydrolysis of elongation factor EF-Tu did not change upon addition of the seleno-*fdhF* mRNA hairpin, demonstrating that the observed activity is specific for SelB (data not shown).

The increase of GTPase activity by the *fdhF* mRNA b-SECIS element depends on the concentration of mRNA added; a 1:1 ratio of SelB, and the mRNA b-SECIS element leads to a maximal stimulation of GTP hydrolysis (data not shown). The mRNA-dependent stimulation of GTP hydrolysis by SelB thereby depends on ribosomes; addition of increasing amounts of ribosomes to SelB in the presence of the *fdhF* mRNA increased the amount of GTP hydrolyzed (Figure 2). To show that this is not a solely ribosome-dependent GTP hydrolysis, as a control, addition of the *fdhF* mRNA to ribosomes in the absence of SelB did not stimulate GTP hydrolysis (Figure 2).

Identification of the Minimal Sequence within fdhF mRNA Sufficient for Stimulation of GTP Hydrolysis by SelB. The *fdhF* mRNA construct used for stimulation of GTP hydrolysis contained a translation initiation region including a Shine-Dalgarno sequence and an AUG initiation codon (described in ref *12*). One explanation for the stimulating effect could be the following: binding of SelB to the *fdhF* mRNA hairpin and simultaneous binding of the mRNA to the ribosome via the Shine-Dalgarno sequence might lead to an increase of the local concentration of SelB on the ribosome. Thus, GTPase activity of SelB would be stimulated. To test this hypothesis, we used several different mRNA constructs to identify the minimal region sufficient for stimulation.

In *E. coli*, three different b-SECIS elements are known which promote selenocysteine insertion; one hairpin is located within the *fdhF* mRNA, and another within the *fdnG* mRNA coding for formate dehydrogenase enzymes H and N, respectively. Therefore, we transcribed *in vitro* regions of the *fdhF* or *fdnG* mRNAs, which lacked the Shine-Dalgarno sequence and the AUG initiation codon (*6*). As another control, two *in vitro* transcribed mRNA fragments containing a Shine-Dalgarno sequence and a translation initiation codon followed by no (AH75 mRNA) or five (AH90 mRNA) phenylalanine codons were used, which were followed by the UGA selenocysteine codon and the *fdhF* mRNA b-SECIS element (*12*; Figure 3).

Addition of all mRNA constructs lacking the Shine-Dalgarno sequence induced about the same degree of stimulation of GTP hydrolysis as did the mRNA constructs with a Shine-Dalgarno motif. Also, the *fdnG* mRNA construct exhibited a comparable stimulation of GTP hydrolysis as did the *fdhF* mRNA (Figure 3). To further narrow down the region necessary for stimulation of GTP hydrolysis by SelB, we tested the minimal region of the *fdhF* mRNA hairpin sufficient to promote binding to SelB (*7*, *15*), which consists of 17 bases only (Figure 3). A comparable increase of GTP hydrolysis by SelB in the presence of ribosomes was observed as with the full-length hairpin (Figure 3). As a control, addition of poly U mRNA or bulk tRNA did not result in a stimulation of GTP hydrolysis, showing that the observed effect is strictly dependent on the b-SECIS element sequence.

Kinetic Parameters of GTP Hydrolysis by SelB. To assess whether the stimulation of GTP hydrolysis of SelB by the mRNA occurs via a change of the affinity for GTP or a change of the rate of the reaction, we determined the kinetic parameters of SelB-ribosome complexes in the absence or presence of mRNA. Table 1 shows the apparent K_m of SelB to GTP in the range of 55 ± 3 μM in the absence or presence of the b-SECIS element. In the absence of the *fdhF* mRNA hairpin, but in the presence of ribosomes, a k_{cat} of 0.05 pmol of GTP/min/pmol of SelB was determined. A 3−4-fold increase in k_{cat} was shown to occur in the presence of the *fdhF* mRNA selenocysteine hairpin and ribosomes (Table 1) consistent with the notion that binding of the selenocysteine mRNA hairpin leads to an increase in the velocity of GTP hydrolysis by SelB. This might be due to the fact that binding of the b-SECIS element renders SelB to a more active enzyme, which is better suited to interact with the ribosome; thereby, the rate of GTP hydrolysis might be increased.

