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Barriers to Heterologous Expression of a Selenoprotein Gene in Bacteria

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The specificity parameters counteracting the heterologous expression in *Escherichia coli* of the *Desulfomicrobium baculatum* gene (*hydV*) coding for the large subunit of the periplasmic hydrogenase which is a selenoprotein have been studied. *hydV'-'lacZ* fusions were constructed, and it was shown that they do not direct the incorporation of selenocysteine in *E. coli*. Rather, the UGA codon is efficiently suppressed by some other aminoacyl-tRNA in an *E. coli* strain possessing a ribosomal ambiguity mutation. The suppression is decreased by the *strA1* allele, indicating that the *hydV* selenocysteine UGA codon has the properties of a "normal" and suppressible nonsense codon. The SelB protein from *D. baculatum* was purified; in gel shift experiments, *D. baculatum* SelB displayed a lower affinity for the *E. coli fdhF* selenoprotein mRNA than *E. coli* SelB did and vice versa. Coexpression of the *hydV'-'lacZ* fusion and of the *selB* and $tRNA^{Sec}$ genes from *D. baculatum*, however, did not lead to selenocysteine insertion into the protein, although the formation of the quaternary complex between SelB, selenocysteyl-$tRNA^{Sec}$, and the *hydV* mRNA recognition sequence took place. The results demonstrate (i) that the selenocysteine-specific UGA codon is readily suppressed under conditions where the homologous SelB protein is absent and (ii) that apart from the specificity of the SelB-mRNA interaction, a structural compatibility of the quaternary complex with the ribosome is required.

The biosynthesis and incorporation of the amino acid selenocysteine into proteins require the function of two *cis*- and four *trans*-acting elements (7). The *cis*-acting elements are a UGA codon which determines the position at which the amino acid is to be inserted and an adjacent secondary structure of the mRNA coding for selenoproteins. This recognition motif of the mRNA determines the specificity of decoding of the particular UGA codon. The *trans*-acting factors are two enzymes involved in selenocysteine biosynthesis, namely, monoselenophosphate synthetase and selenocysteine synthase plus a specific tRNA, $tRNA^{Sec}$, and the specialized translation factor SelB. $tRNA^{Sec}$ is charged with L-serine and serves as an adapter for the conversion of the seryl moiety into selenocysteine. SelB is a translation factor with a function homologous to that of EF-Tu but with exclusive specificity for selenocysteyl-$tRNA^{Sec}$. SelB binds both the charged tRNA and the recognition motif of the mRNA and—according to the present model—thereby donates the selenocysteyl-tRNA in close proximity to the UGA codon at the ribosomal A site (1).

The heterologous expression of genes coding for selenoproteins is of considerable interest, both from a basic and applied view. Attempts to achieve such heterologous expression in prokaryotes have been unsuccessful until now (10, 17, 29). There is only one report that insertion of selenocysteine directed by a UGA codon in a heterologous mRNA could be accomplished, namely, when the recognition motif of the *fdhF* gene was introduced into the *fdhA* gene of *Methanobacterium formicicum* to match the binding specificity of the *Escherichia coli* SelB protein (13). In eukaryotes, the specificity of UGA decoding of selenoprotein mRNAs is determined by a secondary structure in the 3′ untranslated region (5). It has been shown that fusing this untranslated region to the mRNA of an ordinary protein can direct selenocysteine insertion at any UGA codon introduced into the reading frame (6, 32). The yield of selenoproteins synthesized in this way, however, is low.

In this communication we report on the results of the analysis of the specificity barriers of the heterologous expression of a selenoprotein gene in prokaryotes. For a model, we have employed formation of the hydrogenase large subunit from *Desulfomicrobium baculatum* (23), which contains a selenocysteine residue covalently linked to the nickel metallocenter (9, 11).

MATERIALS AND METHODS

Bacterial strains, plasmids, bacteriophages, and media. Bacteria, plasmids, and bacteriophages used in this work are listed in Table 1. Bacteria were grown aerobically in TP medium (12) or LB medium (24) and anaerobically in TGYEP medium (4) supplemented with 0.1% formate when required. Antibiotics were added at the following concentrations: ampicillin, 100 μg/ml; kanamycin, 50 μg/ml; and chloramphenicol, 20 μg/ml. Bacteriophage λ was propagated by the method of Simons et al. (33).

Recombinant DNA techniques. Chromosomal DNA was isolated as described by Murray and Thompson (25). All standard genetic procedures were performed as described by Sambrook et al. (30). Enzymes were used according to the recommendations of the manufacturer.

Preparation of 5′ labeled transcripts. Transcripts were produced by the method of Wyatt et al. (36) using T7 RNA polymerase with either *Bam*HI-linearized plasmid pETWT (*fdhF* mRNA) or the synthetic oligodesoxyribonucleotide DbmRNA (5′-AATTCTTCACCGGTCTCAGCGTGCAGCACGTGCACGGCACAGCCCAGTCACTCTCCCtatagtgagtcgtatta-3′) as the template (Dbm RNA hybridized with oligonucleotide T7-1 [5′-TAATACGACTCACTATAG-3′] in the ϕ10 promoter region [given in lowercase letters] of the oligodesoxyribonucleotide DbmRNA). The sequences of the resulting transcripts are identical to the ones given in Fig. 1. Transcripts were 5′ labeled with [^{32}P]ATP using polynucleotide kinase and purified by gel filtration using Sephadex G50 columns.

β-Galactosidase activity assay. β-Galactosidase activity was determined by the method of Miller (24) with cells from cultures which had been grown aerobically to an optical density at 600 nm (OD_{600}) of 0.5 in TP medium.

