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Expression and Operon Structure of the *sel* Genes of *Escherichia coli* and Identification of a Third Selenium-Containing Formate Dehydrogenase Isoenzyme

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A detailed analysis of the expression of the *sel* genes, the products of which are necessary for the specific incorporation of selenium into macromolecules in *Escherichia coli*, showed that transcription was constitutive, being influenced neither by aerobiosis or anaerobiosis nor by the intracellular selenium concentration. The gene encoding the tRNA molecule which is specifically aminoacylated with selenocysteine (*selC*) proved to be monocistronic. In contrast, the other three *sel* genes (*selA*, *-B*, and *-D*) were shown to be constituents of two unlinked operons. The *selA* and *selB* genes formed one transcriptional unit (*selAB*), while *selD* was shown to be the central gene in an operon including two other genes, the promoter distal of which (*topB*) encodes topoisomerase III. The promoter proximal gene (*orf183*) was sequenced and shown to encode a protein consisting of 183 amino acids (M_r, 20,059), the amino acid sequence of which revealed no similarity to any currently known protein. The products of the *orf183* and *topB* genes were required neither for selenoprotein biosynthesis nor for selenation of tRNAs. *selAB* transcription was driven by a single, weak promoter; however, two major *selD* operon transcripts were identified. The longer initiated just upstream of the *orf183* gene, whereas the 5′ end of the other mapped in a 116-bp nontranslated region between *orf183* and *selD*. Aerobic synthesis of all four *sel* gene products incited a reexamination of a weak 110-kDa selenopolypeptide which is produced under these conditions. The aerobic appearance of this 110-kDa selenopolypeptide was not a consequence of residual expression of the gene encoding the 110-kDa selenopolypeptide of the anaerobically inducible formate dehydrogenase N (FDH_N) enzyme, as previously surmised, but rather resulted from the expression of a gene encoding a third, distinct selenopolypeptide in *E. coli*. A mutant strain no longer capable of synthesizing the 80- and 110-kDa selenopolypeptides of FDH_H and FDH_N, respectively, still synthesized this alternative 110-kDa selenopolypeptide which was present at equivalent levels in cells grown aerobically and anaerobically with nitrate. Furthermore, this strain exhibited a formate- and *sel* gene-dependent respiratory activity, indicating that it is probable that this selenopolypeptide constitutes a major component of the formate oxidase, an enzyme activity initially discovered in aerobically grown *E. coli* more than 30 years ago.

The products of four genes have been identified as being essential for the incorporation of selenium into macromolecules in *Escherichia coli* (8, 44). The *selD* gene encodes a protein which is required for incorporation of selenium into both protein and modified tRNAs (28), whereas the products of the other three genes are required for the synthesis of selenopolypeptides (29). The *selC* gene product is a tRNA (30) which is aminoacylated with serine, and this serine residue is converted to selenocysteine on the tRNA through the joint action of SelD (28) and selenocysteine synthase, the *selA* gene product (17, 19). The product of the *selB* gene encodes a special translation factor which specifically binds $tRNA^{SEC}_{UCA}$ and delivers it to the translating ribosome (18).

In marked contrast to the large amount of information gathered over the last few years concerning the biochemistry of this system, relatively little is known about the transcriptional regulation of the *sel* genes. Since the selenated tRNA molecules, which contain the modified nucleoside 5-methylaminomethyl-2-selenouridine, are synthesized constitutively, it can be assumed that the transcription and consequent synthesis of the SelD protein must be constitutive in order to accommodate the incorporation of selenium into these modified tRNAs. On the other hand, the 80- and 110-kDa selenopolypeptides of formate dehydrogenase H (FDH_H) and FDH_N, respectively, are only synthesized in the anaerobic cell; therefore, the question arises as to whether the *selA*, *selB*, and *selC* genes are also expressed only anaerobically or whether their expression is independent of culture conditions. Immunological data indicate that the SelB protein is present in both aerobic and anaerobic cells (20), strongly suggesting that the *selB* gene is also expressed constitutively.

The four *sel* genes are not clustered on the chromosome but are dispersed over three distinct loci: *selA* and *selB* are at 80 min, *selC* maps to 82 min, and *selD* maps to the 38-min region of the chromosome (29). It is likely, on the basis of sequence data, that the *selA* and *selB* genes form an operon (18, 19). The primary aim of the work presented here was to expand our knowledge of the transcription of the *sel* genes and to clarify their expression and operon structures. These studies have brought several surprising new findings to light, including the discovery that the *selD* gene forms an operon with two other genes, one of which encodes topoisomerase III and the other of which encodes a previously uncharacterized protein. Moreover, data which demonstrate conclusively that *E. coli* possesses a third selenopolypeptide which is synthesized both aerobically and anaerobically in the presence of nitrate and whose presence shows a strong correlation with the formate oxidase activity previously identified in the early 1950s by Pinsent (35) are presented.

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TABLE 1. Strains and plasmids used in this study

Strain or plasmid	Genotype	Reference or source
Strains		
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 ptsF25 deoC1 relA1 flbB530 rpsL150*λ⁻	10
DH1	F⁻ *thi-1 hsdR17 recA1 endA1 relA1 supE44*	21
JM109	*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* F′(*lac-proAB*)	47
K38	HfrC *spoT1 thi-1 phoA4 pit-10 tonA22 ompF627 relA1* λ⁺	31
FM460	MC4100 Δ(*selC*)*400*::Kan	W. Leinfelder
FM932	MC4100 Δ*fdhF mel*::Tn*10*	3
FM934	MC4100 Δ*fdhF rpsL*⁺ *rpsE13* Δ(*srl-recA*)*306*::Tn*10* F′*lacI*ᵠ*Z*::Tn*9 proAB*⁺	F. Zinoni
RH543	Δ(*xthA-pncA*)90 *zdh-201*::Tn*10 relA1*	R. Helling
MN543	MC4100 Δ(*xthA-pncA*)*90 zdh-201*::Tn*10*	This work
WL24	MC4100 *fdh-24*::Mu*cts*d1(Apʳ *lac*)	29
WL9024	MC4100 Δ*fdn* Δ*fdhF mel*::Tn*10*	This work
WL30153	MC4100 Δ(*selAB*)*200*	29
Plasmids		
pACYC184	Cmʳ Tcʳ	11
pUC19	Apʳ *lac′ IPOZ′*	47
pMN302	Cmʳ *selD*⁺	28
pMN81	Apʳ *selC*⁺	30
pMN3021	Cmʳ *orf183*⁺ *selD′*	This work
pMN3022	Cmʳ *selD′ topB*⁺	This work
pGP1-2	Kmʳ T7 gene *1* (RNA polymerase)	45
pT7-5	Apʳ T7 ϕ10	45
pTN304	Apʳ *orf183*⁺	This work
pUC502	Apʳ *orf183*⁺	This work
pWL1	Apʳ *selA*⁺ *selB*⁺	29
pWL107	Cmʳ *selA*⁺ *selB*⁺	29
pWL108	Cmʳ *selA*⁺ *selB*⁺	This work

