JOURNAL OF BACTERIOLOGY, Nov. 2000, p. 6302–6307
0021-9193/00/$04.00+0
Copyright © 2000, American Society for Microbiology. All Rights Reserved.
Vol. 182, No. 22

The Bulged Nucleotide in the *Escherichia coli* Minimal Selenocysteine Insertion Sequence Participates in Interaction with SelB: a Genetic Approach

CHUANG LI, MYRIAM RECHES, AND HANNA ENGELBERG-KULKA*

Department of Molecular Biology, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel

Received 13 July 2000/Accepted 30 August 2000

The UGA codon, which usually acts as a stop codon, can also direct the incorporation into a protein of the amino acid selenocysteine. This UGA decoding process requires a *cis*-acting mRNA element called the selenocysteine insertion sequence (SECIS), which can form a stem-loop structure. In *Escherichia coli*, selenocysteine incorporation requires only the 17-nucleotide-long upper stem-loop structure of the *fdhF* SECIS. This structure carries a bulged nucleotide U at position 17. Here we asked whether the single bulged nucleotide located in the upper stem-loop structure of the *E. coli fdhF* SECIS is involved in the in vivo interaction with SelB. We used a genetic approach, generating and characterizing *selB* mutations that suppress mutations of the bulged nucleotide in the SECIS. All the *selB* suppressor mutations isolated were clustered in a region corresponding to 28 amino acids in the SelB C-terminal subdomain 4b. These *selB* suppressor mutations were also found to suppress mutations in either the loop or the upper stem of the *E. coli* SECIS. Thus, the *E. coli* SECIS upper stem-loop structure can be considered a "single suppressible unit," suggesting that there is some flexibility to the nature of the interaction between this element and SelB.

The UGA codon, which usually acts as a stop codon, can also direct incorporation of the amino acid selenocysteine (for reviews, see references 4, 5, 7, and 25). This UGA decoding process requires a *cis*-acting mRNA element called the selenocysteine insertion sequence (SECIS), which can form a stem-loop structure (4, 9, 15; for reviews, see references 2 and 20). In *Escherichia coli*, a number of genes have been identified in which the UGA directs the incorporation of selenocysteine. These include genes *fdhF* (27) and *fdnG* (3), encoding the selenocysteine-containing enzymes formate dehydrogenase H and N, respectively. Immediately downstream from the selenocysteine-specifying UGA in the mRNA of each of these polypeptides is found a SECIS that has been described as consisting of at least 40 nucleotides capable of forming a stem-loop RNA structure (2, 9). In later work, it was suggested that an extended *fdhF* SECIS was required, consisting of an additional helix of 7 bp in which the U and G residues of the UGA codon are included and the A residue is bulged out (10). After carrying out an extensive mutational analysis of the *fdhF* SECIS DNA, we found that for in vivo UGA-directed selenocysteine incorporation, there is no requirement for the whole stem-loop RNA structure of the *E. coli fdhF* SECIS (including the extended form) (18), as thought previously. Instead, the 17-bp upper stem-loop structure is sufficient to permit selenocysteine incorporation on the condition that it is located 11 nucleotides downstream from the UGA codon (Fig. 1). This mini upper stem-loop structure contains a bulged nucleotide, a U residue, located 17 nucleotides downstream from the UGA (Fig. 1). Selenocysteine incorporation into an *fdhF-lacZ'* fusion polypeptide depends on both (i) the specificity of nucleotide 17 as a U residue and (ii) its presence as a bulged nucleotide (18). The importance of the bulged U_{17} has also been shown using the SELEX procedure (12).

The UGA-directed selenocysteine incorporation into a polypeptide in *E. coli* also requires a number of *trans* elements. These include the *selC*-specified $tRNA^{Sec}$ (16), which is a specialized tRNA that contains a UCA anticodon, and also a special protein elongation factor (EF) called SelB (reviewed in reference 2). SelB binds both GTP and selenocysteyl-$tRNA^{Sec}$ and also binds the mRNA stem-loop structure formed by *fdhF* SECIS mRNA (1, 8, 9, 11, 23). In vitro experiments (13) have shown that selenocysteyl-$tRNA^{Sec}$ binds the N-terminal part of SelB (homologous to EF-Tu) and that the C-terminal subdomain of SelB binds the *fdhF* SECIS. Furthermore, the efficiency of SelB binding is not reduced when the mRNA motif is reduced to a 17-nucleotide-long minihelix. That minihelix is the same 17-bp upper stem-loop structure that we have shown to be the minimal requirement for in vivo selenocysteine incorporation into a polypeptide (Fig. 1) (18). Recently, this interaction between SelB and the loop of the *fdhF* SECIS upper minihelix has also been demonstrated by genetic analysis (14).

