

The function of SECIS RNA in translational control of gene expression in *Escherichia coli*

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The incorporation of selenocysteine into proteins is directed by specific UGA codons and mRNA secondary structures, designated SECIS elements. In bacteria, these elements are positioned within the reading frame of selenoprotein mRNAs immediately downstream of the triplet coding for selenocysteine, and they tether a complex of the selenocysteine-specific elongation factor SelB, GTP and selenocysteyl-tRNA^{Sec} to the site of UGA decoding. A SECIS-like structure was identified in the 5' non-translated region of the *selAB* transcript, encoding selenocysteine synthase and SelB. It specifically binds to SelB and the formation of a SelB·GTP·selenocysteyl-tRNA^{Sec} complex on the SECIS-like element represses expression of the downstream gene. This effect is abolished by mutations preventing formation of the complex. The regulatory pattern observed correlated with the levels of *sel* gene products. As quaternary complex formation on the SECIS-like element did not influence the transcription rate and only slightly reduced the level of *selAB* mRNA, it was concluded that the structure is involved in regulating translation initiation efficiency, thereby coupling selenocysteine biosynthesis to the availability of the trace element selenium.

Keywords: elongation factor/mRNA-binding protein/RNA secondary structure/selenocysteine/translation

Introduction

The amino acid selenocysteine (Sec) has been identified as a component of selected proteins in bacteria, archaea and eukarya (Low and Berry, 1996; Stadtman, 1996; Rother *et al.*, 2001). It is encoded by an in-frame UGA codon interrupting the reading frame of the respective selenoprotein gene and is co-translationally inserted into the nascent polypeptide chain. In *Escherichia coli*, there are three genes coding for selenium-containing formate dehydrogenases, which are expressed constitutively (*fdoG*) or under anaerobic conditions only (*fdhF* and *fdnG*) (Zinoni *et al.*, 1986; Berg *et al.*, 1991; Sawers *et al.*, 1991). The biosynthesis of selenocysteine and its insertion into these proteins require the function of at least four gene products (see Figure 10). *selC* codes for tRNA^{Sec}, which is charged with L-serine by seryl-tRNA synthetase (SerS) and serves as the adapter at which the seryl moiety is converted into the selenocysteyl derivative. The conversion reaction is catalyzed by selenocysteine synthase (the

selA gene product) with the aid of monoselenophosphate as the selenium donor substrate, which is synthesized from selenide and ATP by selenophosphate synthetase (the *selD* gene product). SelB, finally, is a specialized translation factor taking over the function of elongation factor (EF) Tu in selenocysteine insertion. Its N-terminal three domains are homologous to EF-Tu, but it additionally exhibits a C-terminal extension of 272 amino acids, designated domain IV (for reviews, see Hüttenhofer and Böck, 1998; Böck, 2001; Böck *et al.*, 2002).

SelB, in addition to interacting with guanosine nucleotides, binds two RNA ligands, namely Sec-tRNA^{Sec} and the SECIS element of the mRNAs coding for selenoproteins. In bacteria, this mRNA structure is located immediately 3' of the UGA codon that determines the position of selenocysteine incorporation in the nascent polypeptide chain. The binding order is random, but interaction with both RNA substrates leads to a stabilization of the resulting quaternary complex consisting of SelB, GTP, Sec-tRNA^{Sec} and the SECIS element (Baron *et al.*, 1993). Using rapid kinetic analysis, it was shown that the affinity of SelB for the SECIS element is maximized by Sec-tRNA^{Sec} and decreases after its dissociation to allow the translation of codons 3' of the UGA directing selenocysteine insertion (Thanbichler *et al.*, 2000).

The interaction of SelB with the bacterial SECIS element has been additionally probed with a variety of techniques, like truncation of *fdhF* from the 3' side (Zinoni *et al.*, 1990), introduction of single or multiple mutations (Heider *et al.*, 1992; Liu *et al.*, 1998), gel retardation experiments (Baron *et al.*, 1993; Kromayer *et al.*, 1996), footprint analysis (Ringquist *et al.*, 1994) and *in vitro* selection of RNA molecules (aptamers) that tightly bind to SelB (Klug *et al.*, 1997, 1999). The main information gained and relevant for this communication was that: (i) the interaction involves a 17 kDa C-terminal domain of SelB (SelB_{472–614}), designated domain IVb, and the apical part of the SECIS hairpin; (ii) the affinity of the interaction was lower between SelB and the SECIS element as compared with that between domain IVb and the SECIS element, but was equal when SelB was additionally complexed with Sec-tRNA^{Sec}; (iii) finally, and intriguingly, some of the aptamers selected *in vitro* displayed excellent binding affinities but were not functional in the decoding process. Therefore, binding and function in the decoding process could be separated. In the present communication, we report the existence of a SECIS element present in the 5' untranslated region of the mRNA for the bicistronic *selAB* operon. We show that this SECIS structure interacts with SelB and, in the presence of Sec-tRNA^{Sec}, represses translation. By this novel mechanism, it adjusts the level of the gene products to the supply of the trace element selenium. Interestingly, this regulatory SECIS element is

functional in the decoding process, although at reduced efficiency.

Results

The SECIS-like element

The structural genes coding for selenocysteine synthase (*selA*) and translation factor SelB (*selB*) in *E.coli* are organized in a bicistronic transcriptional unit and their reading frames overlap by four bases (Figure 1A). Previous analysis of the transcription of the *selAB* operon has shown that it is transcribed very weakly from a single start site at position -48 relative to the *selA* initiation codon (Sawers *et al.*, 1991). When the 5' non-translated RNA sequence was inspected for regulatory motifs, a putative RNA secondary structure was detected that strikingly resembled the SECIS element directing selenocysteine insertion (Figure 1B). Using mfold 3.1 (Mathews *et al.*, 1999), its free energy was calculated to be -10.6 kcal/mol at 37°C. Since it is located outside the reading frame, it was designated SECIS-like. The putative minimal binding region of the SECIS-like element contains the essential structural and sequence elements that had been shown to be required for SECIS proper (Heider *et al.*, 1992; Hüttenhofer *et al.*, 1996; Liu *et al.*, 1998; Li *et al.*, 2000): the apical helix is closed by two G-C/G-C Watson-Crick base pairs and a preceding A-U/U-A base pair at the basal end and a C-G base pair at the distal end, and contains a bulged-out U residue at a position equivalent to +17 in the SECIS elements. The loop region contains the GU doublet that is supposed to make contact with the SECIS-binding pocket of SelB (Figure 1C).

The SECIS-like structure is positioned at the immediate 5' end of the mRNA and does not include the Shine-Dalgarno motif (Figure 1B).

The SECIS-like element binds SelB and forms a complex with SelB and Sec-tRNA^{Sec}

The striking similarities between the SECIS and the SECIS-like element prompted the analysis on whether the SECIS-like element is a binding target for translation factor SelB. A transcript was synthesized *in vitro* comprising the stem-loop structure at the 5' end of the *selAB* mRNA (see Figure 1B). It was ³²P-labeled at the 5'-terminus and used in gel retardation experiments (Figure 2). Figure 2A shows that the SECIS-like element yields a distinct shift signal at a 5-fold or higher molar excess of protein over the transcript despite the presence of a huge excess of unspecific competitor tRNA. The signal remains unchanged when additional competitor tRNA is added, but disappears completely if an equal amount of cognate SECIS RNA is included in the reactions. This indicates a specific interaction that involves the SECIS-binding site of SelB.