MATERIALS AND METHODS

Strains, plasmids, media, and growth conditions. The bacterial strains and plasmids used in this study are listed in Table 1. Cells were grown either in LB medium or in the buffered rich medium (pH 6.5) described by Begg et al. (4). In both cases the medium was supplemented with 1 μM sodium selenite and 1 μM sodium molybdate unless indicated otherwise. Media were supplemented with 0.4% (wt/vol) glucose and 1% (wt/vol) nitrate as indicated. The minimal medium used for determining the effects of selenium on *sel* gene transcription consisted of 1 mM $MgCl_2$, 15 mM NH_4Cl, 0.1 mM $CaCl_2$, 200 μM Na_2SO_4, 100 mM NaK phosphate buffer (pH 6.5), and 0.8% (vol/vol) glycerol. Special precautions were taken to keep the glassware selenium free; this involved using new 100-ml conical flasks which had been treated for 10 h with concentrated HCl and then thoroughly washed with double-distilled water and autoclaved twice, each time with a fresh change of medium. When required, antibiotics were added to the following final concentrations: ampicillin, 50 μg/ml; chloramphenicol, 30 μg/ml for maintenance of plasmids and 15 μg/ml when the *cat* gene was integrated into the chromosome; kanamycin, 50 μg/ml; and rifampin, 200 μg/ml. The anaerobic growth technique of Balch and Wolfe (2) was used throughout.

Genetic procedures and recombinant DNA techniques. Standard procedures were used for plasmid preparations, restriction enzyme digestion, ligations, transformation, gel electrophoresis, oligonucleotide labelling, and nick translation (32). Transductions with phage P1 *kc* were carried out by the method of Miller (33). Cotransduction of the Δ(*xthA-pncA*) mutation with *zdh*::Tn*10* was screened for by using the benzyl-viologen overlay technique as previously described (4). Cotransduction frequencies were approximately 40%. The *lacZ* gene constructs on pFM320 and pSKS106 (40) were transferred to λRZ5 (M. Berman) by standard procedures (42).

Plasmid constructions. Plasmid pWL108 was constructed by isolating the 4.6-kb *Bam*HI-*Sal*I DNA fragment from cosmid clone pWL1 (29), which contains the *selAB* genes, and performing a three-way ligation with the 3.9-kb *Bam*HI-*Sal*I fragment of vector pACYC184 and the 5.85-kb *Sal*I fragment from pWL107 (29). The resulting construct was checked by restriction analysis and by performing a Southern hybridization with *E. coli* chromosomal DNA as a reference. The *selD* gene contains a single internal *Sal*I restriction site. Restriction of *E. coli* chromosomal DNA with *Sal*I followed by Southern hybridization with the complete *selD* gene as a probe revealed that the promoter proximal portion of the *selD* gene lies on a 3.4-kb chromosomal *Sal*I restriction fragment and the downstream portion lies on an 8-kb *Sal*I restriction fragment. Both of these DNA fragments were cloned into *Sal*I-digested pACYC184 to yield plasmids pMN3021 (3.4-kb insert) and pMN3022 (8.0-kb insert). pUC502 was constructed by cloning the 1.75-kb *Eco*RI fragment, made blunt ended with the Klenow fragment of DNA polymerase, into pUC19 which had been restricted with *Bam*HI and filled in with the Klenow enzyme. pTN304 was derived by cloning the same *Eco*RI fragment into the *Eco*RI site of pT7-5 (45).

DNA sequencing. Sequencing of double-stranded DNA was performed by using the method of Chen and Seeburg (12) and the chain termination method of Sanger et al. (36) with T7 DNA polymerase (46). The 1.75-kb DNA insert of pUC502 was completely sequenced on both DNA strands after successively shortened subclones were generated with exonuclease III (24). DNA sequence analysis was performed

on an IBM AT computer with PC-gene software (version 6) obtained from Genofit SA (Geneva, Switzerland).

RNA preparation and Northern (RNA) analysis of the *selC* gene transcript. Total cellular RNA was prepared by the hot-phenol method of Aiba et al. (1). RNA samples (20 μg) were separated in a 1.5% (wt/vol) agarose gel containing formaldehyde and transferred to GeneScreen membranes exactly as described by the manufacturer (New England Nuclear Research Products, Boston, Mass.). Prehybridization and hybridization of the membrane filter were also performed according to the recommendations of the supplier.

S1 nuclease protection analysis of *selAB* and *selD* transcripts. S1 protection of transcripts was performed essentially as described by Berk and Sharp (6) but with minor modifications. DNA fragments (~0.5 pmol) were hybridized with total RNA (60 μg) as previously described (38); the DNA-DNA hybridization temperature was calculated on the basis of the GC content of the fragment, and DNA-RNA hybridization was carried out at a temperature 2°C higher than calculated. The DNA-RNA hybrids were precipitated in ethanol (subsequent to treatment with S1 nuclease), separated in 0.8% (wt/vol) agarose gels, and transferred to nitrocellulose filters. The migration positions of the hybrids were determined by Southern hybridization with nick-translated probes from the respective genes (see figure legends).

Primer extension of *sel* operon transcripts. Primer extension was performed exactly as previously described (37). Because of the low-level transcription of the *sel* genes, 150 μg of RNA was used for determination of the initiation site of the *selAB* operon and 50 μg of RNA was used for the *selD* operon. The following oligonucleotide primers were used for primer extension and sequence reactions: 5'-GCAACAAT TCCACCACGCGGG-3' for *selAB*, 5'-CTGCTCACTATGC AGGATGGTTTCC-3' for *selD*, and 5'-GGCGCACGCATA CCCGCACGCAGG-3' for *orf183*.