Here we asked whether the single bulged nucleotide in the upper minihelix of the *E. coli fdhF* SECIS is involved in the in vivo interaction with SelB. We used a genetic approach in which we generated and characterized *selB* mutations that suppress mutations in the bulged nucleotide. All the *selB* mutations that we isolated were clustered in a region corresponding to 28 amino acids in the C-terminal 4b subdomain of SelB (31 amino acids before the end of the protein). Our results further support the importance of the bulged nucleotide (U_{17}) of the upper stem-loop of *E. coli* SECIS in the interaction of the SECIS with the SelB elongation factor.

MATERIALS AND METHODS

Bacterial strains and plasmids. The *E. coli* strains and plasmids used in this study are described in Table 1.

Media. Bacteria were grown in liquid or solid Luria-Bertani (LB) medium, M9 minimal medium supplemented with a mixture of amino acids, each at a final concentration of 20 μg/ml (21), or solid LB medium with X-Gal (5-bromo-4-

* Corresponding author. Mailing address: Department of Molecular Biology, The Hebrew University-Hadassah Medical School, Jerusalem 91120, Israel. Phone: 972-2-678-8250. Fax: 972-2-6784010. E-mail: hanita@cc.huji.ac.il.

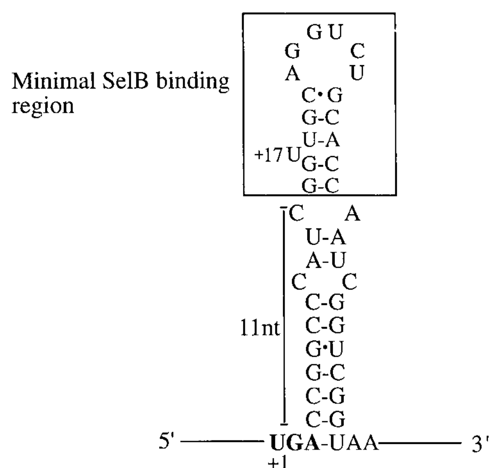

FIG. 1. Minimal *E. coli fdhF* SECIS required for SelB binding and selenocysteine incorporation. The upper stem-loop structure (boxed) is the minimal region required for SelB binding (13). UGA-directed selenocysteine incorporation requires that this structure be located 11 nucleotides (nt) from the UGA codon (bold) (18). The U residue at position 17 is bulged (12, 18). The pairing of boxes C_{20} and G_{27} is questionable (18) and is therefore designated by a dot instead of by a dash.

chloro-3-indolyl-β-D-galactopyranoside, 40 μg/ml). Ampicillin (100 μg/ml) or chloramphenicol (35 μg/ml) was added to the media in which the plasmid-carrying strains were grown. Sodium selenite was obtained from Sigma Chemical Co. (St. Louis, Mo.).

Molecular cloning. All recombinant DNA manipulations were carried out by standard procedures (24). Site-directed mutagenesis was carried out as we have described previously (18, 19). Restriction enzymes and other enzymes used in the recombinant DNA experiments were obtained from New England Biolabs (Beverly, Mass.). DNA sequencing was done using an automated fluorescence DNA 373 sequencer.

Bacterial growth, transformations, and measurements of β-galactosidase activity. *E. coli* cells were transformed (24) by the plasmid of choice. Single colonies of freshly transformed cells were grown on LB plates at 37°C overnight and then in M9 liquid medium at 37°C in a rolling drum for 8 to 10 h until the cultures reached an optical density at 600 nm of 0.7 to 1.0. β-Galactosidase activity was determined as we have described previously (18).

Generation of mutations in *E. coli selB*. Plasmid pLC1(Cm^r) was constructed by cloning the 1,867-bp PCR fragment of the whole *selB* gene from *E. coli* strain MC4100 into the *Hin*dIII and *Bam*HI sites in the tetracycline resistance gene of pACYC184 (Table 1). We used the PCR-based random mutagenesis technique in a reaction mixture including 3.5 mM $MgCl_2$ to introduce random point mutations in the C-terminal part of *selB* (17). The C-terminal part includes the last 872 bp of *selB* from the *Eco*RV site until immediately after the termination codon, corresponding to the end of SelB subdomain 3 and the whole of SelB subdomains 4a and 4b (13). We replaced the last 872 bp of the *selB* in pLC1 with the PCR library-generated mutations in *selB*. We used these ligation mixtures to transform the XL1-Blue strain and picked 4,000 colonies, which we then divided into 40 groups. Each group was grown in 2 ml of LB medium containing chloramphenicol (35 μg/ml) for 6 h. The DNA was extracted from plasmids pLC2^{*1} to pLC2^{*40} bearing the pool of mutated *selB* alleles (Table 1) and used as described below.