From previous experiments, it was known that a crucial requirement for SelB to function in the decoding of UGA is the capacity to undergo a sequence of conformational changes which ultimately result in the release of Sec-tRNA^{Sec} at the ribosomal A-site and in the resolution of the SelB-SECIS complex (Thanbichler *et al.*, 2000). These switches are reflected in the fact that SelB interacts with SECIS elements with a lower affinity and specificity, as does the C-terminal binding domain alone. To assess

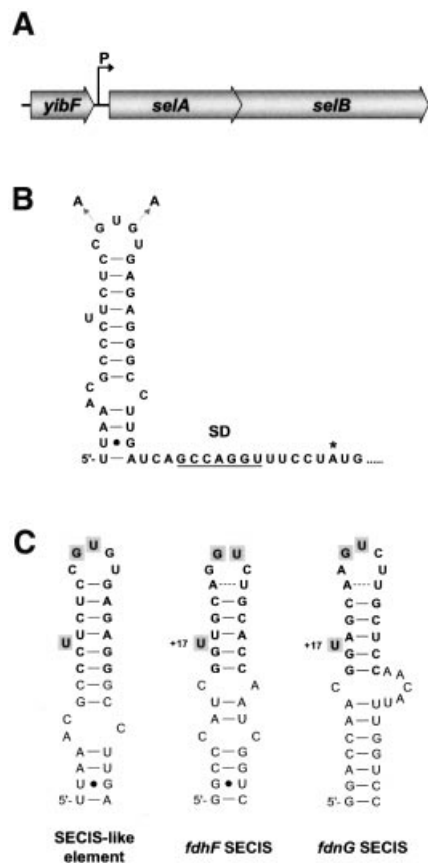


Fig. 1. (A) Organization of the *selAB* operon. (B) Fold of the stem-loop structure at the 5' end of the *selAB* transcript as calculated by the mfold 3.1 program (Mathews *et al.*, 1999). The transcription initiation site as determined by Sawers *et al.* (1991), the Shine-Dalgarno motif (SD), the *selA* start codon (*) and the exchanges introduced to generate a mutant form of the SECIS-like element are indicated. (C) Comparison of the SECIS-like element with the SECIS structures of the *fdhF* and *fdnG* mRNAs. The minimal binding regions are depicted in bold. Bases supposed to make direct contact with SelB (Hüttenhofer *et al.*, 1996) are shaded. The bulged-out U of the SECIS elements at position +17 relative to the selenocysteine-encoding UGA codon is indicated.

whether the SECIS-like element follows the same pattern, mobility shift experiments were conducted by probing the SECIS-like transcript with SelB₄₇₂₋₆₁₄ (Figure 2B). Surprisingly, SelB₄₇₂₋₆₁₄ is unable to form a stable complex with the SECIS-like element, whereas it tightly binds the *fdnG* (data not shown) and *fdhF* SECIS elements (Kromayer *et al.*, 1996; Thanbichler *et al.*, 2000). A weak interaction certainly takes place, as indicated by the tailing of the signal for the transcript, which is absent in the lane containing an excess of specific competitor RNA. There was no interaction detectable between the SECIS-like element and isolated SelB₃₇₉₋₆₁₄, which comprises almost the entire domain IV of SelB (data not shown). These results are different from those obtained with the *fdnG* SECIS element, which binds to SelB₃₇₉₋₆₁₄ with the same affinity as to full-length SelB (our unpublished results). The characteristics of the SECIS-like element are reminiscent of the binding behavior of some of the aptamers selected *in vitro* by Klug *et al.* (1997), which interact well with full-length SelB, but not with isolated SelB₄₇₂₋₆₁₄. When Sec-tRNA^{Sec} is included in the incubation mixture

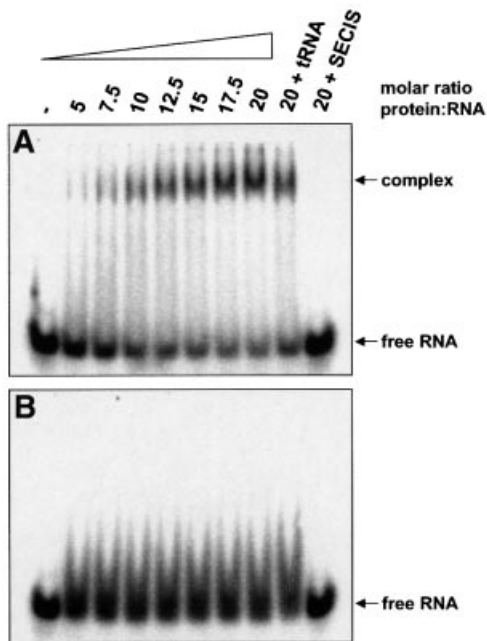


Fig. 2. Interaction of the SECIS-like element with wild-type SelB (A) and SelB₄₇₂₋₆₁₄ (B). ³²P-labeled SECIS-like element (80 nM) was incubated with increasing amounts of protein in the presence of 3.6 μM yeast tRNA. In the last two lanes, additional 3.6 μM unspecific (yeast tRNA) or specific (*fdhF* SECIS) competitor RNA was added to the reactions. After separation of the mixtures in a non-denaturing polyacrylamide gel, radioactivity was detected using a PhosphorImager.

(Figure 3B), a strong retardation signal is already present at a 2.5-fold molar excess of protein, indicating a significant increase in the affinity of SelB for the SECIS-like element. Moreover, the mobility of the complex formed is enhanced, which is due to the additional negative charges introduced by binding of the tRNA. This resembles the binding behavior described previously for the *fdhF* SECIS element; namely, that a tighter and fast migrating complex is formed when both nucleic acid substrates are bound (Baron *et al.*, 1993).

To test whether the interaction between SelB and the SECIS-like element is specific, we have introduced two base changes into the loop region of the hairpin (see Figure 1B). From previous experiments, it was known that the exchange of bases in the terminal loop of the *fdhF* SECIS element is detrimental to SelB binding and thus abolishes its function in the decoding process (Heider *et al.*, 1992; M.Kromayer, unpublished results). Figure 4 indicates that this also holds for the binding capacity of the mutant SECIS-like element, as the ability to interact with SelB disappears.

To obtain qualitative information on the affinity of the interaction between SelB and the SECIS-like element, *in vitro* transcripts of the SECIS-like element and the *fdnG* SECIS element were titrated in parallel with increasing amounts of SelB. Complex formation, as analyzed by gel retardation, is depicted in Figure 5. It appears that the SECIS-like structure has an ~3- to 4-fold lower affinity to the target protein. The K_D value for the interaction of the *fdnG* SECIS with SelB has been determined to be 1.4 nM (Thanbichler *et al.*, 2000). Therefore, the corresponding value for the binding of the SECIS-like element might be in the range of ~10 nM.

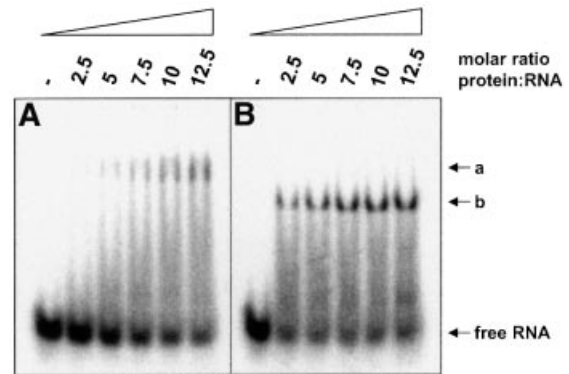


Fig. 3. Effect of Sec-tRNA^{Sec} on complex formation between SelB and the SECIS-like element. ³²P-labeled SECIS-like element (80 nM) was incubated with increasing concentrations of SelB in the presence of 3.6 μM yeast tRNA (A). To analyze the influence of Sec-tRNA^{Sec}, 1.0 μM Sec-tRNA^{Sec} and 100 μM GTP were additionally included in the reactions (B). GTP (100 μM) was added to the gel and electrophoresis buffer for separating the mixtures in both cases. Radioactivity was detected using a PhosphorImager. The positions of the ternary (a) and quaternary (b) complexes are indicated by arrows.

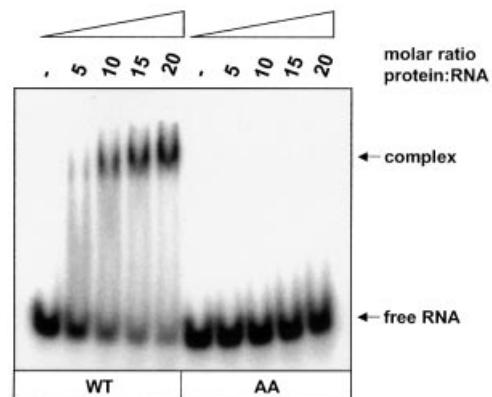


Fig. 4. Specificity of the interaction between the SECIS-like element and SelB. ³²P-labeled wild-type (WT) or mutant (AA) SECIS-like element (80 nM) (see Figure 1B) was mixed with increasing amounts of SelB in the presence of 3.6 μM yeast tRNA as unspecific competitor. Complexes formed were separated from unbound RNA by electrophoresis in a non-denaturing polyacrylamide gel and radioactivity was detected using a PhosphorImager.