Gene expression in the phage T7 promoter-polymerase system and selenium labelling. Expression of the *orf183* gene in the phage T7 promoter-polymerase system was performed in *E. coli* K38(pGP1-2) as previously described (45). The products were separated in a sodium dodecyl sulfate (SDS)–12.5% polyacrylamide gel (27).

Incorporation of ^{75}Se into macromolecules and analysis of the products were carried out as described by Leinfelder et al. (29).

Enzyme assays. β-Galactosidase activity was assayed in cultures of exponentially growing cells, and the specific activity was calculated as described by Miller (33). Formate oxidase enzyme activity in cells which had been grown aerobically at 37°C in medium containing 1% (wt/vol) tryptone, 0.5% (wt/vol) glycerol, 0.1 M KNa phosphate (pH 6.8), 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 10 μM Na_2MoO_4, 1 μM Na_2SeO_3, and further trace elements as given by Neidhardt et al. (34) was determined. Cultures which had reached an optical density at 420 nm of 0.6 to 0.7 were chilled on ice and washed once with cold 0.9% (wt/vol) NaCl and suspended in 0.9% (wt/vol) NaCl at 27.5 mg (wet weight) of cells ml^{-1}. Respiration was assayed at 28°C as oxygen consumption in a Warburg apparatus (Braun, Melsungen, Germany), which was filled with 1 ml of cell suspension, 0.5 ml of 0.5 M KNa phosphate buffer (pH 6.0), 0.2 ml of 20% KOH (center well), and 0.2 ml of 0.5 M sodium formate or 0.2 ml of 0.083 M glucose (side bulb), as described by Pinsent (35). The respiratory quotient for formate was determined by measuring the amount of CO_2 produced in parallel assays with and without KOH, corrected for the amount of oxygen consumed.

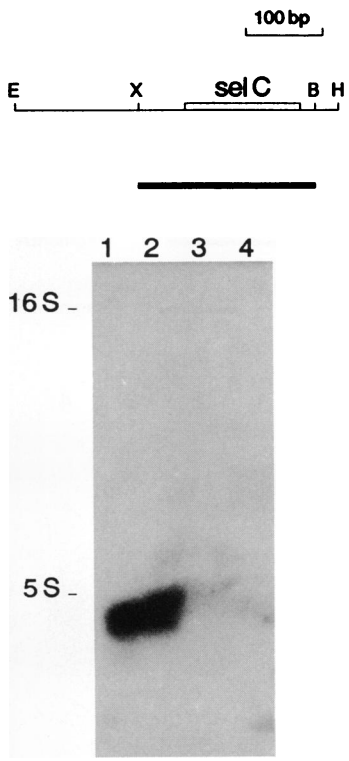

FIG. 1. Northern hybridization of the *selC* gene transcript. The upper portion of the figure shows a schematic representation of pMN81; the raised box represents the *selC* gene. The thick black line indicates the 233-bp *Xho*II-*Bam*HI DNA fragment used as a hybridization probe. Total RNA was isolated from MC4100 grown aerobically (lane 1) and anaerobically (lane 2) and from FM460 (Δ*selC*) grown aerobically (lane 3) and anaerobically (lane 4). The migration positions of 16S and 5S rRNAs are indicated. Abbreviations: B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; X, *Xho*II.

Materials. Sodium [^{75}Se]selenite, L-[^{35}S]methionine, [α-^{32}P]dATP, and [γ-^{32}P]dATP were purchased as aqueous solutions from Amersham-Buchler, Braunschweig, Germany, or New England Nuclear/DuPont, Dreieich, Germany. Enzymes used for recombinant DNA procedures were from Boehringer Mannheim or from Pharmacia, Freiburg, Germany. Isopropyl-β-D-thiogalactopyranoside was from Roth GmbH, Karlsruhe, Germany.

Nucleotide sequence accession number. The sequence data presented in this paper can be obtained from the EMBL data library under accession number M68961.

RESULTS

Transcription of the *sel* genes is independent of both cellular oxygen and selenium status. Transcription of the four *sel* genes, *selA*, *-B*, *-C*, and *-D*, was analyzed with cells grown both aerobically and anaerobically to examine the effects of the oxygen status on transcription and to determine whether the genes are cotranscribed with neighboring genes. A Northern blotting experiment clearly showed that the *selC* gene is transcribed at equivalent levels in both aerobically and anaerobically grown cells (Fig. 1). The radioactive DNA probe from the *selC* gene hybridized specifically with the *selC* gene transcript, as demonstrated by the absence of any hybridization signal with RNA isolated from a *selC* deletion strain (Fig. 1, lanes 3 and 4). No other transcripts which