Selecting mutations in *selB* that can suppress mutations in the bulged nucleotide U_{17} in *E. coli fdhF* SECIS. In previous studies, we generated mutations in the bulged nucleotide U_{17} in the *fdhF-lacZ* fusions of the Amp^r plasmid pRM4 (18). They are on plasmids pZL42 and pZL70 (Table 1 and Fig. 2). The incorporation of selenocysteine was prevented in MC4100 harboring each one of these fusions (18), and the colonies appeared as Lac$^-$ on X-Gal plates. Here, we first used each of these plasmids separately to transform strain WL81300, a Δ*selB* derivative of MC4100 (Table 1). Then we transformed the Amp^r transformants using plasmid DNA prepared from each group (1 to 40) of the pLC2* (Cm^r) plasmid that carried random mutations in the C-terminal part of *selB*. Treated cells were plated on LB agar containing ampicillin (100 μg/ml), chloramphenicol (35 μg/ml), X-Gal (40 μg/ml), and 10^{-6} M sodium selenite. Of the Amp^r Cm^r Lac$^+$ transformants, we selected one colony from each plate. We further confirmed the Lac$^+$ phenotype by measuring β-galactosidase activity. The Lac$^+$ colonies contain two types of plasmids, one carrying the *fdhF-lacZ* fusion mutations in bulged U_{17} (Amp^r) and the second carrying suppresser mutations in *selB* (Cm^r). To isolate the plasmid that was Cm^r Amp^s, we used the plasmid DNA that was extracted from the doubly transformed cells to transform MC4100 cells and selected Cm^r Amp^s Lac$^-$ colonies. The final characterization of suppressor mutations in *selB* was done by DNA sequencing.

RESULTS

Characterizing *selB* mutations that can suppress mutations in the bulged nucleotide at position 17 of the *E. coli fdhF* SECIS. For this work we used our plasmid pRM4 (22), which we constructed previously to carry the TGA codon context of the *E. coli fdhF* gene fused to *lac′Z* (*lacZ* lacking the first eight codons). Selenocysteine incorporation into the gene product of the *lac′Z* fusion was studied in two ways: (i) qualitatively by the appearance of Lac$^+$ colonies on X-Gal plates, and (ii) quantitatively by measuring the UGA-directed (*selC*-dependent) β-galactosidase activity.

To select mutations in *selB* that can suppress mutations in the bulged nucleotide at position 17, two constructs were used. In plasmid pZL70, the nucleotide U_{17} was changed to C, and in pZL42 U_{17} was changed to A (Table 1 and Fig. 2). We prepared plasmid DNA from each of the 40 groups of pCL2* (Cm^r) plasmids that carried random mutations corresponding to the 4a and 4b subdomains of SelB (see Materials and Methods). We used the DNA of each plasmid separately to doubly transform cells that already contained either pZL70 or pZL42. The transformation procedure using pZL70 resulted in 10 Lac$^+$ transformants that were called p70bm[1] to p70bm[10]. Similarly for the other plasmid, pZL42, seven transformants were found from which the plasmids were isolated that were

TABLE 1. Strains and plasmids

Strain or plasmid	Description or relevant genotype	Source or reference
E. coli strain		
MC4100	*araD139* Δ(*argF-lac*)*205 flbB3501 ptsF25 rpsL150 deoC1 relA1*	6
WL81300	MC4100 Δ(*selB*300::Km^r), Δ(*srl-recA*)*306*::Tn*10*	26; A. Böck
RM1	Δ*selC* derivative of MC4100	22
XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE relA1 lac* [F′ *proAB lacI*^{q}ZΔM15 Tn*10*(Tet^r)]	Stratagene
Plasmids		
pRM4	pBR322 derivative (Amp^r) carrying the fused genes λ*cI′-lac′I″Z* into which the TGA region of the SECIS DNA (from −9 to +47) of *E. coli fdhF* gene has been inserted at the junction of λ*cI′-lac′I″Z*	22
pZL38	pRM4 derivative in which bulged nucleotide U_{18} was changed to A_{18}	18
pZL42	pRM4 derivative in which bulged nucleotide U_{17} was changed to A_{17}	18
pZL44	pRM4 derivative in which bulged nucleotide U_{17} was changed to A_{17} and A_{29} to U_{29}	18
pZL70	pRM4 derivative in which bulged nucleotide U_{17} was changed to C_{17}	18
pL24A	pRM4 derivative in which nucleotide U_{24} in loop was changed to A_{24}	This work
pLC1	pACYC184 derivative (Cm^r) in which *selB* was cloned into the *Hin*dIII and *Bam*HI sites of the tetracycline resistance gene	This work
pLC2*	Pool of pLC1 derivatives carrying PCR-generated random mutations in the C-terminal part of *selB*	This work