The SECIS-like element functions in the regulation of *selAB* expression

The characteristics of the SECIS-like element described above showed convincingly that it can form both a complex with SelB alone and a quaternary complex with SelB, Sec-tRNA^{Sec} and presumably GTP, the latter interaction being significantly more stable. A speculation emanating from this is that the formation of the quaternary complex on the SECIS-like structure might affect the expression of the *selAB* mRNA. To follow this assumption, we have correlated the capacity of cells to form this complex with the amount of gene products for the *sel* genes. To this end, the wild-type strains PT90422, MC4100 and EMG29, and derivatives thereof carrying mutations in one or two of these genes, were grown to exponential phase, the cells were harvested and lysed, and the lysates analyzed for the quantity of *sel* gene products by immunoblotting (Figure 6). Intriguingly, a full correlation was detected: synthesis of the *selA* gene product was

suppressed in a genetic background that renders the cells proficient for the formation of Sec-tRNA^{Sec} and the concomitant production of SelB. In contrast, mutations in either *selD*, *selC*, *selB* or *selA* alleviate this suppression.

The same regulatory pattern was observed for the *selB* gene product, although the effect was less pronounced. This correlates with previous results demonstrating that there is an internal translation initiation site upstream of the *selB* gene, which at least partly uncouples *selB* from *selA* mRNA translation and enables expression of *selB* when transcribed as a single gene under the control of the T7Φ10 promoter (Forchhammer *et al.*, 1990). Physiologically, this is meaningful since SelB represents the key regulatory component, whose concentration should not fall below a critical level. The level of SelD remained constant in all strains tested and seems not to be regulated.

Analysis of regulation by the SECIS-like element employing *lacZ* reporter gene fusions

Since immunoblotting yields information on the steady-state level of gene products only and does not integrate the contribution of synthesis and degradation, the studies on

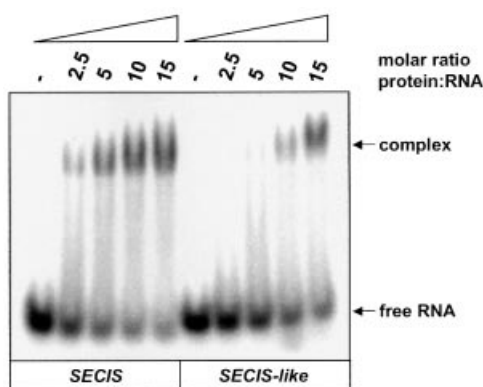


Fig. 5. Affinity of the SECIS-like element for SelB. ³²P-labeled *fdnG* SECIS or SECIS-like element (80 nM) was incubated with increasing amounts of SelB in the presence of 3.6 μM yeast tRNA as unspecific competitor. After separation in a non-denaturing polyacrylamide gel, unbound and complexed RNA was detected using a PhosphorImager.

the regulatory function of the SECIS-like element were extended by the use of *lacZ* reporter gene fusions. Both transcriptional and translational fusions were constructed (Figure 7A). To ensure the correct stoichiometry between regulatory factors and the mRNA, the fusion constructs were integrated into the λ phage and subsequently transferred in single copy to the λ attachment site of the chromosome. Assaying the level of β-galactosidase in cells lysogenized with λRL1, which carries the *selA* upstream region and a 5' fragment of *selA* fused in-frame to *lacZ*, shows that the expression of the reporter gene is repressed in a wild-type genetic background, whereas it is increased ~5-fold in strains with a mutation preventing formation of a functional quaternary complex (Figure 7B). The results obtained fit the regulation pattern as revealed by immunoblot analysis (see Figure 6) and indicate that binding of SelB·GTP·Sec-tRNA^{Sec} to the SECIS-like element is crucial for the repression observed in the wild-type strain. This model is corroborated by the fact that only the derepressed level of β-galactosidase is measured in strains carrying λRL12, a phage whose insert is identical to that of λRL1 except for two mutations in the loop region of the SECIS-like element that have been shown to prevent binding of SelB (see Figure 4). The regulatory pattern of *selB* expression was followed using strains that were lysogenized with phage λRLΔAB carrying the *selA* upstream region, the *selA* open reading frame and a 5' fragment of *selB* fused in-frame to *lacZ*. A deletion that has been shown not to have any polar effects on *selB* expression (data not shown) was introduced into the *selA* gene of the fusion construct to prevent complementation in a Δ*selA* genetic background. As depicted in Figure 7C, synthesis of β-galactosidase is repressed ~3-fold in the wild type, whereas derepression occurs in strains deficient in quaternary complex formation. The effect is less distinct than the one measured for *selA* and again corresponds to the observations made by immunoblot analysis (Figure 6).

To ensure that the increase in β-galactosidase production that was measured in the mutant strains is a specific effect caused by the absence of a factor necessary to form the regulatory complex, complementation studies were performed. It was shown that the introduction of plasmids that lead to a moderate overproduction of SelA (with the

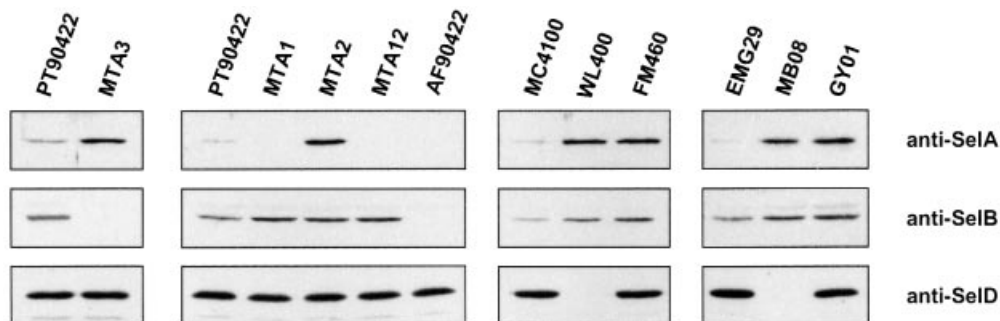


Fig. 6. Immunoblot analysis of the intracellular level of SelA, SelB and SelD in *sel* mutants derived from *E.coli* strains PT90422, MC4100 and EMG29. Cells of the strains PT90422 (wild type), MTA3 (Δ*selB*), MTA1 (Δ*selA*), MTA2 (Δ*selC*), MTA12 (Δ*selA*, Δ*selC*), AF90422 (Δ*selAB*), MC4100 (wild type), WL400 (Δ*selD*), FM460 (Δ*selC*), EMG29 (wild type), MB08 (*selD165*) and GY01 (*selA160*) were grown to an OD₆₀₀ of 1.5, harvested and lysed in sample buffer. The lysates were applied to a 10% SDS-polyacrylamide gel. After separation of the proteins and transfer to a nylon membrane, immunoblot analysis was performed. Note that the point mutation carried by strain GY01 results in the synthesis of a stable but inactive form of SelA, whereas all other mutants do not synthesize stable products of the mutated genes.

SECIS-like element deleted from the upstream region of the plasmid-encoded *selA* gene) or tRNA^{Sec} could completely override the derepression of reporter gene expression that was observed with strains MTA1:: λ RL1 ($\Delta selA$) or MTA2:: λ RL1 ($\Delta selC$), respectively, when they were carrying a control plasmid only (data not shown).

Effect on *selAB* mRNA synthesis and stability

There are several possible mechanisms that could underly the observed regulatory influence exerted by quaternary complex formation at the SECIS-like element, namely transcriptional attenuation, modulation of mRNA stability or regulation of translation initiation. Regulation of transcription by binding of proteins to their own message has emerged to be a general scheme for adjusting protein biosynthesis to the level of a gene product (Yanofsky, 2000). To analyze whether the SECIS-like element might mediate attenuation of *selAB* transcription, the level of β -galactosidase was assayed in strains lysogenized with phage λ RC1, which carries the *selA* upstream region fused to the intact *lacZ* gene (Figure 7D). It appeared that the level of the reporter gene product was constant, independent of the genotype of the infected cells. The same specific β -galactosidase activity was obtained when the wild-type strain PT90422 was lysogenized with λ RC12 instead of λ RC1. This phage carries a similar construct with a mutated form of the SECIS-like element unable to interact with SelB (see Figure 4). These results clearly exclude a regulatory role for the SECIS-like element at the transcriptional level.