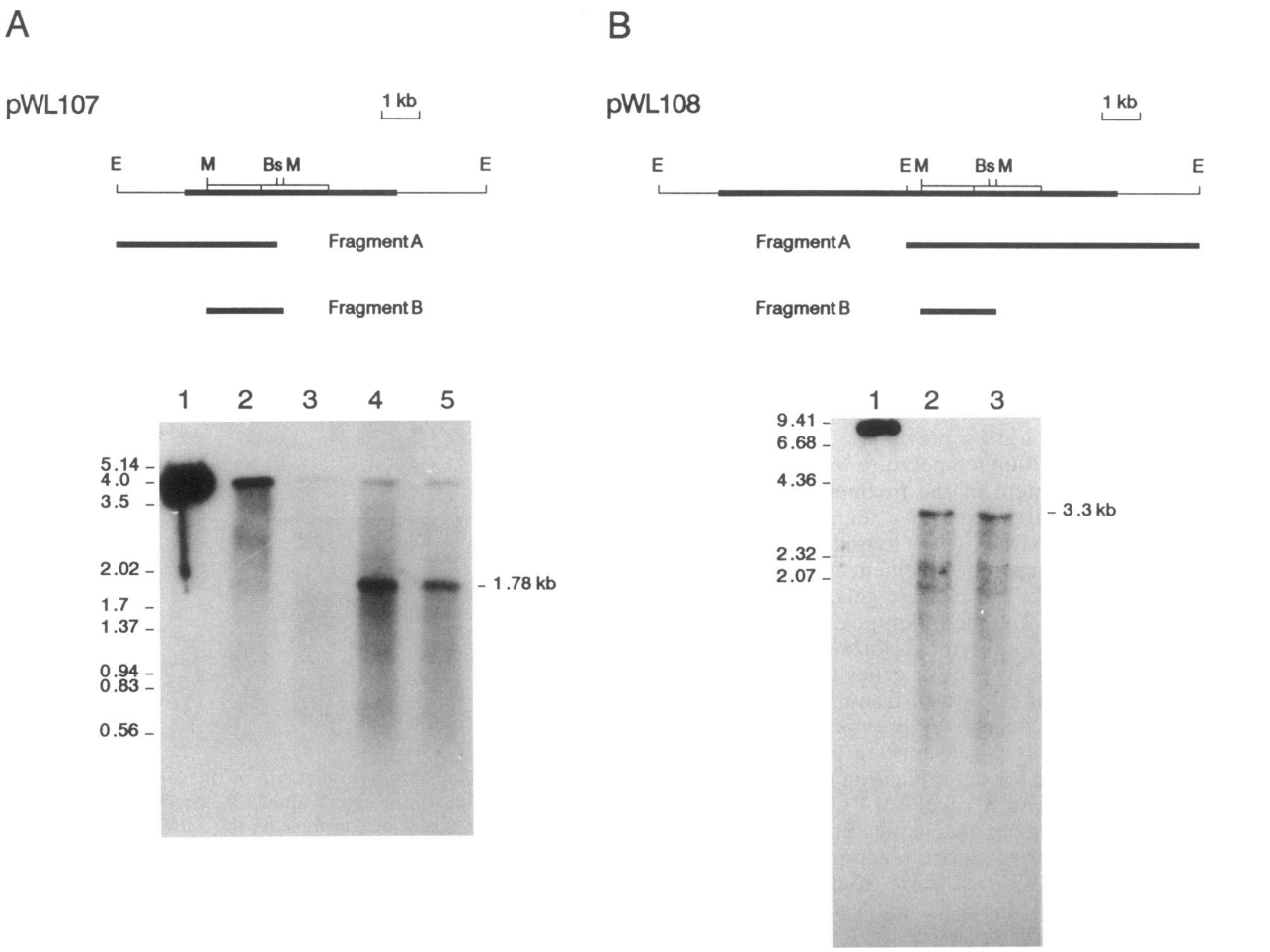

FIG. 2. S1 nuclease transcript mapping of the *selAB* operon. (A) *selA* and *selB* genes on pWL107 (29) and the DNA-RNA hybrid products generated after hybridization and S1 nuclease treatment of fragment A (4.2-kb *Eco*RI-*Bst*EII DNA fragment; lane 1) with total RNA isolated from WL30153 (Δ*selAB*) grown aerobically (lane 2) and anaerobically (lane 3) and MC4100 grown aerobically (lane 4) and anaerobically (lane 5). Fragment B was nick translated with [α-^{32}P]dATP and used to identify the hybrids. (B) Results of a similar experiment in which the 8-kb *Eco*RI fragment (fragment A; lane 1) from pWL108 was hybridized with total RNA isolated from MC4100 grown aerobically (lane 2) and anaerobically (lane 3). The same *Mlu*I fragment (fragment B) as that shown in panel A was employed to identify DNA-RNA hybrids. The DNA size markers were generated by restricting lambda DNA with *Eco*RI and *Hin*dIII (A) or with *Hin*dIII (B). Abbreviations: Bs, *Bst*EII; E, *Eco*RI; M, *Mlu*I.

migrated more slowly in the gel were observed. These results are in agreement with the findings of Burkard and Söll (9).

Analysis of the *selA* and *-B* gene transcripts with a hybridization probe covering the complete *selA* gene and including 400 bp of the 5′ portion of the *selB* gene revealed that, like *selC*, both genes are expressed equally well in aerobic and anaerobic cells and that both genes are cotranscribed (Fig. 2A). The size of the major protected DNA fragment corresponds in length with a transcript initiating just before the *selA* gene; no short protected 400-bp fragment, which would correspond to a *selB* transcript, was observed. The signal observed migrating at 4.2 kb in lanes 2, 3, 4, and 5 is the result of rehybridization of the denatured *Eco*RI-*Bst*EII fragment used as a hybridization probe.

That both genes indeed form an operon was verified by using a DNA fragment covering the complete *selAB* region (Fig. 2B). The length of the protected fragment corresponded to a transcript initiating before the *selA* gene and terminating behind *selB*.

A similar experiment to analyze transcription of the *selD* gene was performed. Surprisingly, two protected species could be identified when DNA fragments covering regions both upstream and downstream of the *selD* gene were used for hybridization (Fig. 3). RNA from both aerobic and anaerobic cells protected a 0.77-kb DNA fragment which correlated in size with that of a transcript initiating at the beginning of the *selD* gene and extending minimally to the *Sal*I restriction site within the *selD* gene (left portion of Fig. 3). A further DNA fragment, which was 640 bp longer, was also protected in both RNA preparations, which is indicative of cotranscription of *selD* with a gene(s) lying upstream. Analysis of transcription downstream of *selD* revealed a transcript which extended approximately 2,000 bases beyond the end of the *selD* gene, together with one which extended approximately 150 bases beyond *selD* (right portion of Fig. 3). The longer transcript was more abundant in anaerobic cells, whereas the shorter transcript was more abundant in aerobic cells. These results indicate that the *selD* gene lies within an operon spanning minimally 3.7 kb of

