Recognition of the mRNA selenocysteine insertion sequence by the specialized translational elongation factor SELB

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In *Escherichia coli* the unusual amino acid selenocysteine is incorporated cotranslationally at an in-frame UGA codon. Incorporation of selenocysteine relies, in part, on the interaction between a specialized elongation factor, the SELB protein, and a *cis*-acting element within the mRNA. Boundary and toeprint experiments illustrate that the SELB-GTP-Sec-tRNA^{Sec} ternary complex binds to the selenoprotein encoding mRNAs *fdhF* and *fdnG*, serving to increase the concentration of SELB and Sec-tRNA^{Sec} on these mRNAs in vivo. Moreover, toeprint experiments indicate that SELB recognizes the ribosome-bound message and that, upon binding, SELB may protrude out of the ribosomal-mRNA track so as to approach the large ribosomal subunit. The results place the mRNA-bound SELB-GTP-Sec-tRNA^{Sec} ternary complex at the selenocysteine codon (as expected) and suggest a mechanism to explain the specificity of selenocysteine insertion. *Cis*-acting mRNA regulatory elements can tether protein factors to the translation complex during protein synthesis.

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The micronutrient selenium is an essential element in mammals and bacteria (Pinsent 1954; Schwarz and Foltz 1957). Selenium has been identified as a covalent base modification in tRNA from bacteria as well as mammals (Chen and Stadtman 1980; Ching and Stadtman 1982; Ching 1984; Wittwer et al. 1984; Wittwer and Ching 1989; Kramer and Ames 1988; Ching 1984) and is incorporated into protein cotranslationally as the amino acid selenocysteine (Cone et al. 1976; Forstrom et al. 1978; Stadtman 1990; Böck et al. 1991). Selenoproteins typically contain one selenocysteine moiety per peptide chain although the mammalian plasma protein, selenoprotein P, has been reported to have as many as 10 selenocysteine residues (Hill et al. 1991, 1993). Other examples of selenoproteins include the three coordinately expressed formate dehydrogenases from *Escherichia coli* (Berg et al. 1991a; Sawers et al. 1991) as well as glutathione peroxidase and type I tetraiodothyronine deiodinase from mammals (Forstrom et al. 1978; Stadtman 1990; Berry et al. 1991). In general, these enzymes catalyze oxidation-reduction reactions, sometimes acting as peroxide scavengers (for recent reviews, see Stadtman 1990; Böck et al. 1991; Burk 1991) and share the common feature that the selenocysteine residue has been implicated during catalysis (Wu and Hilvert 1989; Arkowitz and Abeles 1990; Stadtman 1990; Axley et al. 1991).

Some of the signals directing selenoprotein synthesis in *E. coli* as well as mammals have been identified. Selenocysteine is incorporated cotranslationally at a specific, in-frame UGA codon; recoding of UGA from nonsense to selenocysteine requires the action of the selenocysteine insertion sequence (SECIS), a *cis*-acting mRNA element (Berg et al. 1991a,b; Berry et al. 1991; Heider et al. 1992; Hill et al. 1993). In *E. coli* a number of additional selenocysteine specific factors have also been characterized. Selenoprotein synthesis relies on a unique tRNA (Lee et al. 1989, 1990; Leinfelder et al. 1988a, 1989, 1990; Baron and Böck 1991; Li and Yarus 1992; Baron et al. 1993a) and three protein gene products (Leinfelder et al. 1988b; Böck and Stadtman 1988). One of these, the SELB protein, resembles EFTu in sequence as well as function (Leinfelder et al. 1989; Forchhammer et al. 1989, 1990) and may play a crucial role during translational incorporation of selenocysteine. Moreover, SELB binds the mRNA SECIS (Baron et al. 1993b) and may directly regulate decoding of a specific UGA codon from nonsense to selenocysteine.

The present work uses toeprinting and boundary assays to identify the SELB-binding site on two selenoprotein encoding mRNAs from *E. coli* and investigates the interaction of SELB with the translational initiation complex. We used an RNA construct containing an AUG-UGA immediately adjacent to the SECIS so as to align the AUG-UGA codons with the ribosomal P and A

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sites and mimic the natural selenocysteine accepting complex. In agreement with our previous report (Ringquist et al. 1993a), toeprint experiments indicate that the SECISs from both mRNAs retained their secondary structure when bound to the ribosomal-mRNA track. The data also indicate that SELB protein binds within the highly structured SECIS, even in the presence of prebound ribosome and suggest that formation of the SELB-mRNA-ribosome complex may be an essential step during selenocysteine incorporation in vivo. Binding of SELB to the SECIS may serve to tether it to the translational complex, thus regulating the decoding of a specific, adjacent UGA codon.

Results

The interaction between purified SELB protein, 30S ribosome, and the SECISs from the selenoprotein encoding mRNAs *fdhF* and *fdnG* was examined by toeprinting and boundary assays. The RNAs are illustrated in Figure 1 and contain the naturally occurring SECIS (shaded) along with a strong ribosome-binding site (RBS). The RBSs were designed so that during toeprint experiments the AUG and UGA codons could be aligned with the ribosomal P and A sites, respectively. This arrangement utilizes a bound tRNA^{Met} as the analogue of a peptidyl-tRNA held in the ribosomal P site when the specific UGA is about to be recoded to selenocysteine.