Next, it was investigated whether the stability of the *selAB* mRNA changes when the SECIS-like element is included in a quaternary complex. Many mRNAs have been shown to be protected against degradation by secondary structures positioned at their immediate 5' ends, which are supposed to interfere with the initial endonucleolytic cleavage catalyzed by RNase E (Rauhut and Klug, 1999). Accordingly, binding of SelB-GTP-Sec-tRNA^{Sec} might alter the conformation of the the SECIS-like element or the bordering region and thereby enhance the degradation of the *selAB* mRNA. To address this issue, the steady-state level and half-life of the *selAB* mRNA were analyzed using the S1 nuclease protection assay. Two labeled probes (Figure 8A) hybridizing with the 5' and the 3' end of the *selA* transcript, respectively, were synthesized and used to quantify the transcript levels. As depicted in Figure 8B, there was a reproducible, ~1.5- to 2-fold increase in the steady-state amount of mRNA in the $\Delta selC$ mutant as compared with the wild-type strain. A similar effect was also observed in a $\Delta selA$ or $\Delta selB$ genetic background (data not shown). The ratios between the levels of the 3' and the 5' ends remained approximately constant at 60%. To investigate whether the half-life of the transcript changes under conditions of impeded quaternary complex formation, the decay profile of the *selAB* mRNA was determined for PT90422 (wild type) and MTA2 ($\Delta selC$), which both have the wild-type operon. Figure 8C shows that the 5' end of the transcript is rapidly degraded and reaches a basal level ~6 min after inhibition of transcription. In the $\Delta selC$ genetic background, the mRNA is more stable ($t_{1/2} = 67$ s) than in the wild-type strain

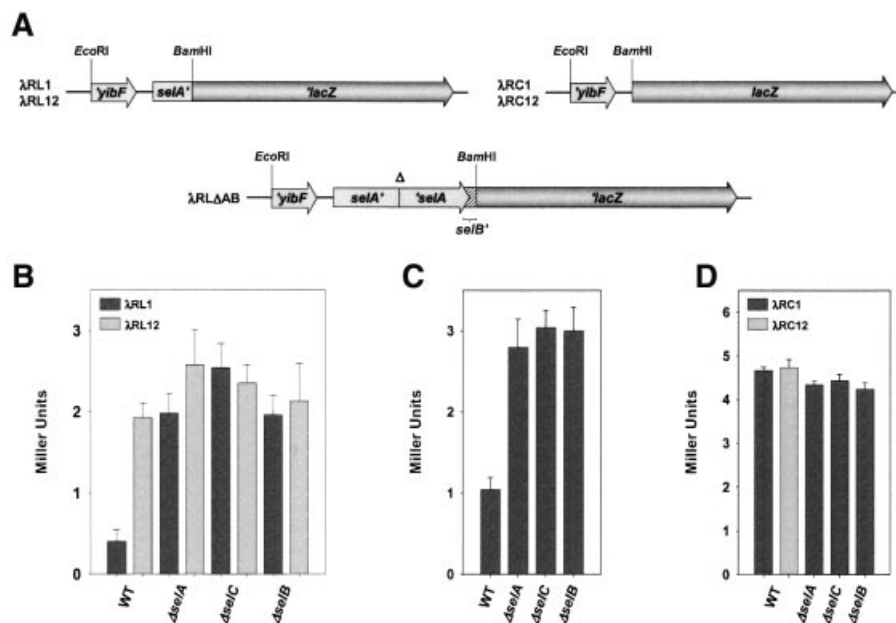


Fig. 7. Regulatory role of the SECIS-like element. (A) Reporter constructs employed to study regulation by the SECIS-like element. The restriction sites used to insert the respective fragment into plasmids pRS551 or pRS552 (Simons *et al.*, 1987) are shown. The position of the deletion introduced into *selA* on the reporter construct harbored by phage λ RL Δ AB is indicated (Δ). λ RL12 and λ RC12 carry the mutated SECIS-like element (see Figure 1B), whereas all other phages contain the wild-type sequence. (B) β -galactosidase activities measured for strains PT90422 (WT), MTA1 ($\Delta selA$), MTA2 ($\Delta selC$) and MTA3 ($\Delta selB$) after lysogenization with phages that carry *selA'*-*lacZ* translational fusions encoding the wild-type (λ RL1) or mutant (λ RL12) SECIS-like element. (C) β -galactosidase activities determined for the same strains, when carrying the *selAB'*-*lacZ* translational fusion harbored by λ RL Δ AB. (D) β -galactosidase activities exhibited by strains PT90422, MTA1, MTA2 and MTA3, when lysogenized with phages λ RC1 and λ RC12, which harbor transcriptional fusions encoding the wild-type and mutant SECIS-like element, respectively.

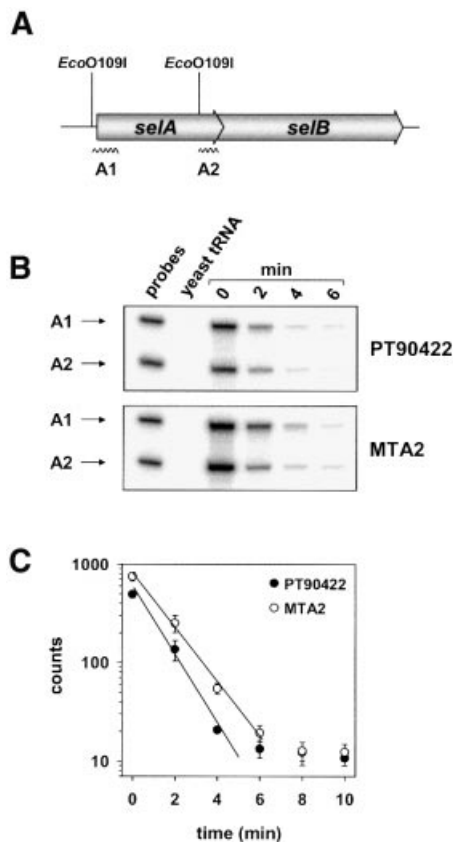


Fig. 8. Steady-state level and half-life of the *selAB* transcript. (A) Localization of probes A1 and A2 directed against the *selA* mRNA. The restriction sites used to generate the probes are indicated. (B) S1 nuclease protection assay. Seventy-five micrograms of yeast tRNA or total RNA that was extracted from cells of strains PT90422 (WT) and MTA2 ($\Delta selC$) harvested before (0 min) or 2, 4 and 6 min after inhibition of transcription by addition of rifampicin were incubated with 10 000 c.p.m. of each probe. After treatment with S1 nuclease, the remaining probes were applied to an 8% urea-polyacrylamide gel, separated and visualized using a PhosphorImager. (C) Half-life of the *selAB* mRNA. The signals of probe A1 obtained in three independent experiments of the type described in Figure 8B were quantified using ImageQuant 2.1 (Molecular Dynamics), normalized to an internal standard and averaged. The straight lines fitted to the linear parts of the curves by regression analysis of the data correspond to half-lives of 67 s in strain MTA2 and 52 s in strain PT90422.

($t_{1/2} = 52$ s), which explains the difference in the steady-state levels observed before. Very similar values were obtained with probe A2, which anneals to the 3' region of *selA* (data not shown).

The SECIS-like element functions as a selenocysteine insertion element

Having resolved the function of the SECIS-like structure as a novel type of regulatory element, it was tempting to assess whether it may still act as a genuine SECIS element, i.e. mediate the decoding of UGA with selenocysteine. To follow this possibility, a plasmid (pWA) was constructed that carries a *lacZ* gene with an in-frame UGA codon followed by the sequence encoding the SECIS-like element inserted into its 5' region (Figure 9A). The distance between the UGA codon and the sequence of the SECIS-like element corresponding to the minimal binding region of SECIS elements was adjusted to 11 bp, which