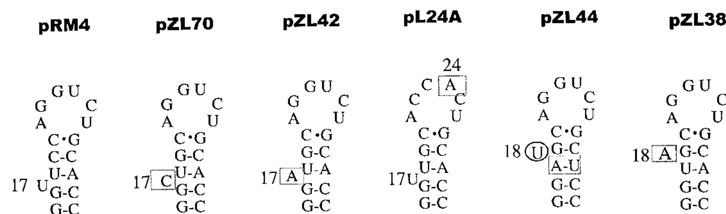

FIG. 2. Location of the mutations in the upper stem and loop of the *E. coli fdhF* SECIS on the plasmids used in this study. The mutated nucleotides are boxed. In pZL44, U_{18} is bulged (circle). The numbers represent the distance of the nucleotide from the UGA codon of *E. coli fdhF* SECIS.

designated p42bm[1] to p42bm[7]. In each case bm stands for bulged mutated. We examined the suppression efficiency of these *selB* mutations quantitatively by measuring the level of UGA-directed (*selC*-dependent) β-galactosidase activity in cells cotransformed by each one of the plasmid groups p70bm [1–10] and p42bm[1–7]. The level of enzymatic activity driven by the unmutated (wild-type) *selB* plasmid pLC1 was only 1%. The presence of the *selB* suppressor mutations that we isolated led to an increase in β-galactosidase activity in the range of 7 to 29% for series p70bm[1–10] and 12 to 40% for series p42bm [1–7] (Table 2).

We separated suppressor plasmids bearing the putative *selB* mutations from the reporter plasmids, p70bm[1–10] from pZL70 and p42bm[1–7] from pZL42 (Materials and Methods). Subsequently, the nature of the mutations in *selB* of each plasmid was characterized by DNA sequencing. We found that p70bm[1], p70bm[2], p70bm[3], p70bm[6], and p70bm[7] each carry a single mutation in *selB*, while p70bm[4], p70bm[5], p70bm[8], and p70bm[10] each carry a double mutation. However, one of the two mutations in the *selB* of p70bm[5] is identical to the single mutation in *selB* of p70bm[7]. In addition, one of the two mutations in p70bm[8] and p70bm[10] causes a change in the amino acid located in the same place as the single mutations in p70bm[2] and p70bm[6], respectively. In all, we isolated six single mutations in *selB* that suppressed bulged nucleotide 17 when it was mutated from U to C. We found that each of these single *selB* mutations was located in a separate specific site of the gene, in the region of nucleotides 1668 to 1747 (Table 2). The corresponding changes in the *selB* amino acids spanned amino acids 556 to 583 (Fig. 3). Thus, we

TABLE 2. *selB* mutations[a]

Plasmid used for screening	Plasmid carrying wt or mutated *selB*[b]	Nucleotide(s) changed in *selB*[c]	Amino acid(s) changed in SelB[d]	Relative β-galactosidase activity[e] (% of control)
pZL70	pLC1	None (wt)	None (wt)	1
	p70bm[1]	GCA→aCA(1747)	Ala→Thr(583)	7
	p70bm[2]	ATG→ATa(1668)	Met→Ile(556)	21
	p70bm[3]	ATC→tTC(1669)	Ile→Phe(557)	17
	p70bm[4]	GAG→GgG(1559)	Glu→Gly(520)	10
		CTC→CcC(1679)	Leu→Pro(560)	
	p70bm[5]	GTA→GaA(1733)	Val→Glu(578)	18
		CTG→CcG(1574)	Leu→Pro(525)	
	p70bm[6]	TGC→cGC(1702)	Cys→Arg(568)	25
	p70bm[7]	GTA→GaA(1733)	Val→Glu(578)	29
	p70bm[8]	GAC→GtC(1820)	Aap→Val(607)	11
		ATG→gTG(1666)	Met→Val(556)	
	p70bm[9]	TTC→TaC(1715)	Phe→Tyr(572)	16
	p70bm[10]	TGC→TtC(1703)	Cys→Phe(568)	16
		GCC→tCC(1249)	Ala→Ser(417)	
pZL42	pLC1	None (wt)	None (wt)	1
	p42bm[1]	ATG→ATa(1668)	Met→Ile(556)	22
	p42bm[2]	GTA→GaA(1733)	Val→Glu(578)	15
		CTG→CcG(1574)	Leu→Pro(525)	
	p42bm[3]	GAT→GAa(1683)	Asp→Glu(561)	17
		GAC→GtC(1532)	Asp→Val(511)	
	p42bm[4]	GTA→GcA(1733)	Val→Ala(578)	40
	p42bm[5]	ATG→tTC(1669)	Ile→Phe(557)	14
	p42bm[6]	TGC→cGC(1702)	Cys→Arg(568)	23
	p42bm[7]	TGC→TtC(1703)	Cys→Phe(568)	12
		GCC→tCC(1249)	Ala→Ser(417)	

[a] Strain *E. coli* WL81300 (a Δ*selB* derivative of MC4100) harboring either pZL70 or pZL42 was freshly transformed by one of each group of plasmids pLC*[1–40]. Lac^+ Amp^r Cm^r transformants were selected, and β-galactosidase activity was determined as described in Materials and Methods. The level of β-galactosidase activity was derived after subtracting the β-galactosidase values of RM1 cells (a Δ*selC* derivative of MC4100) transformed by pRM4, pZL70, or pZL42. The level of β-galactosidase activity in WL81300 cells cotransformed by pRM4 and pLC1 (a pACYC184 derivative carrying the *selB* gene) was designated as 100% activity. wt, wild type.