In vivo labeling with [^{75}Se]selenite. Cells were grown anaerobically to an OD_{600} of 1 in the presence of 1 μM [^{75}Se]selenite. Proteins of whole-cell lysates were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis, and the selenoproteins were visualized by autoradiography by the method of Cox et al. (8).

Construction of plasmids pDBM100, pDBM700, pDSB215, and pDQB216. In order to generate the plasmids pDBM700 and pDBM100 encoding the "selenocysteine-inserting cartridges," the appropriate regions of the *hydV* gene (23) were amplified by PCR (16) using the synthetic oligonucleotides JHD1 (5′-GTTAG

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TABLE 1. Bacterial strains, plasmids, and bacteriophages used in this study

Strain, plasmid, or bacteriophage	Relevant genotype or characteristic[a]	Source or reference
Strains		
D. baculatum DSM 1743	Wild type	DSM[b]
E. coli		
JM109	F$^-$ (*traD36 proAB*$^+$ *lacI*^{q} *lacZ*Δ*M15*) *recA1 endA1 gyrA96 thi-1 hsdR17 relA1 supE44* Δ(*lac-proAB*) λ$^-$	37
FM433	F$^-$ *araD139* Φ(*argF-lac*) *U169 ptsF25 deoC1 relA1 flbB5301 rpsE13* Φ(*srl-recA*)*306*::Tn*10*	39
WL81460	FM433, Δ*selC400*::*kan*	39
WL81300	FM433, Δ*selB300*::*kan*	20
PT91422	F$^-$ *araD139* Φ(*argF-lac*)*U169 ptsF25 deoC1 relA1 flbB5301 strA1* Φ(*srl-recA*)*306*::Tn*10*	This work
PT91460	PT91422, Δ*selC400*::*kan*	This work
FM433::λERM	FM433, Km^r Φ(*fdhF′-′lacZ*)*42*; selenocysteine-inserting cartridge from plasmid pWT in the λ attachment site of the chromosome	34
PT91422::λDRM	PT91422, Km^r, Φ(*hydV′-′lacZ*)*90*; selenocysteine-inserting cartridge from plasmid pDBM100 in the λ attachment site of the chromosome	This work
Plasmids		
pSU2719	Cm^r *lac′IPOZ′a*	22
pUC19	Ap^r	37
pQE32	Ap^r	Qiagen
pREP4	Km^r *lacI*	Qiagen
pSKS106	Ap^r *′lacZ lacYA*	31
pCB2013	Ap^r $selC_{Ec}$	2
pWT	Ap^r Φ(*fdhF′-lacZ*)*42 lacYA*	12
pETWT	Ap^r ϕ10 *fdhF42* (stem-loop structure of the *fdhF* gene from *E. coli*)	1
pFM58	Ap^r Φ(*fdhF′-′lacZ*)hyb423[c] (TCA)	38
pRS552	Km^r Ap^r *′lacZ lacYA*	33
pWL143	Cm^r $selB_{Ec}$, derivative of pWL101 carrying 402 bp of chromosomal DNA between the 3′ end of the *selB* gene and the *Sal*I cloning site	19
pDBM100	pSKS106, Φ(*hydV′-′lacZ*)*90*[d]	This work
pDBM700	pSKS106, Φ(*hydV′-′lacZ*)*645*	This work
pDBC300	pBR322, $selC_{Db}$	35
pDBB213	pBR322, $selB_{Db}$	18
pDSB215	pSu2719, $selB_{Db}$ under the control of the *lac* promoter	This work
pDQB216	pQE32, $selB_{Db}$; under the control of the *lac* promoter, codes for H_6SelB_{Db}	This work
Bacteriophages		
λRS45	*lacZ′YA imm*^{21} *ind*$^+$	33
λERM100	λRS45, Φ(*fdhF′-′lacZ*)*42*	34
λDRM100	λRS45, Φ(*hydV′-′lacZ*)*90*	This work

[a] Ap^r, ampicillin resistance; Km^r, kanamycin resistance; Cm^r, chloramphenicol resistance. Db and Ec superscripts indicate the origin of the respective gene from *D. baculatum* or *E. coli*, respectively.
[b] DSM, Deutsche Stammsammlung für Mikroorganismen.
[c] 423 nucleotides present from *fdhF*.
[d] The numbers indicate the numbers of base pairs from the *hydV* gene cloned into the *lacZ* gene.

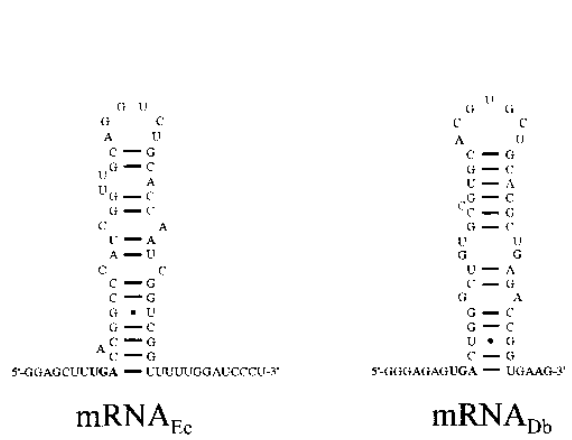
TCAATGTTAACAACGTGTTC-3′) and JHD2 (5′-GAGGGAATTCGATTCC AAGCTGG-3′) or JHD1 and Dbac01 (5′-CCCGTTAACGTGGGGCGCCTG-3′), respectively. In the case of the 701-bp selenocysteine insertion cartridge (JHD1 and JHD2), the PCR fragment was subsequently cut with *Eco*RI and the 5′ overhanging end was filled in with Klenow fragment of DNA polymerase I. After addition of a *Hin*dIII linker (CGAAGCTTCG), the resulting fragment was cleaved with *Hin*dIII and *Hpa*I and ligated with plasmid pSKS106 that had been cut with *Hin*dIII and *Sma*I resulting in the appropriate *hydV-lacZ* fusion. In the case of the 90-bp selenocysteine insertion cartridge (JHD1 and Dbac01), the resulting PCR fragment was cut with *Hpa*I and directly cloned into pSKS106 linearized with *Sma*I.