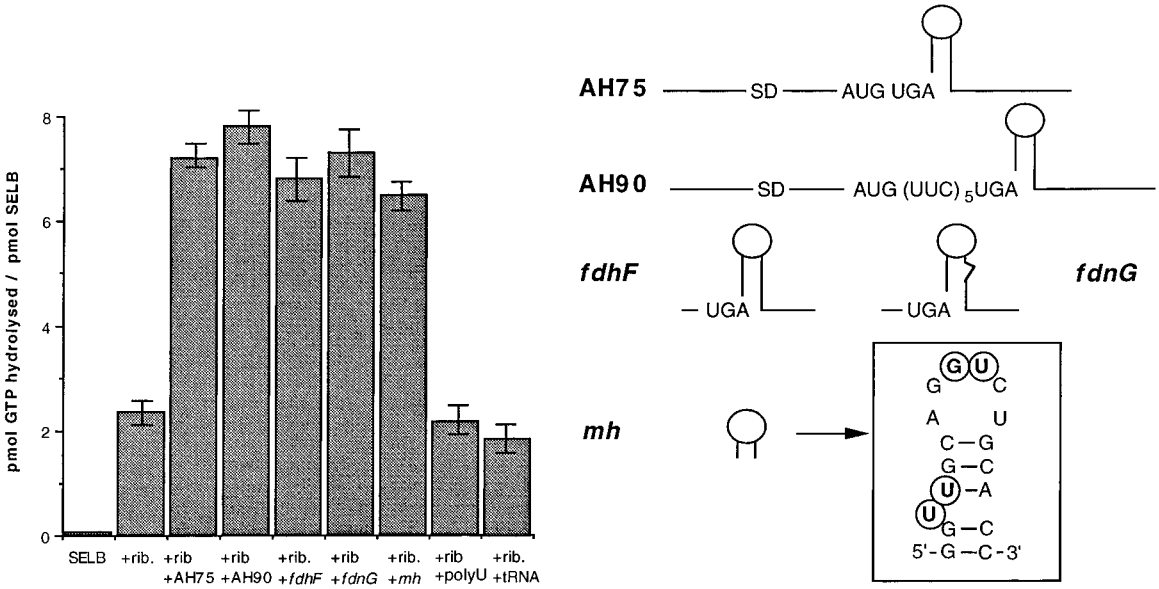

FIGURE 3: (Left) Dependence of GTP hydrolysis by SelB (2.5 μM) in the presence of ribosomes (0.12 μM) and different mRNA constructs either containing the selenocysteine mRNA hairpin (AH75, AH 90, *fdhF*, *fdnG*, mh) or unspecific RNA (poly U mRNA, bulk $tRNA_{yeast}$). mRNAs were addedd at a concentration of 5 μM (AH75, AH 90, *fdhF*, *fdnG*, mh) or 250 mM (polyU mRNA, bulk $tRNA_{yeast}$). Samples were incubated for 60 min at 37 °C. (Right) mRNA constructs used in this study; AH75 and AH 90 mRNA (described in ref *12*) contain a translation initiation region including a Shine-Dalgarno sequence (SD) and a AUG startcodon; this is followed by the UGA selenocysteine codon and the *fdhF* selenocysteine mRNA hairpin (AH75) or a linker region of five phenylalanine codons $(UUC)_5$ and the UGA including the mRNA selenocysteine hairpin; *fdhF*, mRNA fragment of the *fdhF* mRNA containing the *fdhF* selenocysteine mRNA hairpin, but lacking the translation initiation region (described in ref *6*); *fdnG*, mRNA fragment of the *fdnG* mRNA containing the *fdnG* selenocysteine mRNA hairpin, but lacking the translation initiation region (described in ref *6*). *mh*, minimal region of the *fdhF* mRNA hairpin shown to bind to elongation factor SelB (described in ref *7*); bases indicated by circles were demonstrated to directly interact with SelB (*6*).