[b] *E. coli* WL81300 carrying either pZL70 or pZL42 was freshly transformed by plasmids pLC2*[1–40]. Lac^+ Amp^r Cm^r cells carrying plasmids p70bm[1–10] or p42bm [1–7] were selected. Plasmids carrying a single nucleotide change in *selB* are marked in bold letters.

[c] The numbers in brackets represent the position of the changed nucleotide determined by DNA sequencing. Lowercase letters indicate the changed nucleotide.

[d] The numbers in brackets represent the position of the changed amino acid determined according to the DNA sequence.

[e] The β-galactosidase activity directed by the gene fusions in plasmids pZL70 and pZL42 is given relative to that of pRM4. The numbers represent the average of the results of at least three experiments and were derived after subtracting the level of β-galactosidase activity in the Δ*selC* derivative RM1 from that in MC4100.

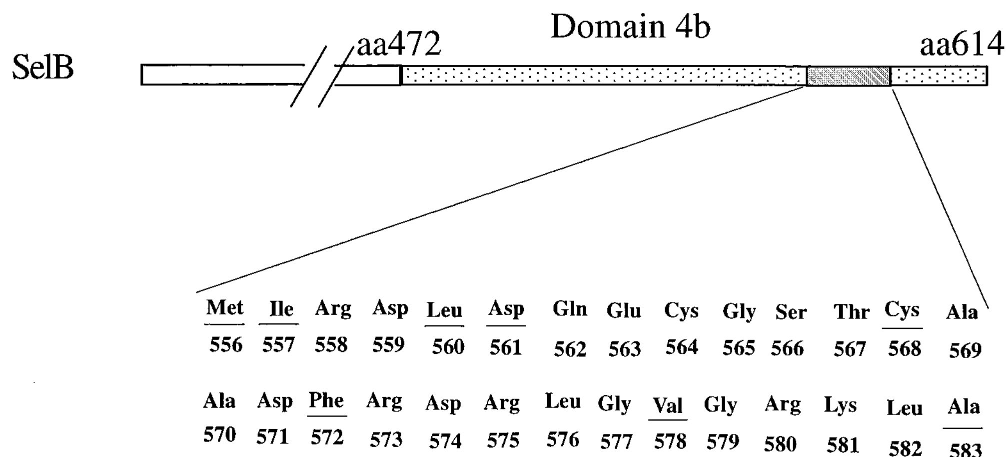

FIG. 3. Schematic representation of the region in SelB in which the mutations are clustered that suppress the mutated U_{17} bulged nucleotide in *E. coli fdhF* SECIS. *selB* DNA corresponding to SelB domains 4a (amino acids [aa] 343 to 474) and 4b (amino acids 472 to 614) (13) (dotted region) was subjected to PCR random mutagenesis. Selection was made for mutations in *selB* that could suppress mutations in the SECIS U_{17} bulged nucleotide; these mutations were subsequently sequenced (Table 2 and Materials and Methods). The selected single mutations are located in 28 amino acids of the 4b region (between 556 and 583 of SelB) (shaded rectangle). Mutated amino acids are underlined.

found that changes in a 28-amino-acid-long region of SelB can suppress the effects of the change from U to C in bulged nucleotide 17. At least one change within this region was found even in the double mutations in *selB* that was obtained in p70bm[5], p70bm[7], and p70bm[10] (Table 2).

When we studied plasmids p42bm[1–7] that carried *selB* mutations that suppressed mutations in the bulged nucleotide from U_{17} to A_{17}, four single mutations in *selB* were isolated (Table 2). It is particularly interesting that these mutations are each identical to one of the single *selB* mutations obtained by selection with pZL70, in which the bulged nucleotide was mutated from U_{17} to C_{17}: p42bm[1] is identical to p70bm[2], p42bm[4] is identical to p70bm[7], p42bm[5] is identical to p70bm[3], and p42bm[6] is identical to p70bm[6]. Thus, selecting with either pZL70 or pZL42 produced mutations located in a region of 28 amino acids of *selB* that can suppress bulged nucleotide U_{17} (Fig. 3).