Determination of the SELB-mRNA binding site

The SELB-mRNA binding site was determined by incubating partially hydrolyzed, [³²P]-end-labeled RNA with SELB protein followed by nitrocellulose filtration to separate bound from unbound RNA. The data, given in Figure 2, identify the 5' and 3' boundaries of the binding sequence within the *fdhF* and *fdnG* mRNAs. Each RNA gave a strong as well as an adjacent, weaker boundary. The strong and weak boundaries probably reflect differences in affinity of SELB for the RNA when the binding site is truncated by one nucleotide. The extra pair of bands observed when the *fdnG* RNA 5' boundary was determined probably represent a contaminating RNase because a band at this position and with increased intensity was also observed upon examination of the 3' boundary. Comparison of the *fdhF* and *fdnG* boundary data indicates that the SELB-binding sites are at homologous positions on these mRNAs even though the sequences within the boundaries are different (Fig. 3). This was surprising first because selenocysteine incorporation in vivo is extremely sensitive to changes in the sequence of the SECIS (Heider et al. 1992) and second because a single base change within the loop region of the *fdhF* SECIS resulted in decreased binding of SELB in vitro (Baron et al. 1993b). Apparently, binding of SELB to the SECIS can be salvaged by multiple, naturally occurring base changes suggesting that it may be an important property of RNA of different sequence to fold into structures with similar function (Tuerk and Gold 1990).

Toeprinting of SELB at the SECIS

Primer-extension inhibition analysis (toeprinting) identifies the position of site-bound protein on an RNA, relying on the ability of protein-RNA complexes to inhibit cDNA synthesis (Hartz et al. 1988). Toeprinting was used to investigate the interaction between the SECIS and SELB protein (Fig. 4). By itself, SELB toeprinted a single site on the SECISs from RNAs *fdhF* and *fdnG*, representing only the second example (after the ribosome) of a toeprintable protein-RNA complex. SELB toeprinted the natural SECISs at the 3'-edge of the boundary sequence (see Fig. 2), and correlated the toeprint site to the 3'-edge of the SELB-mRNA complex. In contrast, the mutant SECIS, G25C, gave no toeprint (Fig. 4). Failure of this sequence to encode selenocysteine in vivo (Heider et al. 1992) and weak binding to SELB in vitro during mobility-shift assays (Baron et al. 1993b) suggest that the toeprint reflects the biologically important complex.

Toeprint experiments were also performed with the SELB-GTP-Sec-tRNA^{Sec} ternary complex (data not shown). These experiments were performed anaerobically because the selenocysteine residue is oxygen labile (Cone et al. 1976). Importantly, the SELB-GTP-Sec-tRNA^{Sec} ternary complex gave the same toeprint observed with SELB alone and did not toeprint the G25C mutant. Moreover, the same concentration of SELB was required to toeprint the *fdhF* SECIS that was reported in the previous experiments (Fig. 4). In contrast, Baron et al. (1993b) observed enhanced binding of SELB to the SECIS in the presence of GTP and Sec-tRNA^{Sec}. These results differ possibly because the mobility-shift assay employed by Baron et al. (1993b) and the toeprint assay may examine different kinetic properties of the SELB-mRNA complex (Ringquist et al. 1993b; Spedding et al. 1993); perhaps, the mobility-shift assay is more sensitive than toeprinting to changes in the off-rate of the SELB-mRNA or SELB-tRNA-mRNA complex.

Toeprinting of the 30S ribosome at the SECIS

Toeprinting was also used to investigate the interaction between the 30S ribosome and the SECIS. RNAs containing a strong RBS adjacent to the SECIS from the *E. coli* messages *fdhF* or *fdnG* were examined (Fig. 5). When tRNA^{Met} was present, and the AUG and UGA codons were aligned with the ribosomal P and A sites, the 30S ribosome toeprinted these RNAs at two positions in agreement with results described previously by Ringquist et al. (1993a). The ribosome toeprinted the mRNAs at +16 and at an extended-toeprint site well beyond the expected limit of the ribosomal-mRNA track (Kang and Cantor 1985). The extended toeprints were sensitive to temperature as well as reverse transcriptase concentration (Ringquist et al. 1993a) and, surprisingly, would have been 14 nucleotides from the start codon if either of the SECIS-associated stem-loops were deleted. The sensitivity of the extended toeprint to reverse transcriptase activity may indicate that multiple toeprints can originate from a single protein-RNA com-

Figure 1. Nucleotide sequence of the RNAs used in this study. In RNAs *fdhF* and *fdnG* the SECIS element is shaded, whereas the UGA codon is underlined. RNA G25C is a mutant of the *fdhF* RNA, and the position of the mutation is indicated.

plex. The +16 toeprint is a hard stop (independent of the reverse transcription activity) suggesting that the mRNA secondary structure unfolds during cDNA synthesis (Ringquist et al. 1993a). Moreover, toeprinting of 70S ribosomes also resulted in an extended-toeprint signal at this position (see Fig. 6). The unusual interaction between ribosome and SECIS was also investigated with the G25C mutant. This RNA exhibited the same extended toeprint as the native *fdhF* SECIS, indicating that the extended toeprint, unlike SELB binding, was unaf-

ected by the mutation (Fig. 5). The multiple toeprint bands associated with the extended toeprint may, in part, be due to heterogeneity in the position of the ribosome-SECIS-tRNA^{Met} ternary complex because the RBS contained unavoidably overlapping AUG and GUG codons.