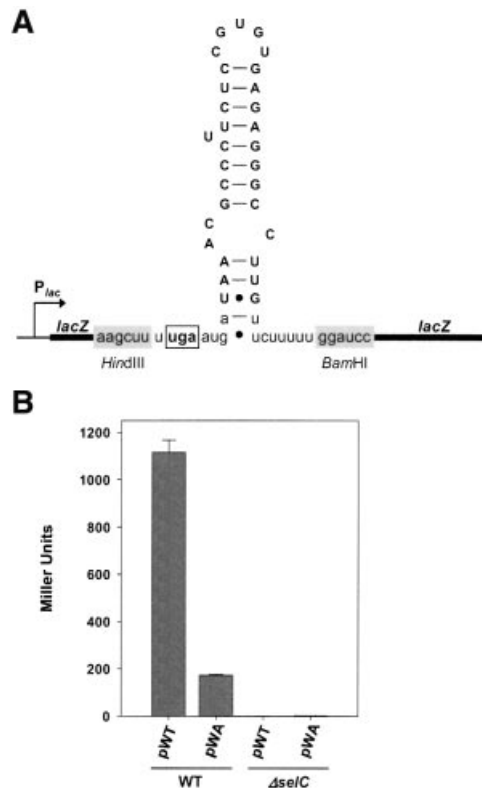


Fig. 9. Ability of the SECIS-like element to mediate SelB-dependent UGA read-through. (A) Schematic representation of the insert carried by plasmid pWA. The sequence of the insert replacing the *fdhF* SECIS cartridge of pWT is depicted as RNA in the predicted stem-loop conformation. Residues identical to those of the SECIS-like element are represented in upper case (B) β -galactosidase activities measured for strains PT90422 (WT) and MTA2 ($\Delta selC$) transformed with pWT (*fdhF* SECIS) and pWA (SECIS-like element).

has been shown to be optimal for SelB activity (Heider *et al.*, 1992; Chen *et al.*, 1993; Liu *et al.*, 1998). As a control, plasmid pWT was employed, which contains the *fdhF* SECIS in exactly the same position. The two plasmids were transferred into *E. coli* PT90422 (wild type) and MTA2 ($\Delta selC$), and β -galactosidase activities were determined as a measure for read-through at the UGA codon (Figure 9B). The results clearly show that the SECIS-like element is able to act as an insertion sequence, but only at a reduced efficiency.

Discussion

Selenocysteine insertion sequences in bacterial selenoprotein mRNAs guide the ribosome to translate in-frame UGA codons with selenocysteine. They do so by the formation of a quaternary complex with translation factor SelB, Sec-tRNA^{Sec} and GTP. Concomitant binding of the SECIS element and the charged tRNA^{Sec} confers to SelB a conformation required to interact with the ribosome and to compete with release factor 2 (for a review, see Böck, 2001; Thanbichler and Böck, 2001). The 'export' of a SECIS element into the 5' untranslated region of the mRNA and its effect on the expression of the *selAB* mRNA represent a novel mechanism of post-transcriptional control. The moderate affinity of the SECIS-like element for SelB alone and the increase in the tightness of

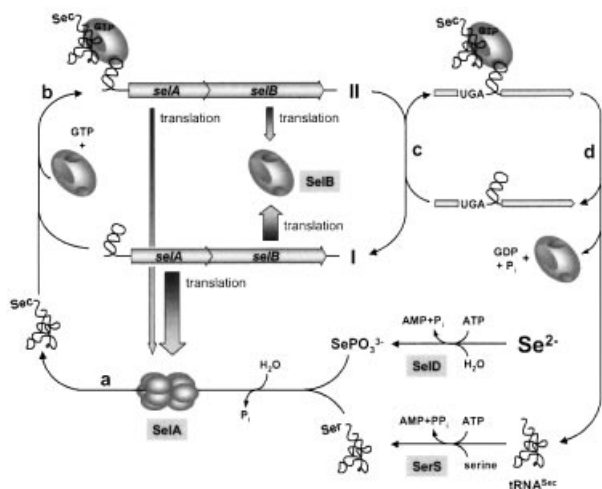


Fig. 10. Proposed model of the reactions involved in the regulation of *selAB* expression. Without the quaternary complex assembled at the SECIS-like element (state I), translation of *selA* and *selB* is derepressed. Biosynthesis of Sec-tRNA^{Sec} (a), which is dependent on a sufficient supply of selenide, enables the formation of the regulatory complex on the 5' mRNA structure of the *selAB* transcript (b), thereby decreasing the rate of translation of *selA* and, to a lesser extent, *selB* (state II). Competition between the SECIS-like element and the SECIS element of selenoprotein mRNAs (c) leads to the dissociation of the regulatory complex and to the formation of the SelB-GTP-Sec-tRNA^{Sec}-SECIS complex, which catalyzes the decoding of UGA as selenocysteine. After A-site interaction and translocation of the ribosome (d), SelB and tRNA^{Sec} are released and ready to re-enter the cycle.

the complex in the presence of Sec-tRNA^{Sec} may be crucial for this function. In this way, the system measures the level of charging of tRNA^{Sec} with selenocysteine and suppresses translation of *selA*, the gene for the main selenocysteine biosynthetic enzyme. What is the physiological need for this control? It may reside in the fact that selenocysteine is a highly toxic molecule because of the reactivity of its selenol group and that it should not be accumulated in the cell (even when attached to tRNA) beyond the level required for selenoprotein formation. Excess Sec-tRNA^{Sec} might cause an increased intracellular level of free selenocysteine by spontaneous hydrolysis of the labile ester bond. It has been shown that selenocysteine can be recycled and serve as a selenium donor for the *de novo* synthesis of Sec-tRNA^{Sec} (Lacourciere, 2002; Mihara *et al.*, 2002). However, selenocysteine also readily infiltrates protein biosynthesis by substituting for cysteine (Müller *et al.*, 1997), which in many cases leads to the inactivation of the affected enzyme due to the considerable difference in the redox potentials of the thiol and the selenol group. The fact that the expression of *selA* is regulated even though the level of the gene product is generally very low might argue for the toxicity of abundant selenocysteine formation. The regulatory function of the SECIS-like element also enables the cell to respond to the availability of the rare trace element selenium: at high supply the formation of the biosynthetic enzyme is suppressed and at limiting supply it is increased. Since no regulation has been detected for *selD* expression, this represents the only way to facilitate scavenging of the substrate under selenium-limiting conditions. Competition between the SECIS-like and the SECIS elements might

additionally allow the cell to quickly adjust selenocysteine biosynthesis to sudden shifts in the intracellular concentration of selenoprotein mRNAs, as induced by abrupt changes between aerobic and anaerobic growth conditions. An increase in the cellular concentration of SECIS elements withdraws SelB-GTP-Sec-tRNA^{Sec} from the regulatory structure and thereby induces the production of additional SelA molecules to meet the elevated need for selenocysteylated tRNA^{Sec}. In contrast, reduced synthesis of selenoprotein mRNAs leads to a higher occupancy of the SECIS-like element and consequently to a decrease in the capacity to synthesize selenocysteine (Figure 10).

The finding that the SECIS-like element also functions as an 'insertion sequence' is intriguing, as its sequence strongly deviates from the SECIS consensus and exhibits exchanges that would cause a total loss of function when introduced into a genuine SECIS element as single mutations (Heider *et al.*, 1992). By *in vitro* selection of aptamers able to bind SelB, it was possible to identify RNA molecules that showed an even higher affinity for SelB than the original SECIS element, although their sequences were only distantly related to that of the SECIS element (Klug *et al.*, 1997). However, in contrast to the SECIS-like element, most of them were inactive in promoting UGA read-through at selenoprotein mRNAs. It was speculated that there exist multiple possibilities to fit RNA molecules into a binding site at the protein, although these molecules do not necessarily include all the features needed for biological activity. The SECIS-like element is a natural example for such a system: the overall binding properties are maintained, but the steric arrangement of determinants that are necessary to trigger the conformational change enabling SelB to interact with the ribosome is different, so that the ability to decode a UGA codon is drastically impaired. This reduction in function is reflected by the inability of the SECIS-like element to stably interact with domain IVb of SelB. Isolated domain IVb shows a very high stringency in the recognition of the SECIS element (Kromayer *et al.*, 1996) and this may be the reason why the SECIS-like structure is partially excluded.