***selB* mutations selected by the suppression of mutations in bulged nucleotide U_{17} of *E. coli* SECIS also suppress other mutations in the SECIS upper stem-and-loop structure.** Here we asked whether the *selB* mutations that were selected by their ability to suppress the bulged nucleotide U_{17} of SECIS can also suppress other mutations in this *cis* element. To answer this question, we used the following plasmids (Fig. 2): (i) in pL24A the U_{24} in the loop was changed to A_{24}; (ii) in pZL44 the wild-type U_{17} bulged nucleotide was mutated to A_{17} so that it paired with a U_{29} that was changed from A_{29}, and the bulged U_{17} nucleotide was replaced with a bulged U_{18} of the wild type; and (iii) in pZL38 we changed U_{18} to A_{18} so that A_{18} became the bulged nucleotide. The level of suppression of the *selB* mutations (selected with U_{17} mutated bulged nucleotide) of these three mutations in the upper stem-loop structure is shown in Fig. 4. It is noteworthy that mutations in *selB* that were selected to suppress a mutation in U_{17} could also suppress mutations in other locations of the SECIS. The mutation in the SECIS loop (pL24A) caused the highest level of suppression. The mutation in the SECIS that generated the bulged U_{18} (pZL44) caused an intermediate level of suppression, and the mutation in which U_{18} was changed to generate a bulged A_{18} (pZL38) caused the lowest level of suppression. The last level of suppression was similar to the level of suppression caused by the two original mutations, U_{17} to C_{17} (pZL70) and U_{17} to A_{17} (pZL42), which we used to select the original *selB* mutations. The latter case is similar to the level of suppression of a mutation in bulged U_{17} to which the *selB* mutations were originally selected.

DISCUSSION

Among the functions of the special elongation factor SelB is that it binds to the *E. coli* mRNA at the SECIS (9, 23). The results of in vitro experiments have shown that subdomain 4b of the SelB C terminus binds to the upper stem-loop structure of the SECIS (13). Previously, we showed that this minihelix is the same 17-bp upper stem-loop structure that we showed is the minimal requirement for in vivo selenocysteine incorporation into a polypeptide (18). This minimal SECIS has a bulged nucleotide that has been shown to be crucial for selenocysteine incorporation (12, 18).

Here, we used a genetic analysis to examine whether this single bulged U_{17} nucleotide is also involved in the in vivo interaction with SelB. We used PCR-based random mutagenesis to generate point mutations in *selB*. These mutations were characterized for their ability to suppress mutations in the bulged nucleotide U_{17} when it was borne on plasmid pZL42 or pZL70 (Table 1 and Fig. 2). DNA sequencing was used to determine the exact position of the nucleotide of the suppressing mutation in *selB*. We found that the changed amino acids in the *selB* suppressor mutants were clustered in a region corresponding to 28 amino acids in the C-terminal subdomain 4b of the SelB protein, the last of which is located 31 amino acids before the end of the protein (Fig. 3).

Kromayer and colleagues (14) obtained similar results using a different genetic approach. Rather than seeking suppressors of mutations in the bulged nucleotide as we did, they isolated *selB* mutations that suppress defined mutations in the loop of *E. coli* SECIS. They found that most of the *selB* mutations correspond to a region of 23 amino acids included in the region that we have described above. Thus, our results reported here combined with those of Kromayer and colleagues (14) suggest that C-terminal subdomain 4b is involved in the interaction with both the SECIS U_{17} bulged nucleotide and the SECIS upper stem-and-loop structure. We found further confirmation for this suggestion in the results of our experiments that