Toeprint experiments were also performed with ribosome and tRNA^{Sec} (Fig. 5). Complex formation was expected to align the UGA codon with the ribosomal P site and might have examined whether secondary structure

Figure 2. Boundary determination of the SELB-binding site on the *fdhF* and *fdnG* RNAs. (A) 3' and 5' boundaries. In each panel, the partial RNase T1 digestion, alkaline hydrolysis, and SELB boundary lanes are indicated. SELB and RNA were present at 20 and 150 nM, respectively. (B) Summary of the 3' and 5' boundary sites on the two RNAs. Closed and open arrows represent the position of the strong and weak boundaries, respectively. The closed circle indicates the position of the possible RNase cleavage.

associated with the SECIS could form when aligned with the A site. Toeprint experiments with tRNA^{Sec} did not result in an extended toeprint or the +16 toeprint when the SECIS-containing mRNA was examined (Fig. 5). However, a control RNA lacking the SECIS did toeprint, perhaps suggesting that mRNA secondary structure associated with the SECIS may extend into the UGA codon and inhibit codon-anticodon pairing. A similar result was observed for a UGA suppressor tRNA (Rafferty et al. 1984) (K. Beyser, unpubl.), indicating that the failure of the ribosome to toeprint the SECIS-containing mRNA reflects the position of the stem-loop structure within the ribosomal-mRNA track and is probably not affected by the selenocysteine specific tRNA.

SELB binding to the preformed ribosome-tRNA^{Met}-SECIS complex

Toeprinting was used to investigate the effect of SELB on the extended toeprint from the ribosome-SECIS-tRNA^{Met} complex when the *fdhF* RNA was presaturated with ribosomes (Fig. 6). Upon addition of SELB protein to the toeprint reaction, the intensity of the extended toeprint signal remained constant, whereas the signal from the +16 toeprint decreased. This may reflect loading of SELB protein onto the ribosome-SECIS-tR-

Figure 3. Comparison of the SELB binding site on the *fdhF* and *fdnG* RNAs. Shaded region denotes the 3' and 5' boundary of the SELB-binding site whereas open boxes emphasize differences between these sequences.

Figure 5. The 30S ribosome toeprinted the natural SECISs from *fdhF* and *fdnG* at two sites and was insensitive to the G25C mutation. (A) Toeprint data for RNAs *fdhF*, *G25C*, and *RNA A*. The ddG sequence, cDNA extension control, and ribosome toeprint lanes are indicated. Toeprinting reactions contained 20 nM 30S ribosome and 8 nM RNA along with either 500 nM tRNA^{Met} or 200 nM tRNA^{Sec} as indicated. cDNA extension used 20 units of M-MLV reverse transcriptase, and the reaction temperature was 23 °C. When tRNA^{Sec} was present, cDNA extension was performed with 2 units of AMV reverse transcriptase at 23 °C. (B) Summary of the ribosome toeprinting data for RNAs *fdhF*, *G25C*, and *fdnG* when tRNA^{Met} was used.

into the channel between the ribosomal subunits and binding of the surrounding sequence within the ribosomal-mRNA track (Fig. 7). This architecture would place site-bound SELB-GTP-Sec-tRNA^{Sec} in a favorable position to interact with the approaching ribosomal A site. Interestingly, Heider et al. (1992) have shown that when the SECIS is truncated it no longer encodes selenocysteine, possibly because the SELB-GTP-Sec-tRNA^{Sec} complex no longer reaches into the ribosomal A site. Longer stems also reduce the efficiency of selenocysteine insertion (Heider et al. 1992), probably by increasing the search time for codon-anticodon pairing. The model also suggests that efficient competition between selenocysteine insertion and release factor RF2-mediated termination is accomplished by increasing the local concentration of the SELB-GTP-Sec-tRNA^{Sec} complex at a specific UGA codon (Zinoni et al. 1987; Baron et al. 1993b).