In the first step of the generation of plasmid pDSB215, an *Nhe*I-*Mlu*I fragment from pDBB213 was cloned into pT7-6 cut with *Xba*I-*Eco*RI. Since *Mlu*I and *Eco*RI are not compatible with each other, these sites were filled in with Klenow fragment reconstituting an *Eco*RI site after ligation. Subsequently, 191 bp upstream of the *selB* gene (positions −14 to −205) were deleted by inverse PCR (26) using the synthetic oligonucleotides Dbac22 (GCTAGGAGAGACGCAT GC) and T7-4 (CTAGAGTCGACCTGCAGC). In the final step, a *Sal*I-*Eco*RI fragment was cloned into pSU2719 cut with the same enzymes. Since pSU2719 carries two *Eco*RI sites, this plasmid was only partially cut with this enzyme.

Plasmid pDQB216 is a pQE32 derivative encoding SelB from *D. baculatum* as a N-terminal His tag fusion. In the first step to construct this plasmid, a *Sph*I fragment carrying the 5′ part of the *selB* gene from plasmid pDSB215 was transferred to pQE32 cut with the same enzyme (pDQB-N). In order to restore the complete *selB* gene, a *Stu*I-*Eco*RI fragment from pDSB215 was cloned into pDQB-N cut with *Stu*I and *Sma*I. Since the *Eco*RI site is not compatible with the *Sma*I site, the *Eco*RI site was filled in with Klenow fragment.

Construction of strain PT91422::λDRM. An *Eco*RI-*Bam*HI fragment from plasmid pDBM100, encoding the N-terminal amino acids of the *lacZ* gene and 90 bp from *hydV* carrying the putative stem-loop structure necessary for selenocysteine insertion, under the control of the *lac* promoter, was transferred to plasmid pRS552. The *Bam*HI site was subsequently filled in with Klenow fragment, thus rendering the selenocysteine insertion cartridge in frame with the downstream *lacZ* gene of plasmid pRS552. The construct encoding the fusion protein was then transferred to the λ attachment site of strain PT91422 with the aid of the lambda derivative λRS45 by the method of Simons et al. (33).

Purification of H_6SelB_{Db}. The fusion protein H_6SelB_{Db} (i.e., SelB from *D. baculatum* possessing a hexahistidine tag at the N terminus) was purified from *E. coli* JM109/pREP4/pDQB216, that was grown to an OD_{600} of 1, induced with 0.5 mM isopropyl-β-D-thiogalactopyranoside and subsequently cultivated for an additional 2 h. The cells were resuspended in 50 mM sodium phosphate (pH 7.2) containing 0.5 M NaCl and 1 mM 2-mercaptoethanol and broken up by passage through a French pressure cell at 16,000 lb/in^2. After centrifugation at 30,000 × *g*, the supernatant was loaded onto a 1-ml Zn^{2+}-chelating Sepharose (Pharmacia) column. The proteins were eluted with an imidazole gradient from 0 to 100 mM in the above buffer. The pure H_6SelB_{Db} protein eluted at about 20 mM



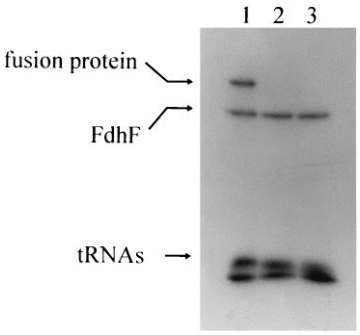


FIG. 2. Effect of the putative mRNA secondary structure of the *hydV* gene from *D. baculatum* on UGA decoding in *E. coli* FM433 as measured by in vivo ^{75}Se labeling experiments. Lane 1, FM433/pWT; lane 2, FM433/pDBM100; lane 3, FM433/pDBM700. The positions of the *fdhF* gene product, *lacZ* fusion protein, and selenated tRNAs are indicated.

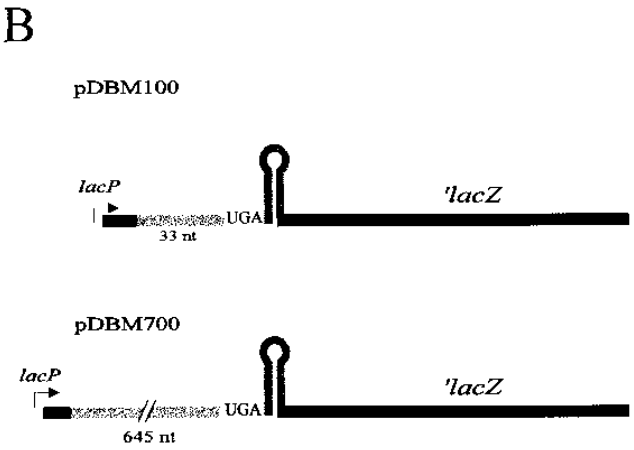


FIG. 1. (A) Comparison of the mRNA secondary structure of the *fdhF* gene of *E. coli* and the putative structure of the *hydV* gene of *D. baculatum* directing selenocysteine incorporation at the UGA selenocysteine codon (shown in boldface type). (B) Schematic representation of selenocysteine insertion cartridges carrying the putative stem-loop structure of the *hydV* gene of *D. baculatum* cloned in frame into the *lacZ* gene of plasmid pSKS106. nt, nucleotides.

imidazole. The protein was stored in a solution containing 50 mM sodium phosphate (pH 7.2), 5 mM $MgCl_2$, 2 mM dithiothreitol, 0.5 mM EDTA, and 50% (wt/vol) glycerol.