Table 1: Kinetics of GTP Hydrolysis by Special Elongation Factor SelB[a]

component(s) added	K_m (μM GTP)	k_{cat} (pmol of Pi/min/pmol of SelB)
none	nd	nd
ribosomes[b]	55.1	0.05
ribosomes[b] + *fdhF* mRNA[c]	58.0	0.16

[a] SelB was present at a final concentration of 2.5 μM. [b] Ribosomes were present at a concentration of 0.12 μM. [c] *fdhF* mRNA was added at a final concentration of 5 μM. Values are the average of those from two independent experiments.

DISCUSSION

In this study, we investigated the influence of multiple interacting factors on the ability of special elongation factor SelB to hydrolyse GTP. Previously, it had been demonstrated that SelB binds GTP with a K_d of 1.7 μM in a molar ratio of 1:1 and GDP with an about 6-fold lesser affinity (*4*). Indeed, like for EF-Tu, we observe a low intrinsic GTPase activity of SelB in the absence of ribosomes or other ligands (Figure 1).

In the presence of ribosomes, GTP hydrolysis of SelB is strongly stimulated in agreement with previous studies on elongation factor EF-Tu. The apparent K_m for ribosome-mediated GTP hydrolysis by SelB was determined to be 55 μM. The fact that GTPase activity of SelB is stimulated by ribosomes may indicate a similar mode of interaction of SelB with the ribosome as observed for EF-Tu (*9*). This is consistent with the finding that SelB exhibits extensive homology to EF-Tu in its N-terminal and central region, including the GTP and tRNA binding domains.

It has been shown previously that the mRNA hairpin structure 3′ to the UGA codon is strictly required for selenocysteine incorporation by binding to special elongation factor SelB (*2*). Here, we demonstrate that the b-SECIS element stimulates ribosome-mediated GTP hydrolysis of SelB by 3−4-fold; this is due to an increase in the apparent k_{cat} of the reaction from 0.05/min in the absence of the mRNA hairpin to 0.16/min in its presence.

We show that the minimal region of the b-SECIS element capable of stimulating GTP hydrolysis by SelB is located in the upper part of the mRNA stem-loop structure (Figure 3). This region of the hairpin is sufficient for binding to SelB (*7*) and distinct bases of this upper region in the loop and in an adjacent bulge of the hairpin directly interact with the elongation factor (Figure 3; *6*). The presence of the mRNA hairpin might either directly or indirectly stimulate GTP hydrolysis of SelB in the presence of ribosomes. We favor a model where binding of SelB to the mRNA hairpin via its C-terminal domain increases the affinity to the ribosome by inducing a conformational change within SelB. This in turn might favor the ribosome-mediated GTP hydrolysis of SelB. Certainly, alternative models cannot be excluded at this point and a structural analysis of SelB in the absence and presence of the b-SECIS element has to be awaited to confirm our model.

But what would be the advantage of a conformational rearrangement within SelB upon binding to the mRNA b-SECIS element? As pointed out above, SelB shares extensive homology to EF-Tu in its N-terminal domain (*4*, *7*). Therefore, this implies the possibility for the SelB-GTP-selenocysteyl-$tRNA^{Sec}$ complex to decode regular UGA stop codons as selenocysteine codons even in the absence of the mRNA hairpin, which could be detrimental for the cell, however (Figure 4, bottom, right side). In that respect, the mRNA hairpin might serve a function in addition to tethering