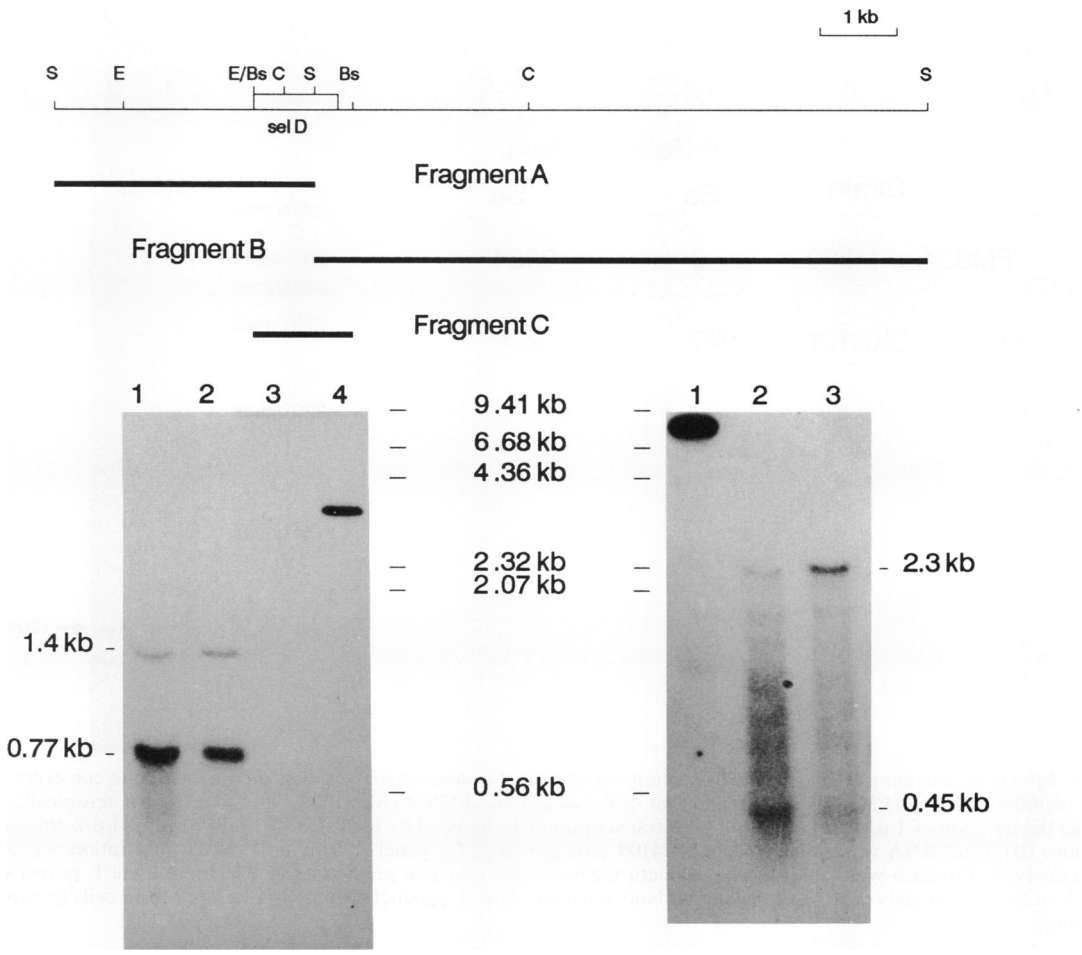

FIG. 3. S1 nuclease transcript mapping of the *selD* gene. The upper part of the figure shows the chromosomal DNA region around the *selD* gene. The 3.4-kb *Sal*I fragment (fragment A) represents the DNA insert in pMN3021, and the 8.0-kb *Sal*I fragment (fragment B) represents that in pMN3022. The autoradiograph on the left shows the DNA-RNA hybrids resulting from hybridization of fragment A (lane 4), which includes the promoter proximal portion of the *selD* gene, with total RNA isolated from MC4100 grown aerobically (lane 1) and anaerobically (lane 2). Lane 3, control in which fragment A was denatured and treated with nuclease S1 in the absence of RNA. The autoradiograph on the right shows the products of hybridization of fragment B (lane 1), including the promoter distal region of the *selD* gene, with total RNA isolated from MC4100 grown aerobically (lane 2) and anaerobically (lane 3). The 1.3-kb *Bst*EII fragment (fragment C) was nick translated with [α-^{32}P]dATP and used to identify the DNA-RNA hybrids. DNA size markers were the same as those described in the legend to Fig. 2. Abbreviations: Bs, *Bst*EII; C, *Cla*I; E, *Eco*RI; S, *Sal*I.

DNA (see below) and that transcription of this operon, the *selAB* operon, and the *selC* gene is not influenced by the oxygen status of the cell.

Another potential factor which could affect transcription of the genes involved in selenium incorporation into macromolecules is selenium availability. This is an important question to address because it has been shown recently that the intracellular concentrations of metal ions such as mercury, iron, and nickel have a major influence on expression of genes encoding biosynthetic and structural components of enzyme systems which respond to, or are dependent upon, these metals (13, 26, 43). Special precautions were taken to ensure that the medium was free of selenium (see Materials and Methods), but to be absolutely sure, a biological control which unequivocally demonstrated that the level of selenium in the medium was below that necessary for translation of UGA_{140} was devised. A 117-bp DNA cartridge containing the internal TGA_{140} triplet from the *fdhF* gene was cloned into the *lacZ* gene (48), such that translation of this mutant *lacZ* gene mRNA is completely dependent upon the presence of selenium in the growth medium. This construct, pFM320 (48), was transferred to λRZ5 and integrated into the chromosome of strain FM934 (Δ*fdhF lacI*^{q}). The results clearly show that the medium was selenium free, since synthesis of β-galactosidase was completely dependent on the addition of 1 μM selenite to the medium (Fig. 4A). Synthesis of β-galactosidase from a similar construct containing the wild-type *lacZ* gene (FM934/λSKS106) was independent of the presence of selenium (Fig. 4A).

RNA isolated from MC4100 grown aerobically in the same medium was used to examine transcription of the *selD* gene. The results show that the intracellular selenium concentration had no influence on *selD* transcription (Fig. 4B). The same result was found for *selAB* and for the transcript initiating 630 bp upstream of the *selD* gene (data not shown).