Gel shift experiments. The gel shift experiments involving translation factor SelB from *E. coli* ($SelB_{Ec}$) were carried out as described previously (1), except a 660-fold excess of competitor tRNA (baker's yeast bulk tRNA) was added. Gel shift experiments with the translation factor SelB from *D. baculatum* ($SelB_{Db}$) were performed in a solution containing 50 mM Tris-acetate (pH 7.0), 50 mM KCl, 2 mM dithiothreitol, 0.5 mM $MgCl_2$, 1 pmol of transcript, and different amounts of SelB. Unspecific aggregation of protein and RNA was suppressed by addition of a 660-fold excess of competitor tRNA (baker's yeast bulk tRNA). The SelB-mRNA complex was resolved in a 4% polyacrylamide gel (cross-link 60:1) in 50 mM Tris-acetate buffer, pH 7.0.

RESULTS

The UGA codon of the *hydV* gene from *D. baculatum* does not direct selenocysteine insertion in *E. coli*. Figure 1a compares the proposed secondary structures bordering the UGA selenocysteine codon of the *fdhF* mRNA from *E. coli* and the *hydV* mRNA of *D. baculatum* at the 3′ side. It is evident that the two recognition motifs differ, especially in the loop region, which has been demonstrated to be crucial for interaction with translation factor SelB in *E. coli* (12, 15, 39). To measure readthrough of the UGA codon, two plasmids were constructed, fusing the *D. baculatum* SelB recognition motif to *lacZ* (Fig. 1b). pDBM700 differs from pDBM100 by containing 645 nucleotides of the *hydV* gene upstream of the UGA codon compared to 33 nucleotides in pDBM100. In both plasmids, expression of the *hydV′-′lacZ* fusion is under control of the *E. coli lac* promoter. The two plasmids were transformed into *E. coli* FM433 (*sel*$^+$), and the capacity for selenoprotein synthesis was analyzed by incorporation of [^{75}Se]selenium. Figure 2 shows that selenium is incorporated into the *fdhF* gene product (lanes 1 to 3) and into an *fdhF′-′lacZ*-encoded fusion protein (lane 1). There was no incorporation into the fusion protein detectable in the case of the cells carrying the recognition motif of the *D. baculatum hydV* gene fused to *lacZ* (lanes 2 and 3).

The UGA codon from *D. baculatum* is suppressed in an *rpsE* mutant of *E. coli*. The rate of β-galactosidase formation expressed from the *hydV′-′lacZ* fusion was determined to assess whether the lack of selenium incorporation is due to efficient termination of protein synthesis at the UGA codon. Surprisingly, the UGA codon is efficiently read in strain FM433 which carries the *rpsE* gene (Table 2) and thus has a diminished proofreading activity during translation (28). This suppression was independent from the presence of a functional *selC* gene product. No misreading of the UGA codon was detected in the case of the homologous system, i.e., the *fdhF′-′lacZ* fusion of plasmid pWT in a Δ*selC* strain.

When the *rpsE* mutant allele was exchanged for the wild-type *rpsE* gene and the strongly restrictive *strA1* mutation was also introduced, the misreading of the UGA codon followed by the mRNA secondary structure from *D. baculatum* was strongly reduced, both in the presence and absence of a func-

TABLE 2. Suppression of the *D. baculatum* selenocysteine insertion cartridges in *E. coli*

Strain	Relevant genotype	β-Galactosidase activity[a] of strain with plasmid:		
		pWT	pDBM100	pDBM700
FM433	*rpsE*	1,600 (±40)	618 (±11)	775 (±20)
WL81460	*rpsE* Δ*selC*	<1	665 (±20)	1,143 (±50)
PT91422	*strA1*	2,050 (±120)	49 (±2)	43 (±2)
PT91460	*strA1* Δ*selC*	<1	44 (±7)	35 (±2)

[a] β-Galactosidase activity is given in Miller units (24). Values in parentheses are standard errors of the mean.