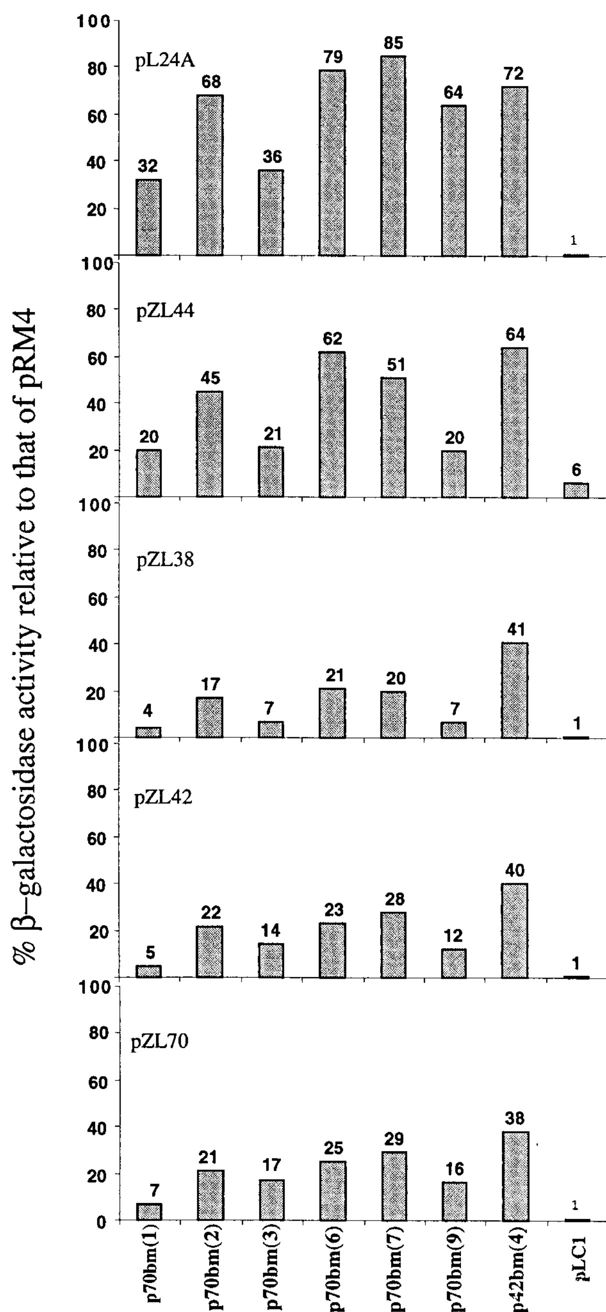

FIG. 4. Selected mutations in *selB* suppress mutations in different positions of the upper stem-loop of *E. coli* SECIS. *E. coli* WL83100 cells were double transformed, first by each of plasmids pL24A, pZL44, pZL38, pZL42, and pZL70 and then by each of plasmids p70bm[1], p70bm[2], p70bm[3], p70bm[6], p70bm [7], p70bm[9], p42bm[4], or the wild-type pLC1. The level of suppression (represented by the number above the columns) was determined by the level of β-galactosidase as described in Table 2, footnote *e*.

showed that the *selB* mutations that were selected by their ability to suppress mutations in the bulged U_{17} were also able to suppress mutations in the loop (pL24A) and even in the stem (pZL38 and pZL44) of the upper minihelix of *E. coli* SECIS (Fig. 4). The mutations with the highest efficiencies of suppression were found in the loop and the intermediate efficiencies in the stem region. The mutation with the lowest level of suppression efficiency was found in the bulged nucleotide either in position 18 or, as in the wild type, in position 17 (Fig. 4). Thus, the *E. coli* SECIS upper stem-loop structure can be regarded as a single suppressible unit, suggesting that there is a flexible interaction between this *cis* mRNA element and SelB.

Our selection procedure allowed us to increase the number of known changes in the amino acids in SelB that can suppress mutations in SECIS (Fig. 3 and Table 2). Both Kromayer and colleagues (14) and our group selected for mutations in the SelB protein in amino acids 556, 568, and 578 (Fig. 3). Furthermore, both groups found similar amino acid changes: Met_{556} to Ile_{556}, Cys_{568} to Arg_{568}, and Val_{578} to Ala_{578}. However, we also found a mutation in which Val_{578} was changed to Glu_{578}. Moreover, in addition to the amino acid changes reported by Kromayer and colleagues (14), we found changes in the 28-amino-acid region at the C terminus of SelB (between amino acids 556 and 583), including Phe_{572} to Tyr_{572}, Ile_{557} to Phe_{557}, and Ala_{583} to Thr_{583} (Table 2). Recall that all of the mutations in *selB* that we have reported here that were able to suppress mutations in *E. coli* SECIS were clustered in the 28-amino-acid region of the 4b subdomain SelB (Fig. 3). Based on in vitro studies (13), the 4b subdomain of SelB has been thought to be the functional domain in the interaction of SelB with the *E. coli* SECIS. However, Kromayer and colleagues (14) have found an additional mutation (Glu_{437} to Lys_{437}) that lies outside 4b, in the 4a subdomain of the SelB protein. We found that single mutations in *selB* suppress mutations in the SECIS loop at higher efficiency than do mutations in the bulged nucleotide position 17 (Fig. 4). It seems possible that the screening test that we used here for *selB* mutations, using the bulged nucleotide in the upper stem-loop structure of *E. coli* SECIS, was more rigid than that used by Kromayer and colleagues (14) to select suppressor mutations in the SECIS loop.

In summary, using a genetic approach, we have increased the repertoire of the amino acids in SelB that are important for a direct interaction between SelB and the 17-nucleotide-long upper stem-loop-structure of the minimal *E. coli* SECIS mRNA. All of these amino acids were clustered in a region of 28 amino acids, the last one of which was 31 amino acids before the C-terminal end of SelB. The direct functional role of each of these amino acids will have to be determined in further experiments by physical means such as X-ray diffraction.

ACKNOWLEDGMENTS

We are deeply grateful to F. R. Warshaw-Dadon (Jerusalem, Israel) for her critical reading of the manuscript.

This research was supported by grants from the United States-Israel Binational Science Foundation (BSF), from the German-Israel Foundation for Scientific Research and Development (GIF), and the Ministry of Science and Culture of the State of Niedersachsen, Germany.