Determination of the 5′ ends of the *selAB* and *selD* operon transcripts. The exact 5′ ends of the transcripts of the *selAB* and selD operons were determined by primer extension

A

E.coli Strain	ß-Galactosidase - Se	ß-Galactosidase + Se
FM934/λFM320	6	2084
FM934/λSKS106	947	984

B

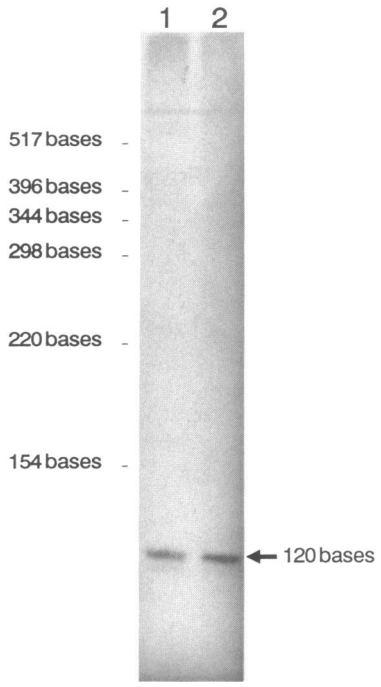

FIG. 4. Effects of selenium on *sel* gene transcription. (A) β-Galactosidase activity produced by a *lacZ* gene construct containing an *fdhF* cartridge including TGA_{140} (FM934/λFM320) and that of the *lacZ* gene (FM934/λSKS106). Cells were grown aerobically without (−Se) and with (+Se) the addition of 1 μM Na_2SeO_3. *lac* gene transcription was induced by inclusion of 1 mM isopropyl-β-D-thiogalactopyranoside in the medium. (B) Total RNA was isolated from MC4100 cells grown as for panel A, and *selD* gene transcription was analyzed by primer extension analysis. Products were analyzed on a denaturing 6% polyacrylamide gel containing 7 M urea. Lane 1, primer extension products from RNA isolated from cells grown aerobically without selenium; lane 2, products from RNA isolated from cells grown aerobically with 1 μM selenium.

analysis. That of the *selC* gene has been determined previously (9). RNA was isolated from aerobically grown cells and was hybridized with radioactively labelled oligonucleotides which were predicted to prime between 100 and 140 bases downstream of the respective initiation sites, on the basis of the results presented in Fig. 2 and 3. Large amounts of RNA had to be used in the experiment to obtain a detectable signal, which indicates that these mRNA species are synthesized at very low levels (Fig. 5). The *selAB* transcript initiated 48 bp upstream of the translational initiation codon of the *selA* gene. The long *selD* transcript had its 5′ end 690 bases upstream of the *selD* translation initiation codon and 24 bases upstream of the initiation codon of a gene which is cotranscribed with the *selD* gene (Fig. 6). Two 5′ ends directly upstream of the *selD* gene which were separated by 4 bp were identified. The exact positions of the 5′ ends of the *selD* operon transcripts are shown in Fig. 6. The −10 and −35 regions upstream of the mapped initiation sites show only poor similarity to the consensus sequences of *E. coli* −10 and −35 promoters, which is consistent with the poor expression of the genes.

***selD* is cotranscribed with two genes, the promoter distal of which encodes topoisomerase III.** A recent detailed mapping analysis of the 38- to 39-min region of the *E. coli* chromosome (23) revealed that the *selD* gene is flanked by the gene encoding topoisomerase III (*topB*) and that encoding signal peptide peptidase (*sppA*). Examination of the DNA sequence directly downstream of the *selD* gene (28) revealed identity with that of *topB* (15); the stop codon of the *selD* gene is separated from the translational initiation codon of *topB* by 4 bp. The *topB* gene is 1,959 bp in length, encoding a protein of 653 amino acids, and is followed by a palindromic sequence characteristic of a rho-independent terminator lying 70 bp downstream of the termination codon of the structural gene (15). The length of the predicted *selD-topB* cotranscript correlates exactly with the length of the transcript observed in Fig. 3.

The *sppA* gene cannot form the other, upstream portion of the *selD* operon, since it is transcribed in the direction opposite to that of the *selD* and *topB* genes (23). Furthermore, the *sppA* gene is 1,854 bp in length and is therefore too long to correspond to the gene which lies upstream of *selD*, which can encode maximally 200 amino acids (Fig. 3). Therefore, in order to identify exactly which gene lies upstream of *selD*, the 1.75-kb *Eco*RI fragment from pMN3021 was cloned into the *Bam*HI site of pUC19 and the sequence of the complete fragment was determined on both strands. The 773 bp of sequence directly upstream of the *selD* gene is shown in Fig. 6. An open reading frame encoding a protein of 183 amino acids (M_r, 20,059) was found to be separated from the *selD* gene by a 116-bp intergenic region (Fig. 6). The gene, which is provisionally termed *orf183*, is predicted to encode a soluble protein which showed no similarity to any protein in the current protein data bases.

The rest of the DNA insert of the *Eco*RI fragment revealed

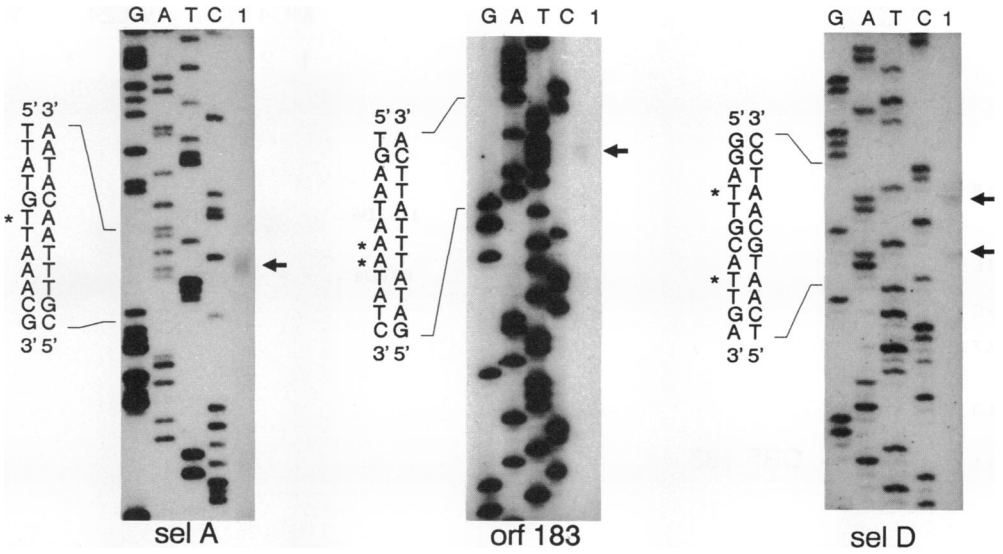

FIG. 5. Primer extension analysis of *sel* gene transcripts. Primer extension reactions were performed on RNA samples isolated from aerobically grown MC4100 cells. The same labelled oligonucleotide used for the primer extension reaction was used to generate the sequence ladders. Products were analyzed on denaturing 8% polyacrylamide gels containing 7 M urea. Asterisks denote the 5′ ends of the transcripts on the DNA sequences. Arrows indicate the positions of the transcripts.

a 160-bp noncoding region upstream of *orf183*, part of which is shown in Fig. 6, and the first 900 bp of the *sppA* gene (25) (data not shown). Transcription of the *sppA* gene was opposite to that of the *orf183-selD-topB* operon, which is in agreement with the findings of Helling (23).