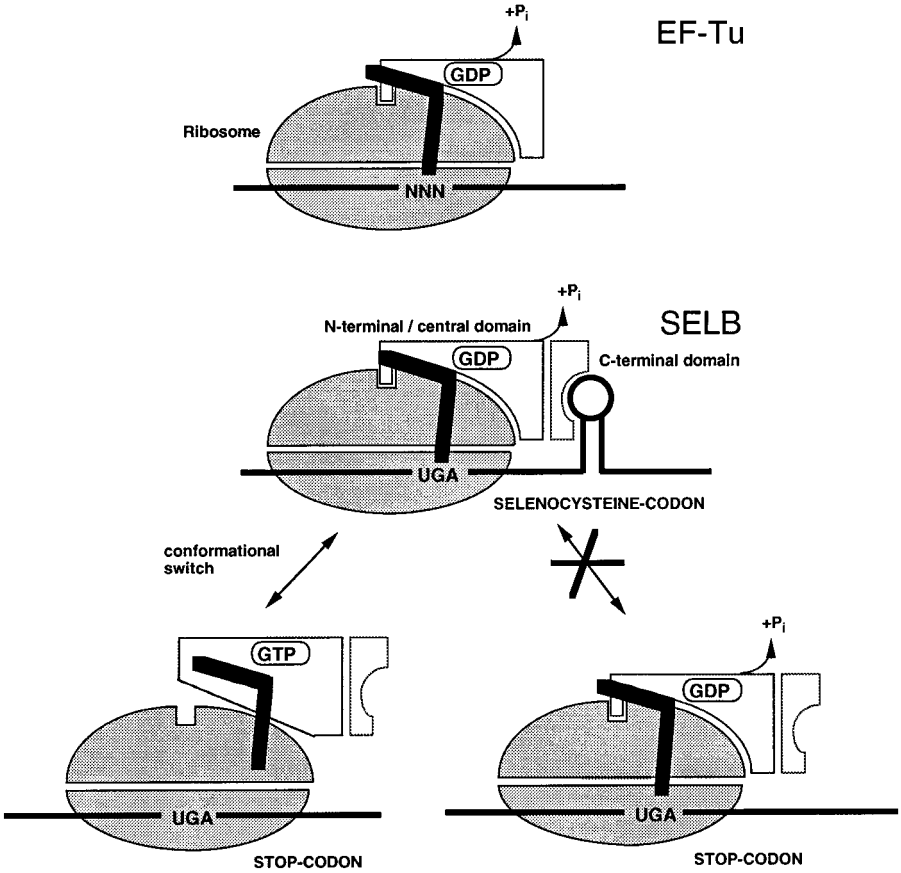

FIGURE 4: Model of the interaction of EF-Tu and SelB with the ribosome; NNN, any codon except UAA, UGA, or UAG; The C-terminal extension of SelB interacting with the mRNA hairpin structure is indicated as a separate domain, not found in EF-Tu. The elongation factor EF-Tu/GTP complex is able to bind to all canonical aminoacyl-tRNAs (except selenocysteyl-tRNA^{Sec}) which enables the respective tRNAs to decode any sense codon (NNN); during that process GTP is hydrolyzed. The special elongation factor SelB/GTP complex (middle) recognizes specifically selenocysteyl-tRNA^{Sec} which is able to decode UGA codons; prerequisite to decoding is the presence of the b-SECIS element, a stable mRNA stem loop structure located 3′ to the UGA codon. The b-SECIS element binds to the C-terminal domain of the SelB factor; it is assumed that GTP is hydrolyzed by SELB during decoding of the UGA codon, analogous to EF-Tu. (Bottom right) Since SelB shares extensive homology to EF-Tu in its N-terminal domain, this would imply the possiblity to decode regular UGA stop codons as selenocysteine. (Bottom left) A conformational switch is induced within SelB upon binding to the b-SECIS mRNA element; this prevents decoding of regular UGA stop codons as selenocysteine codons (bottom right) as only upon this conformational switch, SelB is able to interact with the ribosome and hydrolyse GTP.

of selenocysteyl-tRNA^{Sec} to the UGA codon by simultaneous binding of SelB to both ligands. It might act also as an antideterminant feature within SelB, which prevents decoding of regular UGA stop codons as selenocysteine codons (Figure 4, bottom left side). Only at the UGA selenocysteine codon, which is followed by the mRNA hairpin structure, the conformational switch within SelB would be induced, leading to a more productive interaction between the elongation factor and the ribosome (Figure 4, middle).