What other signals are required for recoding of the UGA codon from nonsense to selenocysteine? Surprisingly, the SELB-binding site on the *fdhF* and *fdnG* messages are substantially different. Yet, SELB binds these

RNAs tightly while unable to recognize single-site mutations within the *fdhF* message (Heider et al. 1992; Baron et al. 1993b). Multiple, naturally occurring base changes between these messages may compensate for binding. Of course, the ability of message sequences to form stem-loop structures that have the same or similar roles in translation in spite of having very different base sequences need not be viewed as a result of multiple mutations. A similar situation has been described by Tuerk and Gold (1990) for RNA ligands generated in vitro to the bacteriophage T4 DNA polymerase suggesting that it may be a potential property of RNAs of different sequence to fold into homologous structures with similar function. Other features that may affect the interaction between site-bound SELB and the ribosomal A site include the length of the stem region of the SECIS as well as its position relative to the UGA codon (Heider et al. 1992). Each of these variables is likely to affect the rate of interaction between the SELB ternary complex and the ribosomal A site.

Other examples of *cis*-acting mRNA elements that

Figure 6. Complex formation between 70S ribosome-tRNA^{Met}-RNA and SELB. (A) Toeprint data when 50S ribosome, 30S ribosome, and tRNA^{Met} were present at 50, 20, and 200 nM, respectively. Experiments were performed with *fdhF* or G25C as indicated. The concentration of SELB was varied between 1000 and 1 nM as indicated. (B) Summary of the toeprint data when SELB, ribosome, mRNA, and tRNA^{Met} were present.

suppress nonsense codons have been described (Engelberg 1981; Dahlberg 1989; Brown et al. 1990; Rein and Levin 1992). For instance, the suppressible, in-frame UGA codon in the *E. coli* RF2 message is dependent on an adjacent Shine-Dalgarno-like element (Craig et al. 1985; Curran and Yarus 1988), whereas the mammalian type C retroviruses also rely on a *cis*-acting mRNA element for recoding an in-frame UGA codon from nonsense to glutamate during synthesis of the gag-pol gene product (Rein and Levin 1992). Like selenocysteine, the type C retroviruses use a unique tRNA (Rein and Levin 1992) although specialized translation factors have not been identified. Elucidation of the mechanism by which a *cis*-acting mRNA element and a specialized elongation factor conspire to recode a UGA codon from nonsense to selenocysteine will lead to an enhanced understanding of

the molecular mechanism by which the translation complex decodes the nucleotide message into protein.

Materials and methods

Materials

M-MLV reverse transcriptase was purchased from Bethesda Research Laboratories, AMV reverse transcriptase was from Life Sciences, Inc., T7 RNA polymerase was from U.S. Biochemicals Corporation, and Taq polymerase was from Promega Corporation. T4 polynucleotide kinase, alkaline phosphatase, and RNA ligase were purchased from New England Biolabs. tRNA^{Met} was purchased from Subriden RNA (Rolling Bay, WA) whereas tRNA^{Sec} was purified as described by Baron and Böck (1991). Purified SELB was obtained as described previously (Forchhammer et al. 1990). 30S ribosomes were a gift from R. Traut (Univ.

Figure 7. Model for the ribosome-SECIS-SELB complex. The ribosome structure was taken from Noller (1991) and is oriented so that the 30S subunit (shaded) is in front. The plausible location of the ribosomal E, P, and A sites as well as the mRNA track and the SELB-SECIS complex are indicated.

of Calif., Davis). Other reagents used in this work were of the highest quality obtainable.

Nucleic acids

RNAs were generated *in vitro* by transcription of PCR-amplified DNA oligonucleotides with T7 RNA polymerase. DNA oligonucleotides (Table 1) were synthesized on an Applied Biosystems Model 394 DNA/RNA synthesizer and purified by 6% preparative polyacrylamide gel electrophoresis. PCR amplification was performed in 50 mM KCl, 10 mM Tris-HCl (pH 8.6), 2.5 mM MgCl₂, and 170 μg/ml BSA, and dNTPs were present at 1

mM each. Taq polymerase was used at 100 units per 0.1 ml of reaction and the 5' and 3' primers were present at 1 μM. Transcription was performed in 8% PEG 8000, 5 mM DTT, 40 mM Tris-HCl (pH 8.0), 12 mM MgCl₂, 1 mM spermidine, 0.002% Triton X-100 and contained 2 mM NTPs. T7 RNA polymerase was present at a final concentration of 1 U/μl. The reaction was incubated at 37°C for 2 hr and then treated with 20 units of DNase I (New England Biolabs, Inc.) for an additional 5 min at 37°C. The reaction was stopped by the addition of an equal volume of loading buffer [72% formamide, 120 mM EDTA (pH 8.0)] and heated to 60°C for 3 min prior to electrophoresis on a 6% polyacrylamide, 8 M urea denaturing gel. The RNA transcript was visualized by UV shadowing, and the RNA band was excised from the gel and eluted into dH₂O. The RNA was then ethanol precipitated, dried under vacuum, and redissolved in dH₂O. The concentration of RNA was determined assuming one A₂₆₀ unit is equal to 40 μg/ml.