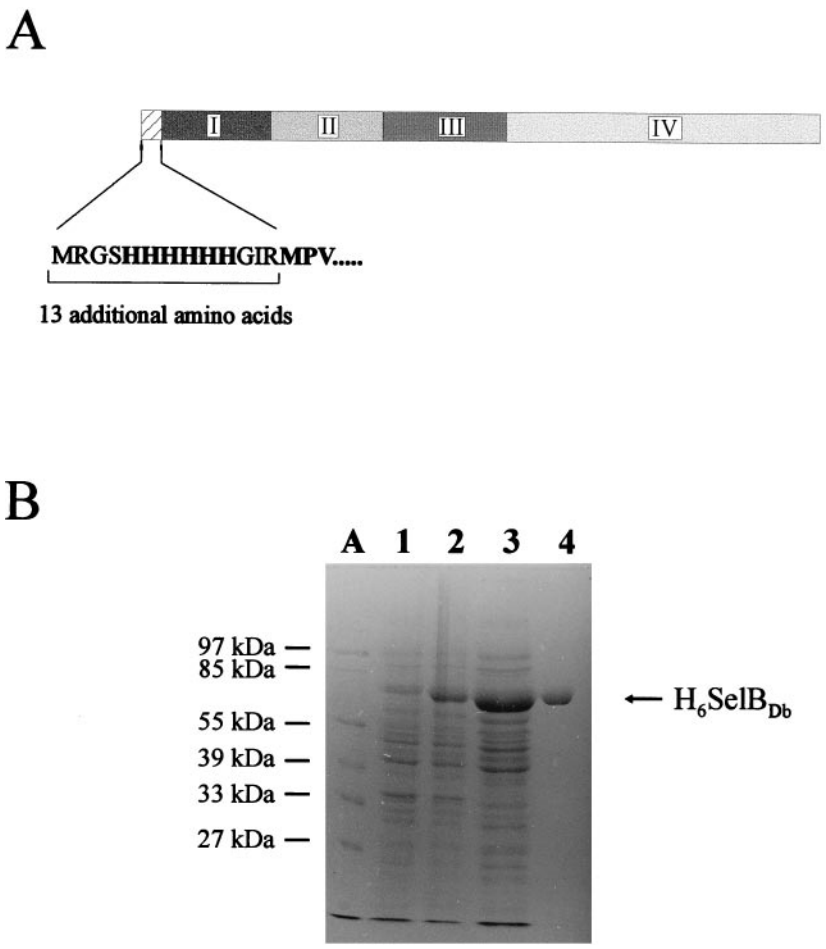


FIG. 3. Purification of translation factor SelB from *D. baculatum* as a His tag fusion protein (H_6SelB_{Db}). (A) Schematic representation of the His tag fusion protein carrying 13 additional amino acids at its N terminus. Structural domains I, II, III, and IV of the protein are indicated. (B) Purification of H_6SelB_{Db} followed by SDS-polyacrylamide gel electrophoresis. Lanes 1 and 2, SDS lysates of *E. coli* JM109/pDQB2166/pREP4 before and after induction with isopropyl-β-D-thiogalactopyranoside; lane 3; S30 extract; lane 4, affinity chromatography pool; lane A, molecular mass standards (positions shown to the left of the gel).

tional *selC* gene. Again, the efficiency of the decoding of the UGA codon from the homologous *fdhF'-'lacZ* was unaffected and fully dependent on a functional *selC* gene.

Purification of SelB from *D. baculatum* and analysis of its interaction with the homologous and heterologous recognition sequence. The failure to translate the *D. baculatum* selenoprotein mRNA in *E. coli* could reside in the inability of the *E. coli* SelB to interact with the heterologous mRNA. To test whether the *D. baculatum* SelB protein can interact in vitro with its cognate recognition motif, the protein was purified. To this end, the *selB* gene previously cloned (18) was fused with a DNA segment encoding 13 additional amino acids including a His tag at the N terminus (Fig. 3A). The gene fusion was overexpressed, and the product was purified in a single step by immobilized metal affinity chromatography (Fig. 3B).

The purified protein containing the N-terminal His tag (H_6SelB_{Db}) was tested for interaction with the ^{32}P-labeled stem-loop structures of the *fdhF* and *hydV* mRNAs (Fig. 1). The reciprocal experiment employing SelB protein (without His tag) from *E. coli* was also performed. Figure 4 shows that the two translation factors preferentially—but not exclusively—interact with the homologous mRNA; the heterologous one is bound only at excessive protein/RNA ratios. Note that in the experimental set using SelB from *D. baculatum*, the mobility of the complex changes with increasing SelB concentration. This effect is also observed using SelB from *E. coli* in an experiment without the addition of competitor RNA. To date, we have no explanation for this phenomenon.

Interaction of SelB from *D. baculatum* with selenocysteyl-tRNA^{Sec}. The functionality of SelB is dependent on the interaction with two different RNA species. Apart from the mRNA recognition motif, SelB has to bind the specific tRNA^{Sec}. We have, therefore, analyzed whether SelB in its native form (without His tag; pDSB215) can interact with selenocysteyl-tRNA in the heterologous host. We exploited the fact that in the *E. coli* system, overproduction of $SelB_{Ec}$ and the consequent high ratio of $SelB_{Ec}$ relative to tRNA^{Sec} and mRNA impairs the statistics of interaction of $SelB_{Ec}$ with both RNA species and thereby suppresses selenoprotein synthesis (34). This suppression can be relieved by concomitant overproduction of tRNA^{Sec}. Table 3 demonstrates that overproduction of SelB from *D. baculatum* indeed reduces readthrough of the UGA codon of the *fdhF'-'lacZ* fusion almost completely as it is observed with SelB from *E. coli*(pWL143). This reduction is also reversed by concomitant overproduction of tRNA^{Sec} (pCB2013). In the light of the results obtained for the SelB protein from *E. coli* (34), this reduction strongly indicates that