Are there other experimental data to corroborate this model? The "tether model" implies that simultaneous binding of SelB to selenocysteyl-tRNA^{Sec} and to the mRNA hairpin increases the local concentration of the SelB-GTP-selenocysteyl-tRNA^{Sec} complex at the UGA codon on the ribosome. If this would be the only function of the b-SECIS element, overexpression of SelB and tRNA should increase the local concentration of SelB on the ribosome as well which in turn should result in decoding of regular stop codons as selenocysteine; this, however, is not observed (S. Müller and A. Böck, manuscript in preparation). Further support comes from a SELEX approach which recently identified mRNA hairpin structures binding to SelB with a comparable affinity than wild-type mRNA but which failed to promote selenocysteine incorporation into proteins in *E. coli* (*16*). This might be due to the fact that these RNA structures, while still binding to SelB, were unable to trigger the conformational switch within SelB prerequisite for its interaction with the ribosome. Thus, binding of the mRNA hairpin to SelB and the promotion of selenocysteine incorporation may be two separate functions. Lastly, in eukaryotes, specific RNA structures promote selenocysteine incorporation into proteins. These RNA structures locate in the 3′ untranslated regions of mRNAs coding for seleno-proteins and are believed to bind to a yet unidentified SelB homologue. The RNA structures, designated as b-SECIS elements, are able to promote selenocysteine incorporation even when supplied *in trans*, thereby implying additional functions of these b-SECIS elements other than the "tethering effect" (*17*). Thus, in the eukaryotic system, a conformational switch within a putative SelB homologue may also promote selenocysteine incorporation.

Certainly, it would be desirable to obtain data on SelB-GTP hydrolysis during codon−anticodon interaction between the anticodon of selenocysteyl-tRNA^{Sec} and the UGA codon in the ribosomal A-site. Is was shown previously, that by this codon−anticodon interaction GTPase activity of EF-Tu

is stimulated dramatically by 10^4-fold in a full system containing in addition poly U mRNA and phenylalanyl-tRNA^{Phe} (*18*−*20*). This approach, however, cannot be used for the SelB system as we demonstrated previously that the b-SECIS element has to be partly unfolded at the decoding of the UGA codon during the dynamic process of elongation. No codon anticodon interaction between tRNA^{Sec} and the UGA codon could be observed *in vitro* by binding of a selenocysteine encoding mRNA to the ribosome via a Shine-Dalgarno sequence (*12*); this might be due to the fact that the UGA codon was shown to be entrapped within an intricate tertiary structure in b-SECIS element containing mRNAs in solution (*6*). In that respect, an *in vitro* translation approach will have to be considered to address the question of GTP hydrolysis of SelB in an codon−anticodon-dependent fashion.

In conclusion, our study shows for the first time that SelB exhibits a low intrinsic GTPase activity and that ribosomes are able to stimulate the GTPase activity of SelB, like observed for EF-Tu. Unlike for EF-Tu, the presence of the mRNA selenocysteine hairpin structure 3′ to the UGA codon increases GTP hydrolysis of SelB additionally by 3−4-fold. Thus, SelB and ET-Tu share a similar structure and function, as well as exhibit a distinct difference: the molecular switch within SelB which we propose to occur upon binding to the b-SECIS element is not unprecendented within elongation factors. It was shown previously that EF-Tu undergoes a dramatic conformational change upon binding to GTP (*21*, *22*) and to the ribosome upon codon−anticodon interaction (*19*). Taken into consideration that the tRNA and GTP binding domains of SelB are very similar to EF-Tu, SelB might undergo several conformational changes: one upon binding to GTP and the other upon binding to the b-SECIS element.

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