Boundary experiments

The determination of the 5' and 3' boundaries of the SELB binding site was performed as described by Tuerk et al. (1990). Briefly, the RNA transcript was 5'-end-labeled by incubation with alkaline phosphatase (1 unit) at 37°C 30 min and subsequent treatment with polynucleotide kinase (2 units) in the presence of [γ-³²P]ATP for 30 min at 37°C. Labeling of the 3'-end with [5'-³²P]pCp was performed with RNA ligase for 30 min at 37°C. The 5'- as well as 3'-labeled RNAs were band purified on a 6% polyacrylamide, 8 M urea gel. End-labeled RNA (75 pmol) was hydrolyzed in 50 mM Na₂CO₃ (pH 9.0) and 1 mM EDTA in a volume of 100 μl for 10 min at 90°C. The hydrolysis reaction was stopped by addition of 11 μl of 3 M Na-acetate (pH 5.2). Binding reactions were done with 20 nM SELB protein in binding buffer [50 mM K₂HPO₄/KH₂PO₄ (pH 7.5), 2 mM Mg-acetate, 0.1 mM EDTA, 1 mM DTT, 0.5 mM GTP, 0.02% Tween-20] at 37°C for 5 min. The concentration of hydrolyzed RNA was 150 nM. Bound, end-labeled RNA was isolated from unbound RNA by filtration through nitrocellulose membranes (Millipore, Inc.), and rinsing with 5 ml of wash buffer [50 mM Tris-HCl (pH 7.5)]. The RNA was eluted from the filters by

Table 1. DNA oligonucleotides used in this study

Oligonucleotide ^a	Sequence ^b
5G3	5'-CCGAAGCTTAATACGACTCACTATAGGGAGATGCCTGTCGAGCATGCTG-3'
3G3	5'-CCCGTCGACAAAGCTGTTAGCTAC-3'
fdhF	5'-CCCGTCGACAAAGCTGTTAGCTACTGCAGTTCGTTAATAGCATTGCTCGTTGCGCCATTACCGACCGATTGGTGCG AGACCTGCAACCGATGGGCCGTGTCACAT(T) ₆ CCTCCTTA(T) ₈ AGATCTCAGCATGCTCGACAGGCATCTCCCTATAG TGAGTCGTATTAAGCTTCGG-3'
fdnG	5'-CCCGTCGACAAAGCTGTTAGCTACTGCAGTTCGTTATATCCACCCAGTGGTTGGTCATCGCACCGCGACCAAAATGT TGGAGCAAGACTTGCTACCGTTGGTCCGTGTCACAT(T) ₆ CCTCCTTA(T) ₈ AGATCTCAGCATGCTCGACAGGCATCTC CC-3'
G25C	5'-CCCGTCGACAAAGCTGTTAGCTACTGCAGTTCGTTAATAGCATTGCTCGTTGCGCCATTACCGACCGATTGGTGCA GAGCTGCAACCGATGGGCCGTGTCACAT(T) ₆ CCTCCTTA(T) ₈ TAGATCTCAGCATGCTCGACAGGCATCTCCCTATAG TGAGTCGTATTAAGCTTCGG-3'
RNA A	5'-CCCGTCGACAAAGCTGTTAGCTACTGCAGTTCGTTAATAGCATTGCTCGTTGCTCACAT(T) ₆ CCTCCTTA(T) ₈ AGATC TCAGCATGCTCGACAGGCATCTCCCTATAGTGAGTCGTATTAAGCTTCGG-3'
T7pts	5'-CTTAATACGACTCACTATAGGG-3'
fdhF-b	5'-TTGCGCCATTACCGACCGATTGGTGCGAGACCTCAACCGATGGGCCGTGTCACATTCTCCCTATAGTGAGTCGTATT AAG-3'
fdnG-b	5'-GTTGGTCATCGCACCGCGACCAAAATGTTGGAGCAAGACTTGCTACCGTTGGTCCGTATTCTCCCTATAGTGAGTCG TATTAAG-3'

^aDNA oligonucleotides 5G3 and 3G3 are the 5' and 3' PCR primers, respectively. 3G3 was also used for priming cDNA synthesis during sequencing and toeprinting. T7pts is the 5' primer for transcription of DNA oligonucleotides fdhF-b and fdnG-b.

^bThe symbol (T)_n represents the number of times T is repeated.

incubation in an emulsion of 200 μ l of 7 M urea, 400 μ l of phenol [equilibrated against 1 M Tris-HCl (pH 8.0)] for 30 min at room temperature. The urea/phenol emulsion was separated into phases by the addition of 200 μ l of chloroform followed by centrifugation. The aqueous phase was removed, and the RNA was precipitated upon the addition of 20 μ l of 3 M Na-acetate, 20 μ g of carrier tRNA, and 2 volumes of ethanol. The pellet was washed once with 70% ethanol, dried, and resuspended in 5 μ l of dH₂O followed by the addition of 5 μ l formamide loading dye [72% formamide, 120 mM EDTA (pH 8.0)] prior to electrophoresis.

A sequence ladder of the *fdhF* and *fdnG* RNAs was generated by partial RNase T1 digestion. The RNase T1 digest was performed in a 10- μ l reaction containing 0.5 pmoles of end-labeled RNA and 10 units of RNase T1 (New England Biolabs, Inc.) in 7 M urea, 20 mM Na-citrate (pH 5.0) and 1 mM EDTA. The RNA was incubated for 10 min at 50°C without enzyme and then another 10 min after addition of enzyme. The reaction was stopped by adding 10 μ l of loading buffer.