Although the finding that a translation factor complexed with a specific tRNA regulates gene expression is novel, the underlying mechanism bears some resemblance to the way translational control is achieved in the biosynthesis of threonyl-tRNA synthetase (Sacerdot *et al.*, 1998) and several ribosomal proteins (reviewed by Zengel and Lindahl, 1994). These proteins repress translation by binding to the 5' region of their own mRNA, which mimics the structure of the cognate RNA substrate, and thereby modulate the accessibility and quality of the translation initiation region (TIR). The exact mechanism enabling post-transcriptional control of *selA* and *selB* expression by the SECIS-like element in its complex with SelB and Sec-tRNA^{Sec} is still open. Attenuation of transcription can be excluded as quaternary complex formation on the SECIS-like element showed no influence on the transcription rate. An ~1.5-fold increase in the steady-state level of the *selAB* mRNA was observed in the absence of the functional complex, which can be explained by the elevated stability of the transcript observed under the same condition. A direct influence of quaternary complex formation at the SECIS-like element on mRNA stability

Table I. Relevant bacterial strains, phages and plasmids used in this study

	Genotype	Reference
Strains		
PT90422	F ⁻ , <i>araD139</i> , $\Delta(\textit{argF-lac})\textit{U169}$, <i>ptsF25</i> , <i>deoC1</i> , <i>relA1</i> , <i>flbB5301</i> , <i>rpsE</i> ⁺ , <i>strA1</i>	P.Tormay, unpublished
MTA1	PT90422, $\Delta\textit{selA}$	This study
MTA2	PT90422, $\Delta\textit{selC}$	This study
MTA3	PT90422, $\Delta\textit{selB}$	This study
MTA12	PT90422, $\Delta\textit{selA}$, $\Delta\textit{selC}$	This study
AF90422	PT90422, $\Delta(\textit{selAB})$	A.Friebel, unpublished
MC4100	F ⁻ , <i>araD193</i> , $\Delta(\textit{argF-lac})\textit{U169}$, <i>ptsF25</i> , <i>relA1</i> , <i>deoC1</i> , <i>flbB5301</i> , <i>rpsL150</i> , λ ⁻	Casadaban and Cohen (1979)
FM460	MC4100, $\Delta\textit{selC400::neo}$	Sawers <i>et al.</i> (1991)
WL400	MC4100, $\Delta\textit{selD204::cat}$	Leinfelder <i>et al.</i> (1990)
EMG29 (NCIB 10241)	F ⁻ , <i>proC23</i> , <i>trp-30</i> , <i>his-51</i> , <i>lac-28</i> , <i>rpsL101</i> , λ ⁺	NCIB
GY01	EMG29, <i>selA160</i>	Mandrand-Berthelot <i>et al.</i> (1978)
MB08	EMG29, <i>selD165</i>	Graham (1980)
Phages		
λ RS45	<i>lacZYA</i> , <i>imm</i> ²¹ , <i>ind</i> ⁺	Simons <i>et al.</i> (1987)
λ RL	λ RS45, Kn ^R , <i>lacZYA</i>	This study
λ RL1	λ RS45, Kn ^R , $\Phi(\textit{selA}'\textit{-lacZ})$, <i>lacYA</i>	This study
λ RL12	λ RS45, Kn ^R , $\Phi(\textit{selA}'\textit{-lacZ})$, <i>lacYA</i> , mutant SECIS-like element	This study
λ RL Δ AB	λ RS45, Kn ^R , $\Phi(\textit{selA}_{\Delta(490-936)}\textit{B}'\textit{-lacZ})$, <i>lacYA</i>	This study
λ RC	λ RS45, Kn ^R , <i>lacZYA</i>	This study
λ RC1	λ RS45, Kn ^R , <i>selA</i> upstream region, <i>lacZYA</i>	This study
λ RC12	λ RS45, Kn ^R , <i>selA</i> upstream region, <i>lacZYA</i> , mutant SECIS-like element	This study
Plasmids		
pWT	ColE1 <i>ori</i> , Ap ^R , (<i>lacZ-fdhF</i>) ₄₂ , <i>lacYA</i>	Heider <i>et al.</i> (1992)
pWA	pWT derivative, SECIS-like element instead of <i>fdhF</i> SECIS	This study
pUAB	ColE1 <i>ori</i> , Ap ^R , <i>selAB</i>	This study

is improbable as the capability to form the complex did not influence the synthesis of β -galactosidase in strains harboring a transcriptional fusion of the *selA* upstream region with *lacZ*. Rather, increased translation initiation rates in the absence of the complex might lead to a more frequent occupancy of the TIR and thereby to a more efficient masking of internal RNase target sites, as shown for several other systems (Baumeister *et al.*, 1991; Yarchuk *et al.*, 1991; Jain and Kleckner, 1993; Braun *et al.*, 1998).

It is unlikely that this small influence on the mRNA level fully accounts for the 5-fold stimulation of LacZ production observed in strains carrying the *selA'*-*lacZ* translational fusions. Therefore, the main function of quaternary complex formation on the SECIS-like element is presumably the regulation of translation initiation efficiency. In the presence of tRNA^{fMet}, the ribosomal 30S subunit covers ~54 bases around the initiation codon of an mRNA, ranging from position -35 to +19 (Hüttenhofer and Noller, 1994). The regulatory structure encompasses bases -48 to -16 of the *selAB* transcript and, therefore, overlaps with the binding site of the initiating ribosome. Either the stem-loop has to be melted completely to allow translation initiation to occur or it can be adopted by the ribosomal mRNA track, as shown for the mRNA structures mediating translational control of *thrS* expression (Sacerdot *et al.*, 1998). In both situations, loading of the SECIS-like element with SelB-GTP-Sec-tRNA^{Sec} might pose additional thermodynamic or steric constraints on the TIR-30S subunit

interaction, leading to a decreased efficiency of initiation complex formation.

Strain MTA3, which lacks *selB*, did not show higher levels of β -galactosidase than strains with mutations in other *sel* genes, when lysogenized with λ RL1. Consequently, association of the SECIS-like element with SelB alone does not seem to have any repressive effect on *selA* translation, which is reasonable as the structure is likely to be permanently occupied by SelB under the conditions prevalent in the cell. The distinct regulatory function of the quaternary complex can be explained by its increased stability, which might impede the displacement of SelB from its mRNA-binding site. However, it cannot be excluded that the formation of the translation initiation complex is sterically hindered by SelB and that this effect depends on the interaction of SelB with the bulky tRNA ligand or on a particular conformational state of SelB that is adopted in the quaternary complex only. Association of SelB-GTP-Sec-tRNA^{Sec} with the SECIS-like element leads to the formation of a complex that is able to functionally interact with the ribosome and to decode UGA as selenocysteine. An interesting and unprecedented mechanism might, therefore, involve binding of a ribosome to the quaternary complex by A-site interaction, which may block translation initiation. Further experimentation, which is beyond the scope of this communication, is required to solve this issue.

Interestingly, there are some parallels between the SECIS-like element of the *selAB* mRNA and the SECIS

element of eukaryotic cells. Both structures are located in an untranslated region and both of them are able to mediate the decoding of UGA as selenocysteine by functionally interacting with components of the selenocysteine incorporation machinery, when positioned in a functional context. Since SECIS elements are thought to be responsible for the hierarchy of selenoprotein formation in higher eukaryotes (Low *et al.*, 2000), both structures additionally serve as regulatory elements that adjust the level of gene expression to the supply of the trace element selenium.

Materials and methods

Materials

[γ -³²P]ATP (6000 Ci/mmol) was purchased from NEN Life Science Products (Boston, MA). Oligonucleotides were synthesized by MWG Biotech (Ebersberg, Germany). Chromosomal DNA from *E. coli* MC4100 and T7 RNA polymerase, purified according to the method of Wyatt *et al.* (1991), were a generous gift from S. Leonhartsberger. Nucleotides, tRNA from baker's yeast and S1 nuclease were obtained from Roche Biochemicals (Mannheim, Germany). All other enzymes were from MBI Fermentas (St Leon-Roth, Germany) and New England Biolabs (Frankfurt, Germany).

Bacterial strains, phages, plasmids and standard procedures

The bacterial strains, phages and plasmids used in this study are listed in Table I. Generally, *E. coli* DH5 α (F⁻, *gyrA96*, *recA1*, *relA1*, *endA1*, *thi-1*, *hsdR17*, *ghnV44*, *deoR*, Δ (*lacZYA-argF*)U169, [Φ 80d Δ (*lacZ*)M15]) (Woodcock *et al.*, 1989) was used for the propagation of plasmids. Standard procedures were performed as described by Ausubel *et al.* (1997) and Sambrook *et al.* (1989). Owing to length limitations, details on the construction of plasmids, strains and recombinant phages are given in the Supplementary data available at *The EMBO Journal* Online.