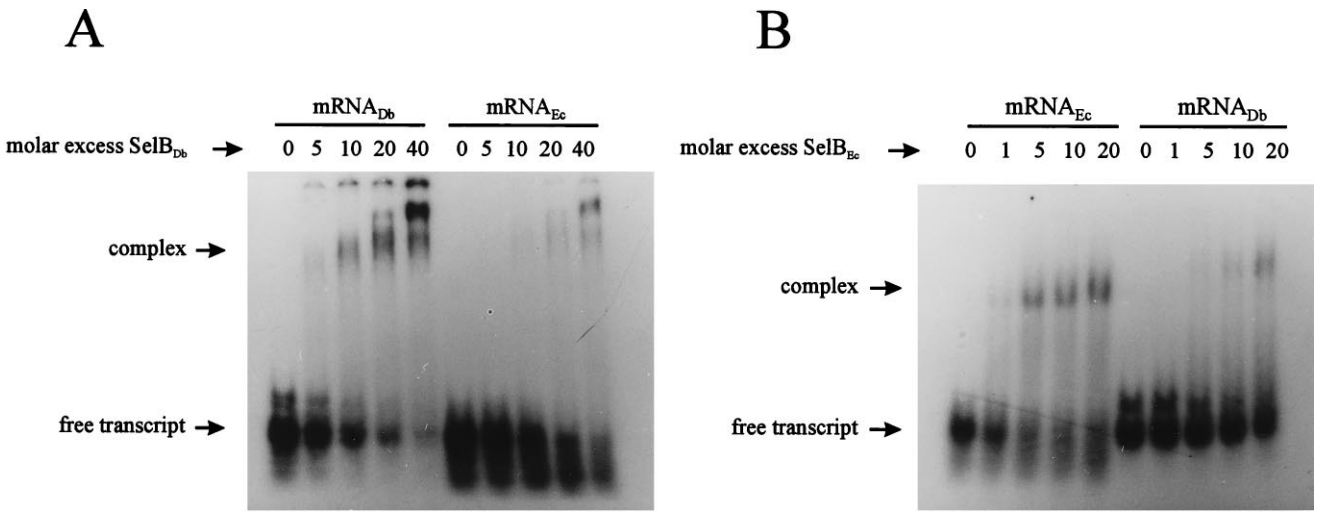


FIG. 4. Specificity of the SelB-mRNA interaction as visualized by gel shift titration experiments. Interaction of SelB from *D. baculatum* (SelB$_{Db}$) (A) or SelB from *E. coli* (SelB$_{Ec}$) (B) with either the mRNA stem-loop structure of the *hydV* (mRNA$_{Db}$) or *fdhF* (mRNA$_{Ec}$) gene. The molar excess of the SelB from the respective organism is indicated above the lanes. The migration position of the SelB-mRNA complex is indicated.

the translation factor from *D. baculatum* is capable of binding selenocysteyl-tRNA^{Sec} in the heterologous host in vivo.

Incompatibility between the *D. baculatum* SelB–selenocysteyl-tRNA^{Sec}–mRNA complex and the *E. coli* translational system. Having shown that SelB from *D. baculatum* synthesized in the heterologous host can interact with selenocysteyl-tRNA and also binds the *D. baculatum* mRNA recognition sequence in vitro, we tested whether the *D. baculatum* UGA codon plus the recognition motif can be decoded as selenocysteine in *E. coli* when *selB* and the tRNA^{Sec} gene from this organism are also expressed. For this purpose, strain PT91422::λDRM which contains the chromosomally encoded *hydV′-′lacZ* fusion was transformed with plasmid pDSB215 (carrying the *D. baculatum selB* gene) and either pDBC300 (*D. baculatum selC*) or pCB2013 (*E. coli selC*). Readthrough of the UGA codon as measured by β-galactosidase activity was not detectable (data not shown). In contrast, the overexpression of *selB* from *D. baculatum* has a reducing effect on the high degree of suppression of the *hydV′-′lacZ* fusion as tested in WL81300 transformed with pDBM100. The concomitant *selB$_{Db}$* expression reduces the β-galactosidase activity by 55% from 708 to 325 Miller units. No effect is seen when the *E. coli selB* gene is coexpressed.

TABLE 3. Ability of SelB from *D. baculatum* to bind to tRNA^{Sec} in vivo

SelB variant	Plasmid	tRNA^{Sec} overproduction[a]	
		− (pUC19)	+ (pCB2013)
SelB$_{Db}$	pDSB215	<1	90 (±4)
SelB$_{Ec}$	pWL143	<1	97 (±3)
—[b]	pSU2719	100 (±5)	201 (±6)

[a] Symbols: −, no overproduction; +, overproduction. The plasmids in parentheses are the plasmids used. The values are β-galactosidase activities (given in Miller units [24]). Values in parentheses are standard errors of the mean. See text for details.

[b] —, no SelB overproduction.

DISCUSSION

In the genetic code, UGA can possess a number of different functions (27). UGA directs cysteine insertion in the genus *Euplotes*, tryptophan insertion in *Mycoplasma* and mitochondria, selenocysteine insertion in selected organisms, and finally, UGA can signify polypeptide chain termination. Whereas the decoding of the UGA codon as cysteine and tryptophan is characteristic of only a few biological systems, functioning as a selenocysteine and functioning as a stop codon are traits of the genetic code in all three lines of descent (7, 21).

In prokaryotes, the decision on whether UGA is read as a selenocysteine codon rests on the existence of an mRNA secondary structure immediately 3′ to the codon (12, 39). Since selenocysteine occurs in the active site of functionally very different enzymes (7, 21), it was not surprising to find that heterologous expression of selenoprotein genes cannot be accomplished (10, 17, 29) (Fig. 2). Since the genes for tRNA^{Sec} from a wide range of organisms can complement the *selC* lesion of an *E. coli* mutant (3, 14, 35), it was clear that charging of the heterologous tRNA and biosynthesis of selenocysteyl-tRNA cannot be the bottleneck for heterologous expression. More likely, the recognition of the mRNA motif determining the decoding of UGA as selenocysteine by heterologous SelB protein was a likely candidate for the expression barrier. Indeed, the purified SelB translation factors from *E. coli* and *D. baculatum* showed a 5- to 10-fold-higher affinity for the homologous mRNA recognition motif than for the heterologous one. The interaction of the selenocysteine-specific translation factor from *D. baculatum* and the stem-loop structure of the *hydV* mRNA in vitro substantiates the notion that it is this mRNA secondary structure that renders the UGA codon a selenocysteine codon, as proposed by computer analysis (39).

The specificity as measured by the mobility shift assays, however, is not exclusive. One should, therefore, expect that an increased amount of the heterologous protein should overcome the lowered affinity, which is, however, not the case. This is consistent with the mutational analysis of the *fdhF* mRNA secondary structure where the exchange of a single nucleotide in the loop region abolishes in vivo selenocysteine incorporation but only quantitatively reduces the affinity for SelB (1, 12).