Formation of the SELB-GTP-Sec-tRNA^{Sec} ternary complex

The SELB-GTP-Sec-tRNA^{Sec} ternary complex was generated in an anaerobic atmosphere (95% N₂, 5% H₂) as described previously by Baron and Böck (1991). Briefly, seryl-tRNA^{Sec} was converted into selenocysteinyl-tRNA^{Sec} by incubating 200 pmoles of [¹⁴C]seryl-tRNA^{Sec} (337 cpm/pmole) with 25 μ g/ml SELA protein and 38 μ g/ml SELD protein in 100 mM HEPES (pH 7.0), 20 mM KCl, 5 mM Mg-acetate, 2 mM DTT, 20 μ M ATP, and 100 μ M SeO₃ in a total volume of 200 μ l. The reaction was performed at 37°C for 20 min, and the efficiency of serine to selenocysteine conversion was monitored by thin layer chromatography (Baron and Böck 1991). Formation of the SELB-GTP-Sec-tRNA^{Sec} ternary complex was performed by incubation of the individual components in the toeprinting buffer for 15 min at 37°C. Complex formation was monitored by SELB-dependent protection of selenocysteinyl-tRNA^{Sec}, but not seryl-tRNA^{Sec}, to pancreatic RNase digestion (Baron and Böck 1991).

Toeprinting

Primer-extension inhibition (toeprinting) was performed as described by Hartz et al. (1988) with M-MLV or AMV reverse transcriptase as indicated in the Results. The [³²P]-labeled primer 3G3, which is complementary to the 3'-end of the various RNA transcripts was annealed as described in Hartz et al. (1988). Toeprint reactions were performed in VDG buffer [10 mM Tris-acetate (pH 7.4), 60 mM NH₄Cl, 10 mM Mg-acetate, 1 mM GTP, 6 mM β -mercaptoethanol] and contained 8 nM RNA in a volume of 10 μ l along with 30S ribosome or SELB protein as specified in the Results. The reaction components were combined on ice and preincubated as indicated in the Results before addition of reverse transcriptase and incubation for an additional 15 min. When selenocysteinyl-tRNA^{Sec} was present toeprinting experiments were performed in an anaerobic atmosphere (95% N₂, 5% H₂).

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References

- Arkowitz, R.A. and R.H. Abeles. 1990. Isolation and characterization of a covalent selenocysteine intermediate in the glycine reductase system. *J. Am. Chem. Soc.* **112**: 870–872.
- Axley, M.J., A. Böck, and T.C. Stadtman. 1991. Catalytic properties of an *Escherichia coli* formate dehydrogenase mutant in which sulfur replaces selenium. *Proc. Natl. Acad. Sci.* **88**: 8450–8454.
- Baron, C. and A. Böck. 1991. The length of the aminoacyl-acceptor stem of the selenocysteine-specific tRNA^{Sec} of *Escherichia coli* is the determinant for binding to elongation factors SELB and Tu. *J. Biol. Chem.* **266**: 20375–20379.
- Baron, C., E. Westhof, A. Böck, and R. Giege. 1993a. Solution structure of selenocysteine-inserting tRNA^{Sec} from *Escherichia coli*: Comparison with canonical tRNA^{Ser}. *J. Mol. Biol.* **231**: 274–292.
- Baron, C., J. Heider, and A. Böck. 1993b. Interaction of translation factor SELB with the FDHF selenopolypeptide mRNA. *Proc. Natl. Acad. Sci.* **90**: 4181–4185.
- Berg, B.L., J. Li, J. Heider, and V. Stewart. 1991a. Nitrate-inducible formate dehydrogenase in *Escherichia coli* K-12 I. Nucleotide sequence of the *fdnGDI* operon and evidence that opal (UGA) encodes selenocysteine. *J. Biol. Chem.* **266**: 22380–22385.
- Berg, B.L., C. Baron, and V. Stewart. 1991b. Nitrate-inducible formate dehydrogenase in *Escherichia coli* K-12 II. Evidence that a mRNA stem-loop structure is essential for decoding opal (UGA) as selenocysteine. *J. Biol. Chem.* **266**: 22386–22391.
- Berry, M.J., L. Banu, Y. Chen, S.J. Mandel, J.D. Kieffer, J.W. Harney, and P.R. Larsen. 1991. Recognition of UGA as a selenocysteine codon in type I diiodinase requires sequences in the 3' untranslated region. *Nature* **353**: 273–276.
- Böck, A. and T.C. Stadtman. 1988. Selenocysteine, a highly specific component of certain enzymes, is incorporated by a UGA-directed co-translational mechanism. *BioFactors* **1**: 245–250.
- Böck, A., K. Forchhammer, J. Heider, and C. Baron. 1991. Selenoprotein synthesis: An expansion of the genetic code. *Trends Biochem. Sci.* **16**: 463–467.
- Brown, C.M., P.A. Stockwell, N.A. Trotman, and W.P. Tate. 1990. The signal for the termination of protein synthesis in procaryotes. *Nucleic Acids Res.* **18**: 2079–2086.
- Burk, R.F. 1991. Molecular biology of selenium with implication for its metabolism. *FASEB J.* **5**: 2274–2279.
- Chen, C.S. and T. Stadtman. 1980. Selenium-containing tRNAs from *Clostridium stricklandii*: Cochromatography of one species with L-prolyl-tRNA. *Proc. Natl. Acad. Sci.* **77**: 1403–1407.
- Ching, W.M. 1984. Occurrence of selenium-containing tRNAs in mouse leukemia cells. *Proc. Natl. Acad. Sci.* **81**: 3010–3013.
- Ching, W.M. and T. Stadtman. 1982. Selenium-containing tRNA^{Glu} from *Clostridium stricklandii*: Correlation of aminoacylation with selenium content. *Proc. Natl. Acad. Sci.* **79**: 374–377.
- Cone, J.E., M. Del Rio, J.N. Davis, and T.C. Stadtman. 1976. Chemical characterization of the selenoprotein component of clostridial glycine reductase: Identification of selenocysteine as the organoselenium moiety. *Proc. Natl. Acad. Sci.*