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1    TAATGACCCAACCGGTACTTGTCAGCAGGAATTGCGGACTGTGCTGTAACAAAATCCACT

61   CTCGTGTTAATTTTGTGAATAAATATCACGACAGGAGTTAATCAAATGGATGCACTCGAA
                                                    M  D  A  L  E

121  CTATTGATCAATCGCCGTAGCGCCTCCCGCTTGGCTGAACCCGCGCCAACGGGTGAACAA
     L  L  I  N  R  R  S  A  S  R  L  A  E  P  A  P  T  G  E  Q

181  CTGCAAAACATCCTGCGTGCGGGTATGCGTGCGCCGGACCATAAGTCCATGCAACCGTGG
     L  Q  N  I  L  R  A  G  M  R  A  P  D  H  K  S  M  Q  P  W

241  CATTTTTTTGTGATTGAAGGGGAAGGGCGCGAGCGTTTCAGCGCCGTACTGGAACAGGGG
     H  F  F  V  I  E  G  E  G  R  E  R  F  S  A  V  L  E  Q  G

301  GCGATTGCTGCCGGTAGTGATGACAAAGCTATCGACAAAGCCCGTAATGCGCCGTTCCGC
     A  I  A  A  G  S  D  D  K  A  I  D  K  A  R  N  A  P  F  R

361  GCACCGCTCATCATCACGGTGGTGGCGAAATGCGAAGAGAATCATAAAGTCCCGCGCTGG
     A  P  L  I  I  T  V  V  A  K  C  E  E  N  H  K  V  P  R  W

421  GAACAGGAAATGTCTGCCGGATGCGCGGTCATGGCGATGCAAATGGCAGCAGTTGCCCAG
     E  Q  E  M  S  A  G  C  A  V  M  A  M  Q  M  A  A  V  A  Q

481  GGGTTTGGCGGCATCTGGCGCAGTGGCGCATTAACTGAAAGTCCGGTAGTGCGTGAAGCA
     G  F  G  G  I  W  R  S  G  A  L  T  E  S  P  V  V  R  E  A

541  TTCGGTTGCCGTGAGCAGGATAAAATTGTCGGTTTTCTCTACCTCGGTACGCCGCAGCTG
     F  G  C  R  E  Q  D  K  I  V  G  F  L  Y  L  G  T  P  Q  L

601  AAAGCATCTACGTCGATTAACGTCCCGGACCCGACGCCGTTTGTAACTTATTTCTGATAA
     K  A  S  T  S  I  N  V  P  D  P  T  P  F  V  T  Y  F  -