Heterologous expression could not be observed even under the condition that selenocysteyl-tRNA, mRNA, and SelB were all from the heterologous organism.

All the experimental data point to the conclusion that the postulated quaternary complex also forms between these heterologous components. First, SelB from *D. baculatum* reduces the programmed readthrough of the UGA codon in *E. coli*. This effect could be unambiguously attributed to the sequestering of selenocysteyl-tRNA^{Sec} in the *E. coli* system (34). Second, the SelB protein from *D. baculatum* is capable of binding to the *hydV* mRNA secondary structure in vitro and apparently also in vivo. Thus, although the quaternary complex between SelB, selenocysteyl-tRNA, GTP, and mRNA seems to form in the heterologous organism, it is not compatible for translation. An obvious explanation is that the heterologous SelB does not properly interact with the *E. coli* ribosome. Whether this is due to the SelB structure itself or the overall structure of the quaternary complex, including the critical distance of the UGA codon relative to the complex, remains to be determined.

A crucial difference of the *D. baculatum* SelB "decoding complex" relative to the *E. coli* one is also indicated by the finding that the UGA codon of the heterologous mRNA is easily suppressed in an *E. coli* strain harboring a ribosomal ambiguity mutation (*rpsE* [Table 2]) (28) and that misreading is reduced in a *strA1* genetic background. This suppression is independent of the presence of a functional *selC* gene, indicating the insertion of another amino acid, possibly tryptophan (28). The UGA codon of the *E. coli fdhF* mRNA, in contrast, is not suppressed at all. The decoding of the UGA codon from the *D. baculatum hydV* gene, therefore, is affected by the fidelity control of the *E. coli* ribosome; that of the *fdhF* mRNA, however, is not. It is plausible to assume that this suppressibility of the heterologous UGA codon is a consequence of the failure of the specific selenocysteine insertion, since it is higher in the absence of the appropriate SelB protein. If this were the case, the binding of the translation factor would also be involved in preventing RF2 action at this codon.

A high level of readthrough was also observed when the *grdA* gene from *Clostridium sticklandii* encoding the selenoprotein A of glycine reductase is expressed in *E. coli* (10). Again, no specific selenocysteine insertion but a high level of suppression of the UGA codon was detected. About 10% of the produced protein carried selenocysteine, as determined by enzyme activity. The researchers assume that this selenocysteine is incorporated via tRNA^{Cys} incorrectly charged with selenocysteine. This unspecific incorporation of selenocysteine cannot be observed upon expression of the *hydV* gene from *D. baculatum* in *E. coli.*

The number of steps controlling the fidelity of selenocysteine insertion directed by the UGA codon are impressive. First, charging of tRNA^{Sec} is limited compared to the other serine isoacceptors to control the flux into this trace element pathway. Second, selenocysteine synthase discriminates between the two kinds of seryl-tRNAs, and SelB binds only the selenocysteylated one. Furthermore, there is some not yet fully analyzed cooperativity in tRNA and mRNA binding by SelB which appears to be crucial for decoding (1, 34), and finally and at least for the two organisms investigated here, SelB (or the SelB quaternary complex) from the heterologous organism does not function with the *E. coli* ribosome. It will be a challenge to analyze the basis of this incompatibility in detail and to overcome it, possibly by mutational adjustments.