- 73: 2659–2663.
- Craigien, W.J., R.G. Cook, W.P. Tate, and C.T. Caskey. 1985. Bacterial peptide chain release factors: Conserved primary structure and possible frameshift regulation of release factor 2. *Proc. Natl. Acad. Sci.* **82**: 3616–3620.
- Curran, J.F. and M. Yarus. 1988. Use of tRNA suppressors to probe regulation of *Escherichia coli* release factor 2. *J. Mol. Biol.* **203**: 75–83.
- Dahlberg, A.E. 1989. The functional role of ribosomal RNA in protein synthesis. *Cell* **57**: 525–529.
- Engelberg, K.H. 1981. UGA suppression by normal tRNA^{Trp} in *Escherichia coli*: Codon context effects. *Nucleic Acids Res.* **9**: 983–991.
- Forchhammer, K., W. Leinfelder, and A. Böck. 1989. Identification of a novel translation factor necessary for the incorporation of selenocysteine into protein. *Nature* **342**: 453–456.
- Forchhammer, K., K.-P. Rucknagel, and A. Böck. 1990. Purification and biochemical characterization of SELB, a translation factor involved in selenoprotein synthesis. *J. Biol. Chem.* **265**: 9346–9350.
- Forstrom, J.W., J.J. Zakowski, and A.L. Tappel. 1978. Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. *Biochemistry* **17**: 2639–2644.
- Frank, J., P. Penszek, R. Grassucci, and S. Srivastova. 1991. Three-dimensional reconstruction of the 70S *Escherichia coli* ribosome in ice: The distribution of ribosomal RNA. *J. Cell Biol.* **115**: 597–605.
- Gold, L. 1988. Posttranscriptional regulatory mechanisms in *Escherichia coli*. *Annu. Rev. Biochem.* **57**: 199–233.
- Hartz, D., D.S. McPheeters, R. Traut, and L. Gold. 1988. Extension inhibition analysis of translation initiation complexes. *Methods Enzymol.* **164**: 419–425.
- Heider, J., C. Baron, and A. Böck. 1992. Coding from a distance: Dissection of the mRNA determinants required for the incorporation of selenocysteine into protein. *EMBO J.* **11**: 3759–3766.
- Hill, K.E., R.S. Lloyd, J.G. Yang, R. Read, and R.F. Burk. 1991. The cDNA for rat selenoprotein P contains 10 TGA codons in the open reading frame. *J. Biol. Chem.* **266**: 10050–10053.
- Hill, K.E., R.S. Lloyd, and R.F. Burk. 1993. Conserved nucleotide sequences in the open reading frame and 3'-untranslated region of selenoprotein P mRNA. *Proc. Natl. Acad. Sci.* **90**: 537–541.
- Kang, C. and C.R. Cantor. 1985. Structure of ribosomal-bound messenger RNA as revealed by enzymatic accessibility studies. *J. Mol. Biol.* **181**: 241–245.
- Kramer, G.F. and B.N. Ames. 1988. Isolation and characterization of a selenium metabolism mutant of *Salmonella typhimurium*. *J. Bacteriol.* **170**: 736–743.
- Lee, B.J., P.J. Worland, J.N. Davis, T.C. Stadtman, and D. Hatfield. 1989. Identification of selenocysteinyl-tRNA^{Ser} in mammalian cells that recognizes the nonsense codon, UGA. *J. Biol. Chem.* **264**: 9724–9727.
- Lee, B.J., M. Rafagopalan, Y.S. Kim, K.H. You, K.B. Jacobson, and D. Hatfield. 1990. Selenocysteine tRNA^{Ser} gene is ubiquitous within the animal kingdom. *Mol. Cell. Biol.* **10**: 1940–1949.
- Leinfelder, W., E. Zehelein, M.-A. Mandrand-Berthelot, and A. Böck. 1988a. Gene for a novel tRNA species that accepts L-serine and cotranslationally inserts selenocysteine. *Nature* **331**: 723–725.
- Leinfelder, W., K. Forchhammer, F. Zinoni, G. Sawers, M.-A. Mandrand-Berthelot, and A. Böck. 1988b. *Escherichia coli* genes whose products are involved in selenium metabolism. *J. Bacteriol.* **170**: 540–546.
- Leinfelder, W., T.C. Stadtman, and A. Böck. 1989. Occurrence *in vivo* of selenocysteyl-tRNA^{Ser}_{UGA} in *Escherichia coli*: Effect of *sel* mutations. *J. Biol. Chem.* **264**: 9720–9723.