REFERENCES

1. **Baron, C., J. Heider, and A. Böck.** 1993. Interactions of translation factor SelB with the formate dehydrogenase H selenopolypeptide mRNA. Proc. Natl. Acad. Sci. USA **90:**4181–4185.
2. **Baron, C., and A. Böck.** 1995. *In* D. Söll and U. RhajBhandary (ed.), tRNA: structure, biosynthesis and function, p. 529–544. ASM Press, Washington, D.C.
3. **Berg, B. L., J. Li, J. Heider, and V. Stewart.** 1991. Nitrate-inducible formate dehydrogenase in *Escherichia coli* K-12. I. Nucleotide sequence of the *fdnGHI* operon and evidence that opal (UGA) encodes selenocysteine. J. Biol. Chem. **266:**22380–22385.
4. **Berry, M. J., and P. R. Larsen.** 1993. Recognition of UGA as a selenocysteine codon in eukaryotes: a review of recent progress. Biochem. Soc. Trans. **21:**827–832.
5. **Böck, A., K. Fochhammer, J. Heider, W. Leinfelder, G. Sawers, B. Veprek, and F. Zinoni.** 1991. Selenocysteine: the 21st amino acid. Mol. Microbiol. **5:**515–520.
6. **Casadaban, M. J., and S. N. Cohen.** 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.
7. **Chambers, I., and P. R. Harrison.** 1987. A new puzzle in selenoprotein biosynthesis: selenocysteine seems to be encoded by the 'stop' codon, UGA. Trends Biochem. Sci. **12:**255–256.
8. **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.
9. **Heider, J., C. Baron, and A. Böck.** 1992. Coding from a distance: dissertion of the mRNA determinants required for the incorporation of selenocysteine into protein. EMBO J. **11:**3759–3766.
10. **Hüttenhofer, A., E. Westhof, and A. Böck.** 1996. Solution structure of mRNA hairpins promoting selenocysteine incorporation in *Escherichia coli* and their base-specific interactions with special elongation factor SelB. RNA **2:**354–366.
11. **Hüttenhofer, A., J. Heider, and A. Böck.** 1996. Interaction of the *Escherichia coli fdhF* mRNA hairpin promoting selenocysteine incorporation with the ribosome. Nucleic Acids Res. **24:**3903–3910.
12. **Klug, S. J., A. Huttenhofer, M. Kromayer, and M. Famulok.** 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.
13. **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.
14. **Kromayer, M., B. Neuhierl, A. Friebel, and A. Böck.** 1999. Genetic probing of the interaction between the translation factor SelB and its mRNA binding element in *Escherichia coli*. Mol. Gen. Genet. **262:**800–806.
15. **Lee, B. J., P. J. Worland, J. N. Davis, T. C. Stadtman, and D. L. Hatfield.** 1989. Identification of a selenocysteyl-tRNA (Ser) in mammalian cells that recognizes the nonsense codon, UGA. J. Biol. Chem. **264:**9724–9727.
16. **Leinfelder, W., E. Zehelein, M. A. Mandrand-Berthelot, and A. Böck.** 1988. Gene for a novel tRNA species that accepts L-serine and cotranslationally inserts selenocysteine. Nature **331:**723–725.
17. **Lin-Goerke, J. L., D. J. Robbins, and J. D. Burczak.** 1997. PCR-based random mutagenesis using manganese and reduced dNTP concentration. Biotechniques **23:**409–412.
18. **Liu, Z., M. Reches, I. Groisman, and H. Engelberg-Kulka.** 1998. The nature of the minimal "selenocysteine insertion sequence" (SECIS) in *Escherichia coli*. Nucleic Acids Res. **26:**896–902.
19. **Liu, Z., M. Reches, and H. Engelberg-Kulka.** 1999. A sequence in the *Escherichia coli fdhF* "selenocysteine insertion sequence" (SECIS) operates in the absence of selenium. J. Mol. Biol. **294:**1073–1086.
20. **Low, S. C., and M. J. Berry.** 1996. Knowing when not to stop: selenocysteine incorporation in eukaryotes. Trends Biochem. Sci. **21:**203–208.
21. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
22. **Reches, M., C. Zhao, and H. Engelberg-Kulka.** 1994. A bio-assay based on recombinant DNA technology for determining selenium concentration. J. Appl. Environ. Microbiol. **60:**45–50.
23. **Ringquist, S., D. Schneider, T. Gibson, C. Baron, A. Böck, and L. Gold.** 1994. Recognition of the mRNA selenocysteine insertion sequence by the specialized translational elongation factor SELB. Genes Dev. **8:**376–85.
24. **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.
25. **Stadtman, T. C.** 1996. Selenocysteine. Annu. Rev. Biochem. **65:**83–100.
26. **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:**1253–9.
27. **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.