REFERENCES

1. **Baron, C., J. Heider, and A. Böck.** 1993. Interaction of translation factor SelB with the formate dehydrogenase H selenopolypeptide messenger RNA. Proc. Natl. Acad. Sci. USA **90:**4181–4185.
2. **Baron, C., J. Heider, and A. Böck.** 1990. Mutagenesis of *selC*, the gene for the selenocysteine-inserting tRNA-species in *E. coli*: effects on *in vivo* function. Nucleic Acids Res. **18:**6761–6766.
3. **Baron, C., C. Sturchler, X. Q. Wu, H. J. Gross, A. Krol, and A. Böck.** 1994. Eukaryotic selenocysteine inserting tRNA species support selenoprotein synthesis in *Escherichia coli*. Nucleic Acids Res. **22:**2228–2233.
4. **Begg, Y. A., J. N. Whyte, and B. A. Haddock.** 1977. The identification of mutants of *Escherichia coli* deficient in formate dehydrogenase and nitrate reductase activities using dye indicator plates. FEMS Microbiol. Lett. **2:**47–50.
5. **Berry, M. J., L. Banu, Y. 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 deiodinase requires sequences in the 3′ untranslated region. Nature (London) **353:**273–276.
6. **Berry, M. J., L. Banu, J. W. Harney, and P. R. Larsen.** 1993. Functional characterization of the eukaryotic SECIS elements which direct selenocysteine insertion at UGA codons. EMBO J. **12:**3315–3322.
7. **Böck, A., K. Forchhammer, J. Heider, W. Leinfelder, G. Sawers, B. Veprek, and F. Zinoni.** 1991. Selenocysteine: the 21st amino acid. Mol. Microbiol. **5:**515–520.
8. **Cox, J. C., E. S. Edwards, and J. A. DeMoss.** 1981. Resolution of distinct selenium-containing formate dehydrogenases from *Escherichia coli*. J. Bacteriol. **145:**1317–1324.
9. **Eidsness, M. K., R. A. Scott, B. Pickril, D. V. DerVartanian, J. LeGall, I. Moura, J. J. G. Moura, and H. D. Peck, Jr.** 1989. Evidence for selenocysteine coordination to the active site nickel in the (NiFeSe) hydrogenase from *Desulfovibrio baculatus*. Proc. Natl. Acad. Sci. USA **86:**147–151.
10. **Garcia, G. E., and T. C. Stadtman.** 1992. *Clostridium sticklandii* glycine reductase selenoprotein A gene: cloning, sequencing, and expression in *Escherichia coli*. J. Bacteriol. **174:**7080–7089.
11. **He, S.-H., M. Teixeira, J. LeGall, D. S. Patil, D. V. DerVartanian, B. H. Huynh, and H. D. Peck, Jr.** 1989. EPR studies with ^{77}Se enriched (NiFeSe) hydrogenase of *Desulfovibrio baculatus*. Evidence for a selenium ligand to the active-site nickel. J. Biol. Chem. **264:**2678–2682.
12. **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.
13. **Heider, J., and A. Böck.** 1992. Targeted insertion of selenocysteine into the α subunit of formate dehydrogenase from *Methanobacterium formicicum*. J. Bacteriol. **174:**659–663.
14. **Heider, J., W. Leinfelder, and A. Böck.** 1989. Occurrence and functional compatibility within Enterobacteriaceae of a tRNA species which inserts selenocysteine into protein. Nucleic Acids Res. **17:**2529–2540.
15. **Hüttenhofer, A., E. Westhof, and A. Böck.** 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.
16. **Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White.** 1990. PCR protocols: a guide to methods and applications. Academic Press Ltd., London, England.
17. **Kreimer, S., and J. R. Andreesen.** 1995. Glycine reductase of *Clostridium litorale*—cloning, sequencing, and molecular analysis of the *grdAB* operon that contains two in-frame TGA codons for selenium incorporation. Eur. J. Biochem. **234:**192–199.
18. **Kromayer, M., R. Wilting, P. Tormay, and A. Böck.** 1996. Domain structure of the prokaryotic selenocysteine-specific elongation factor SelB. J. Mol. Biol. **262:**413–420.
19. **Leinfelder, W., K. Forchhammer, F. Zinoni, G. Sawers, M.-A. Mandrand-Berthelot, and A. Böck.** 1988. *Escherichia coli* genes whose products are involved in selenium metabolism. J. Bacteriol. **170:**540–546.
20. **Leinfelder, W.** 1989. Ph.D. thesis. University of Munich, Munich, Germany.
21. **Low, S. C., and M. J. Berry.** 1996. Knowing when not to stop: selenocysteine incorporation in eukaryotes. Trends Biochem. Sci. **21:**203–208.
22. **Martinez, E., B. Bartolomé, and F. D. L. Cruz.** 1988. pACYC184-derived cloning vectors containing the multiple cloning site and *lacZ*α reporter gene of pUC8/9 and pUC18/19 plasmids. Gene **68:**159–162.
23. **Menon, N. K., H. D. Peck, Jr., J. Le Gall, and A. E. Przybyla.** 1987. Cloning and sequencing of the genes encoding the large and small subunits of the periplasmic (NiFeSe) hydrogenase of *Desulfovibrio baculatus*. J. Bacteriol. **169:**5401–5407. (Erratum, **170:**4429, 1988.)
24. **Miller, J. H.** 1992. A short course in bacterial genetics, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Murray, M. G., and W. F. Thompson.** 1980. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. **8:**4321–4325.
26. **Ochman, H., A. S. Gerber, and D. L. Hartl.** 1988. Genetic applications of an inverse polymerase chain reaction. Genetics **120:**621–623.
27. **Osawa, S., T. H. Jukes, K. Watanabe, and A. Muto.** 1992. Recent evidence for evolution of the genetic code. Microbiol. Rev. **56:**229–264.

28. **Parker, J.** 1989. Errors and alternatives in reading the universal genetic code. Microbiol. Rev. **53:**273–298.
29. **Rocher, C., C. Faucheu, F. Herve, C. Benicourt, and J. L. Lalanne.** 1991. Cloning of murine SeGpx cDNA and synthesis of mutated GPx proteins in Escherichia coli. Gene **98:**193–200.
30. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
31. **Shapira, S. K., J. Chou, F. V. Richaud, and M. J. Casadaban.** 1983. New versatile plasmid vectors for expression of hybrid proteins coded by a cloned gene fused to *lacZ* gene sequences encoding an enzymatically active carboxy-terminal portion of beta-galactosidase. Gene **25:**71–82.
32. **Shen, Q. C., F. F. Chu, and P. E. Newburger.** 1993. Sequences in the 3′-untranslated region of the human cellular glutathione peroxidase gene are necessary and sufficient for selenocysteine incorporation at the UGA codon. J. Biol. Chem. **268:**11463–11469.
33. **Simons, R. W., F. Houman, and N. Kleckner.** 1987. Improved single and multicopy *lac*-based cloning vectors for protein and operon fusions. Gene **53:**85–96.
34. **Tormay, P., A. Sawers, and A. Böck.** 1996. Role of stoichiometry between mRNA, translation factor SelB and selenocysteyl-tRNA in selenoprotein synthesis. Mol. Microbiol. **21:**1253–1259.
35. **Tormay, P., R. Wilting, J. Heider, and A. Böck.** 1994. Genes coding for the selenocysteine-inserting tRNA species from *Desulfomicrobium baculatum* and *Clostridium thermoaceticum*: structural and evolutionary implications. J. Bacteriol. **176:**1268–1274.
36. **Wyatt, J. R., M. Chastain, and J. D. Puglisi.** 1991. Synthesis and purification of large amounts of RNA oligonucleotides. BioTechniques **11:**764–769.
37. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequence of M13mp18 and pUC19 vectors. Gene **33:**103–109.
38. **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. USA **84:**3156–3160.
39. **Zinoni, F., J. Heider, and A. Böck.** 1990. Features of the formate dehydrogenase mRNA necessary for decoding of the UGA codon as selenocysteine. Proc. Natl. Acad. Sci. USA **87:**4660–4664.