- Leinfelder, W., K. Forchhammer, B. Veprek, E. Zehelein, and A. Böck. 1990. *In vitro* synthesis of selenocysteinyl-tRNA_{UGA} from seryl-tRNA_{UGA}: Involvement and characterization of the *selD* gene product. *Proc. Natl. Acad. Sci.* **87**: 543–547.
- Li, W.-Q. and M. Yarus. 1992. Bar to normal UGA translation by the selenocysteine tRNA. *J. Mol. Biol.* **233**: 9–15.
- Noller, H.F. 1991. Ribosomal RNA and translation. *Annu. Rev. Biochem.* **60**: 191–227.
- Pinsent, J. 1954. The need for selenite and molybdate by members of the coli-aerogenes group of bacteria. *Biochem. J.* **57**: 10–16.
- Rafferty, L.A., J.B. Egan, S.W. Cline, and M. Yarus. 1984. Defined set of cloned termination suppressors: *in vivo* activity of isogenetic UAG, UAA, and UGA suppressor tRNAs. *J. Bacteriol.* **158**: 849–859.
- Rein, A. and J.G. Levin. 1992. Readthrough suppression in the mammalian type C retroviruses and what it has taught us. *New Biol.* **4**: 283–289.
- Ringquist, S., M. MacDonald, T. Gibson, and L. Gold. 1993a. Nature of the ribosomal mRNA track: Analysis of ribosome binding sites containing different sequences and secondary structure. *Biochemistry* **32**: 10254–10262.
- Ringquist, S., P. Cunningham, C. Weitzmann, L. Formenoy, C. Pleij, J. Ofengand, and L. Gold. 1993b. Translation initiation complex formation with 30S ribosomal particles mutated at conserved positions in the 3'-minor domain of 16S RNA. *J. Mol. Biol.* **234**: 14–27.
- Sawers, G., J. Heider, E. Zehelein, and A. Böck. 1991. Expression and operon structure of the *sel* genes of *Escherichia coli* and identification of a 3rd selenium containing formate dehydrogenase isoenzyme. *J. Bacteriol.* **173**: 4983–4993.
- Schwarz, K. and C.M. Foltz. 1957. Selenium is an integral part of factor-3 against dietary necrotic liver degeneration. *J. Am. Chem. Soc.* **79**: 3292–3293.
- Spedding, G., G. Gluick, and D.E. Draper. 1993. Ribosome initiation complex formation with the pseudoknotted α operon messenger RNA. *J. Mol. Biol.* **229**: 609–622.
- Stadtman, T.C. 1990. Selenium biochemistry. *Annu. Rev. Biochem.* **59**: 111–128.
- Tuerk, C. and L. Gold. 1990. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science* **249**: 505–510.
- Tuerk, C., S. Eddy, D. Parma, and L. Gold. 1990. Autogenous translational operator recognized by bacteriophage T4 DNA polymerase. *J. Mol. Biol.* **213**: 749–761.
- Wittwer, A.J. and W.-M. Ching. 1989. Selenium-containing tRNA^{Glu} and tRNA^{Lys} from *Escherichia coli*: Purification, codon specificity and translational activity. *BioFactors* **2**: 27–34.
- Wittwer, A.J., L. Tsai, W.-M. Ching, and T.C. Stadtman. 1984. Identification and synthesis of a naturally occurring selenonucleoside in bacterial tRNAs: 5-[(methylamino)methyl]-2-selenouridine. *Biochemistry* **23**: 4650–4655.
- Wu, Z.-P. and D. Hilvert. 1989. Conversion of a protease into an acyl transferase: selenosubtilisin. *J. Am. Chem. Soc.* **111**: 4513–4514.
- Zinoni, F., A. Birkmann, W. Leinfelder, and A. Böck. 1987. Cotranslational insertion of selenocysteine into formate dehydrogenase from *Escherichia coli* directed by a UGA codon. *Proc. Natl. Acad. Sci.* **84**: 3156–3160.
- Zinoni, F., J. Heider, and J. Böck. 1990. Features of the formate dehydrogenase mRNA necessary for decoding of the UGA codon as selenocysteine. *Proc. Natl. Acad. Sci.* **87**: 4660–4664.

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