Growth conditions and β -galactosidase activity assay

To measure the efficiency of UGA read-through, the test strains, carrying either pWT or pWA, were grown aerobically in TP medium (Heider *et al.*, 1992) at 37°C. Strains lysogenized with λ RS45 derivatives were cultivated aerobically at 37°C in TP2 medium, which contains 1% peptone, 0.6% glucose, 100 mM potassium phosphate pH 7.0, 1 mM MgCl₂, 0.1 mM CaCl₂, 1 μ M sodium selenite, and trace elements (Neidhardt *et al.*, 1974). When reaching the mid-exponential phase (OD₆₀₀ ~ 1.5), the cells were chilled on ice and used for the determination of β -galactosidase activities as described by Miller (1972). The results of at least three independent measurements, which were each performed in triplicate, were averaged and corrected for the background values, which were measured with strain PT90422 lysogenized with λ RL or λ RC, respectively.

Immunoblot analysis

Cells were grown aerobically in TP2 medium to the mid-exponential phase, harvested by centrifugation, resuspended in SDS sample buffer (Ausubel *et al.*, 1997) to an OD₆₀₀ of 10 and lysed by incubation for 15 min at 95°C. The proteins were separated in a 10% SDS-polyacrylamide gel (Laemmli, 1970), transferred to a nitrocellulose membrane (BioTrace NT; Pall Corporation, Ann Arbor, MI) and probed with rabbit antisera raised against SelA, SelB or SelD. Immunocomplexes were detected using horseradish peroxidase-protein A conjugate (Bio-Rad Laboratories, Munich, Germany) and the ECL system from Roche Biochemicals (Penzberg, Germany).

Purification of proteins

Wild-type SelB from *E. coli* was purified as described previously (Thanbichler *et al.*, 2000). SelB₄₇₂₋₆₁₄ fused with an N-terminal His₆ affinity peptide was prepared according to Kromayer *et al.* (1996).

Purification and aminoacylation of tRNA^{Sec}

tRNA^{Sec} was purified according to the method of Leinfelder *et al.* (1990). Aminoacylation of tRNA^{Sec} with serine and conversion of the seryl into a selenocysteyl residue were carried out as described previously (Thanbichler *et al.*, 2000).

Mobility shift assay

The *fdnG* stem-loop was prepared as described previously (Thanbichler *et al.*, 2000). The *fdhF* stem-loop and the SECIS-like elements were prepared by *in vitro* transcription (Milligan *et al.*, 1987; Wyatt *et al.*, 1991) using oligo T7-top (5'-TAATACGACTCACTATAG-3') and oligos fdhF-sl (5'-GACCGATTGGTGCAGACAGCTCAACCGATGG-GCCTATAGTGAGTCGTATTA-3'), SLE-wt (5'-TCAAGGCCCTCT-CACACGGAGAAGGGCGTTTAAACATAACCTATAGTGAGTCGTATTA-3') or SLE-aa (5'-TCAAGGCCCTCTCATATGGAGAAGG-GCGTTTAAACATAACCTATAGTGAGTCGTATTA-3') to assemble the partially double-stranded templates. Quantification of the transcripts was performed as described elsewhere (Thanbichler *et al.*, 2000). The transcripts were purified, folded and assayed for their interaction with SelB as described previously (Thanbichler and Böck, 2002). Radioactivity was detected by exposure of the dried gels on a phosphor screen (Molecular Dynamics, Sunnyvale, CA). After scanning the screen with a PhosphorImager (Storm 860 Laser Scanner; Molecular Dynamics), the signals were visualized using ImageQuant 2.1 (Molecular Dynamics).

S1 nuclease protection assay

To determine the steady-state concentration and half-life of the *selAB* mRNA, cells were grown in TP2 medium at 37°C under aerobic conditions. At an OD₆₀₀ of 1.0, a 10 ml sample was withdrawn, immediately mixed with 1.25 ml of ice-cold 5% phenol (in ethanol) and stored on ice. Subsequently, rifampicin (240 μ g/ml) was added to the cultures and additional samples were withdrawn at regular intervals. The cells were pelleted by centrifugation at 4°C and frozen in liquid nitrogen. Total RNA was extracted by the acid hot phenol method (Aiba *et al.*, 1981). Oligos S1-selA1 (5'-GCTCGCCCAAGGTTGGTATGCAGCAC-3') and S1-selA2 (5'-CGACCAATCACCGGCACTGGCAATTC-3') were labeled at the 5' end using [γ -³²P]ATP and T4 polynucleotide kinase. Probes annealing with the 5' and 3' end of the *selA* transcript, respectively, were synthesized by PCR in reactions containing either of the two labeled oligos and *Eco*O109I-treated plasmid pUAB as template and gel purified. RNA was co-precipitated with 10 000 c.p.m. of each probe, washed with 80% ethanol and dissolved in 20 μ l of hybridization buffer (80% formamide, 0.5 M NaCl, 1 mM EDTA, 40 mM PIPES pH 7.0). The solution was heated for 5 min at 95°C, immediately transferred to a 58°C water bath and incubated for 12 h. After addition of 200 μ l of S1 digestion buffer (300 mM NaCl, 2 mM ZnSO₄, 30 mM sodium acetate pH 5.5, 1 U/ μ l S1 nuclease) and incubation for 45 min at 37°C, the reaction was stopped by addition of 5.5 μ l of 10% SDS and 2 μ l of 10 mg/ml yeast tRNA. The nucleic acids were precipitated, dissolved in formamide loading dye and separated on an 8% urea-polyacrylamide gel. The signals were detected as described above.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

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References

- Aiba, H., Adhya, S. and de Crombrughe, B. (1981) Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. *J. Biol. Chem.*, **256**, 11905–11910.
- Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidmann, J., Smith, J. and Struhl, K. (eds) (1997) *Current Protocols in Molecular Biology*. J. Wiley & Sons, New York, NY.
- Baron, C., Heider, J. and Böck, A. (1993) Interaction of translation factor SELB with the formate dehydrogenase H selenopolyprotein mRNA. *Proc. Natl Acad. Sci. USA*, **90**, 4181–4185.
- Baumeister, R., Flache, P., Meleforts, O., von Gabain, A. and Hillen, W. (1991) Lack of a 5' non-coding region in *Tn1721* encoded *tetR* mRNA is associated with a low efficiency of translation and a short half-life in *Escherichia coli*. *Nucleic Acids Res.*, **19**, 4595–4600.
- Berg, B.L., Li, J., Heider, J. and Stewart, V. (1991) Nitrate-inducible formate dehydrogenase in *Escherichia coli* K-12. *J. Biol. Chem.*, **266**, 22380–22385.
- Böck, A. (2001) Selenium metabolism in bacteria. In Hatfield, D.L. (ed.),

- Selenium: Its Molecular Biology and Role in Human Health*. Kluwer Academic, Dordrecht, The Netherlands, pp. 7–22.
- Böck,A., Thanbichler,M., Rother,M. and Resch,A. (2002) Selenocysteine. In Ibbá,M., Francklyn,C. and Cusack,S. (eds), *Aminoacyl-tRNA Synthetases*. Landes Bioscience, Georgetown, TX.
- Braun,B., Le Derout,J. and Régnier,P. (1998) Ribosomes inhibit an RNase E cleavage which induces the decay of the *rpsO* mRNA of *Escherichia coli*. *EMBO J.*, **17**, 4790–4797.
- Casadaban,M.J. and Cohen,S.N. (1979) Lactose genes fused to exogenous promoters in one step using a Mu-*lac* bacteriophage: *in vivo* probe for transcriptional control sequences. *Proc. Natl Acad. Sci. USA*, **76**, 4530–4533.
- Chen,G.T., Fang,L. and Inouye,M. (1993) Effect of the relative position of the UGA codon to the unique secondary structure in the *fdhF* mRNA on its decoding by selenocysteinyl tRNA in *Escherichia coli*. *J. Biol. Chem.*, **268**, 23128–23131.
- Forchhammer,K., Rücknagel,K. and Böck,A. (1990) Purification and biochemical characterization of SELB, a translation factor involved in selenoprotein synthesis. *J. Biol. Chem.*, **265**, 9346–9350.
- Graham,A. (1980) The synthesis of formate dehydrogenase and nitrate reductase proteins in various *fdh* and *chl* mutants of *Escherichia coli*. *FEMS Microbiol. Lett.*, **7**, 145–151.
- Heider,J., Baron,C. and Böck,A. (1992) Coding from the distance: dissection of the mRNA determinants required for the incorporation of selenocysteine into proteins. *EMBO J.*, **11**, 3759–3766.
- Hüttenhofer,A. and Böck,A. (1998) RNA structures involved in selenoprotein synthesis. In Grunberg-Manago,M. and Simons,R.W. (eds), *RNA Structure and Function*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 603–639.
- Hüttenhofer,A. and Noller,H. (1994) Footprinting mRNA–ribosome complexes with chemical probes. *EMBO J.*, **13**, 3892–3901.
- Hüttenhofer,A., Westhof,E. and Böck,A. (1996) Solution structure of mRNA hairpins promoting selenocysteine incorporation in *Escherichia coli* and their base-specific interaction with special elongation factor SELB. *RNA*, **2**, 354–366.
- Jain,C. and Kleckner,N. (1993) S10 mRNA stability and steady state levels in *Escherichia coli*: indirect effects of translation and role of *rne* function. *Mol. Microbiol.*, **9**, 233–247.
- Klug,S.J., Hüttenhofer,A., Kromayer,M. and Famulok,M. (1997) *In vitro* and *in vivo* characterization of novel mRNA motifs that bind special elongation factor SelB. *Proc. Natl Acad. Sci. USA*, **94**, 6676–6681.
- Klug,S.J., Hüttenhofer,A. and Famulok,M. (1999) *In vitro* selection of RNA aptamers that bind special elongation factor SelB, a protein with multiple RNA-binding sites, reveals one major interaction domain at the carboxyl terminus. *RNA*, **5**, 1180–1190.
- Kromayer,M., Wilting,R., Tormay,P. and Böck,A. (1996) Domain structure of the prokaryotic selenocysteine-specific elongation factor SelB. *J. Mol. Biol.*, **262**, 413–420.
- Lacourciere,G. (2002) Selenium is mobilized *in vivo* from free selenocysteine and is incorporated specifically into formate dehydrogenase H and tRNA nucleosides. *J. Bacteriol.*, **184**, 1940–1946.
- Laemmli,U. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**, 680–685.
- Leinfelder,W., Forchhammer,K., Veprek,B., Zehelein,E. and Böck,A. (1990) *In vitro* synthesis of selenocysteinyl-tRNA_{UCA} from seryl-tRNA_{UCA}: involvement and characterization of the *selD* gene product. *Proc. Natl Acad. Sci. USA*, **87**, 543–547.
- Li,C., Reches,M. and Engelberg-Kulka,H. (2000) The bulged nucleotide in the *Escherichia coli* minimal selenocysteine insertion sequence participates in interaction with SelB: a genetic approach. *J. Bacteriol.*, **182**, 6302–6307.
- Liu,Z., Reches,M., Groisman,I. and Engelberg-Kulka,H. (1998) The nature of the minimal 'selenocysteine insertion sequence' (SECIS) in *Escherichia coli*. *Nucleic Acids Res.*, **26**, 896–902.
- Low,S. and Berry,M. (1996) Knowing when not to stop: selenocysteine incorporation in eukaryotes. *Trends Biochem. Sci.*, **21**, 203–208.
- Low,S.C., Grundner-Culemann,E., Harney,J.W. and Berry,M.J. (2000) SECIS–SBP2 interactions dictate selenocysteine incorporation efficiency and selenoprotein hierarchy. *EMBO J.*, **19**, 6882–6890.
- Mandrand-Berthelot,M., Wee,M.Y.K. and Haddock,B.A. (1978) An improved method for the identification and characterization of mutants of *Escherichia coli* deficient in formate dehydrogenase activity. *FEMS Microbiol. Lett.*, **4**, 37–40.
- Mathews,D., Sabina,J., Zuker,M. and Turner,D. (1999) Expanded sequence dependence of thermodynamic parameters improves prediction of RNA secondary structure. *J. Mol. Biol.*, **288**, 911–940.
- Mihara,H., Kato,S., Lacourciere,G., Stadtman,T., Kennedy,R., Kurihara,T., Tokumoto,U., Takahashi,Y. and Esaki,N. (2002) The *iscS* gene is essential for the biosynthesis of 2-selenouridine in tRNA and the selenocysteine-containing formate dehydrogenase H. *Proc. Natl Acad. Sci. USA*, **99**, 6679–6683.
- Miller,J. (1972) *Experiments in Molecular Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Milligan,J.F., Groebe,D.R., Witherell,G.W. and Uhlenbeck,O.C. (1987) Oligoribonucleotide synthesis using T7 RNA polymerase and synthetic DNA templates. *Nucleic Acids Res.*, **15**, 8783–8798.
- Müller,S., Heider,J. and Böck,A. (1997) The path of unpecific incorporation of selenium in *Escherichia coli*. *Arch. Microbiol.*, **168**, 421–427.
- Neidhardt,F., Bloch,P. and Smith,D. (1974) Culture medium for enterobacteria. *J. Bacteriol.*, **119**, 736–747.
- Rauhut,R. and Klug,G. (1999) mRNA degradation in bacteria. *FEMS Microbiol. Rev.*, **23**, 353–370.
- Ringquist,S., Schneider,D., Gibson,T., Baron,C., Böck,A. and Gold,L. (1994) Recognition of the mRNA selenocysteine insertion sequence by the specialized translational elongation factor SELB. *Genes Dev.*, **8**, 376–385.
- Rother,M., Resch,A., Wilting,R. and Böck,A. (2001) Selenoprotein synthesis in archaea. *Biofactors*, **14**, 75–83.
- Sacerdot,C., Caillet,J., Graffe,M., Eyermann,F., Ehresmann,B., Ehresmann,C., Springer,M. and Romby,P. (1998) The *Escherichia coli* threonyl-tRNA synthetase gene contains a split ribosomal binding site interrupted by a hairpin structure that is essential for autoregulation. *Mol. Microbiol.*, **29**, 1077–1090.
- Sambrook,J., Fritsch,E. and Maniatis,T. (eds) (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Sawers,G., Heider,J., Zehelein,E. and Böck,A. (1991) Expression and operon structure of the *sel* genes of *Escherichia coli* and identification of a third selenium-containing formate dehydrogenase isoenzyme. *J. Bacteriol.*, **173**, 4983–4993.
- Simons,R., Houman,F. and Kleckner,N. (1987) Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. *Gene*, **53**, 85–96.
- Stadtman,T. (1996) Selenocysteine. *Annu. Rev. Biochem.*, **65**, 83–100.
- Thanbichler,M. and Böck,A. (2001) Functional analysis of prokaryotic SELB proteins. *Biofactors*, **14**, 53–59.
- Thanbichler,M. and Böck,A. (2002) Selenoprotein biosynthesis: purification and assay of components involved in selenocysteine biosynthesis and insertion in *Escherichia coli*. *Methods Enzymol.*, **347**, 3–16.
- Thanbichler,M., Böck,A. and Goody,R.S. (2000) Kinetics of the interaction of translation factor SelB from *Escherichia coli* with guanosine nucleotides and selenocysteine insertion sequence RNA. *J. Biol. Chem.*, **275**, 20458–20466.
- Woodcock,D., Crowther,P., Doherty,J., Jefferson,S., DeCruz,E., Noyer-Weidner,M., Smith,S., Michael,M. and Graham,M. (1989) Quantitative evaluation of *Escherichia coli* host strains for tolerance to cytosine methylation in plasmid and phage recombinants. *Nucleic Acids Res.*, **17**, 3469–3478.
- Wyatt,J.R., Chastain,M. and Puglisi,J.D. (1991) Synthesis and purification of large amounts of RNA oligonucleotides. *Biotechniques*, **11**, 764–769.
- Yanofsky,C. (2000) Transcription attenuation: once viewed as a novel regulatory strategy. *J. Bacteriol.*, **182**, 1–8.
- Yarchuk,O., Iost,I. and Dreyfus,M. (1991) The relation between translation and mRNA degradation in the *lacZ* gene. *Biochimie*, **73**, 1533–1541.
- Zengel,J. and Lindahl,L. (1994) Diverse mechanisms for regulating ribosomal protein synthesis in *Escherichia coli*. *Prog. Nucleic Acid Res. Mol. Biol.*, **47**, 331–370.
- Zinoni,F., Birkmann,A., Stadtman,T.C. and Böck,A. (1986) Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*. *Proc. Natl Acad. Sci. USA*, **83**, 4650–4654.
- Zinoni,F., Heider,J. and Böck,A. (1990) Features of the formate dehydrogenase mRNA necessary for decoding of the UGA codon as selenocysteine. *Proc. Natl Acad. Sci. USA*, **87**, 4660–4664.

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