661  TTGTCGCGAAACTGTCTGGATTGTGAGCAAAGGCGCAGGAATTCAGACACTCTCACTTAT

721  CACTTCACGGAATGAGGGTTACCATACCCGGAATTGCAATTGACAGGAGATGTCCATGAGCG
                                                    selD  M  S
```

FIG. 6. Nucleotide sequence of the *orf183* gene. The nucleotide and deduced amino acid sequences of the first gene in the *selD* operon, *orf183*, are shown together with the first two codons of the *selD* gene lying downstream (28). The underlined sequences signify potential ribosome binding sites (41), and ⊓ indicates the positions of the mapped 5′ ends of the *orf183* and *selD* gene transcripts (Fig. 5).

DiGate and Marians have already demonstrated that the *topB* gene encodes a protein and that it has a potent decatanase activity (14, 15). It was important to demonstrate that the *orf183* gene also encodes a protein. This was done by cloning the 1.7-kb *Eco*RI fragment from pMN3021 into pT7-5 and analyzing incorporation of [^{35}S]methionine into protein. The results clearly show that the *orf183* gene encodes a protein product and that its migration position correlated well with that predicted from the amino acid sequence (Fig. 7).

Neither the *orf183* gene product nor topoisomerase III is required for incorporation of selenium into macromolecules in *E. coli*. Strain MN543 (Δ*xthA-pncA*) (Table 1), which is deleted for the complete *orf183-selD-topB* operon, synthesized both the 80- and the 110-kDa selenopolypeptides, as well as the selenated tRNA species when transformed with a plasmid containing only the *selD* gene (data not shown). Furthermore, a strain specifically deleted for the *orf183* gene, but retaining the *selD* and *topB* genes, was fully competent in selenoprotein and seleno-tRNA synthesis (data not shown). Identification of the physiological functions of Orf183 and topoisomerase III is currently under investigation.

Constitutive expression of the *sel* genes is necessary for synthesis of an aerobic formate oxidase. The findings presented above clearly demonstrate that all the *sel* genes are expressed in aerobic cells. The selenopolypeptides of both FDH_H and FDH_N, however, are synthesized only anaerobically, and this has been recently shown to occur through transcriptional regulation of the structural genes of both enzymes (5, 7). We have often observed a faint 110-kDa selenopolypeptide in aerobically grown cells (48) (Fig. 8, lane 1), which was assumed to be low-level aerobic synthesis of the 110-kDa selenopolypeptide of FDH_N. Recent data from Stewart and Berg (5) rule out this possibility, since the structural genes of FDH_N are not expressed aerobically. This raises the possibility that the aerobic 110-kDa selenopolypeptide is encoded by a gene other than the *fdnG* gene, which encodes the 110-kDa selenopolypeptide of FDH_N (5).

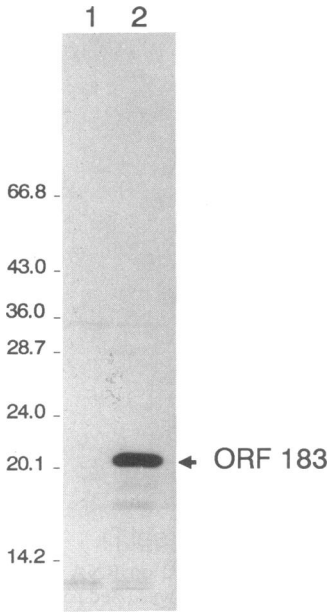

FIG. 7. Synthesis of the *orf183* gene product in a T7 promoter-polymerase system. The products of an in vivo labelling experiment (see Materials and Methods) were separated in an SDS–12.5% polyacrylamide gel. Lane 1, pT7-5; lane 2, pTN304. The migration positions of molecular weight marker proteins are indicated.

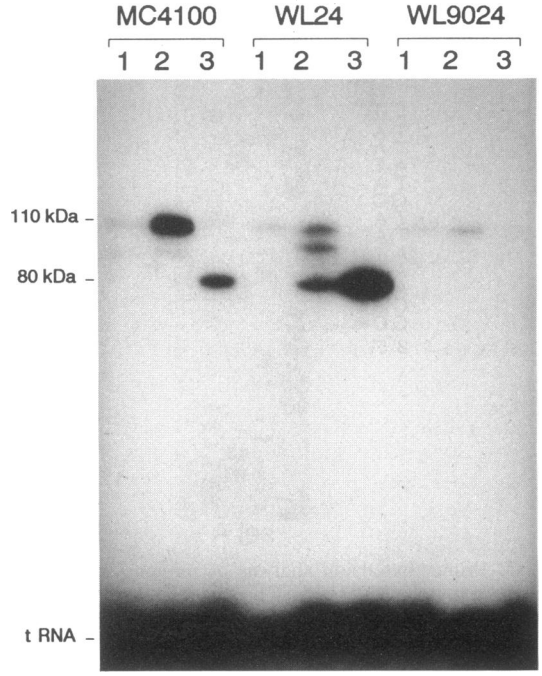

FIG. 8. ^{75}Se incorporation into a 110-kDa polypeptide in aerobically grown *E. coli* cells. Autoradiogram of an SDS–9% polyacrylamide gel in which cells of MC4100, WL24 (*fdn*::Mud1), and WL9024 (Δ*fdhF* Δ*fdn*) were labelled is shown. Cells were grown aerobically (lane 1), anaerobically with glucose plus nitrate (lane 2), and anaerobically with glucose (lane 3). Three times more protein was applied to lanes 2 and 3 of WL24 than those containing the other samples.

Two lines of evidence support this: first, in the 1950s it was observed that aerobically growing *E. coli* cells exhibit a formate oxidase enzyme activity of relatively high specific activity which is unlikely to be catalyzed by FDH_N (35); and second, a mutant strain which we isolated previously (29), WL24, has a Mud1 insertion in the structural gene encoding the 110-kDa selenopolypeptide of FDH_N (5) and produces a truncated selenopolypeptide but still synthesizes a weak 110-kDa selenopolypeptide both aerobically and when grown anaerobically with nitrate (29) (Fig. 8). This indicates that a second gene which encodes a 110-kDa selenopolypeptide must exist. Interestingly, strain WL24 is derepressed for the synthesis of the 80-kDa selenopolypeptide of FDH_H when the strain is grown anaerobically in the presence of nitrate (Fig. 8). This results from an increase in the intracellular formate concentration, and under these circumstances the *fdhF* gene, encoding FDH_H, is no longer repressed by nitrate (35a).

Proof that *E. coli* actually does have a third selenoprotein came from the isolation of a temperature-resistant, ampicillin-sensitive derivative of WL24 which had deleted the Mud1 phage plus neighboring sequences and produced no truncated selenopolypeptide. This *fdn* mutant was transduced to Δ*fdhF* and thus lacked both the 80-kDa selenopolypeptide of FDH_H and the 110-kDa selenopolypeptide of FDH_N. This strain, WL9024, still produced a selenopolypeptide when grown aerobically and when grown anaerobically with nitrate but produced barely detectable amounts of the polypeptide when grown anaerobically with glucose alone (Fig. 8).

Measurement of the formate oxidase activity of aerobically grown cells revealed that when formate was used as a substrate, both the wild-type strain MC4100 and WL9024 (Δ*fdn* Δ*fdhF*) had identical activities (Table 2). The calculated respiratory quotient of 1.72 (CO_2 produced/O_2 consumed) is in close agreement to the value of 2.0 which would be expected for respiration at the expense of formate. Analysis of the activity in strain FM460 (Δ*selC*) showed a 15-fold decrease over that observed in WL9024, which indicates that the *selC* gene is required for synthesis of active enzyme and strongly suggests that the aerobic 110-kDa selenopolypeptide is a component of the formate oxidase activity. The residual activity observed in FM460 with formate as a substrate was the same as MC4100 without substrate and probably represents respiration of endogenous products (Table 2).

TABLE 2. Formate oxidase enzyme activity of aerobically grown *E. coli*

Strain[a]	Substrate[b]	Respiration rate (μl of O_2 consumed min^{-1} mg of $cells^{-1}$)
MC4100 (wild type)	None	0.011
MC4100	Glucose	0.114
MC4100	Formate	0.151
WL9024 (Δ*fdn* Δ*fdhF*)	Formate	0.142
FM460 (Δ*selC*)	Glucose	0.114
FM460	Formate	0.009

[a] Strains were grown and harvested as described in Materials and Methods.
[b] Glucose was added to a final concentration of 8 mM, and formate was added to 50 mM.

DISCUSSION

The synthesis of selenoproteins in *E. coli* necessitates readthrough of a UGA codon. This observation led to considerable speculation which suggested that the UGA suppression mechanism may be controlled not only by codon context but also by the cellular oxygen status (16). Recent reports from our laboratory have demonstrated that UGA readthrough occurs very efficiently in both aerobically and anaerobically grown *E. coli* cells (22, 48), implying that all the *sel* genes products are synthesized under both conditions. In this communication we have shown that the expression of the *sel* genes is constitutive. Moreover, we have discovered that *E. coli* possesses a third selenoprotein which is synthesized with equal efficiency when the cultures are grown both aerobically and anaerobically with nitrate. The synthesis of this newly identified selenoprotein is also dependent upon the *selC* gene product, strongly suggesting that its gene bears an in-frame TGA codon. Several points argue in favor of the weak 110-kDa protein being a component of a third FDH isoenzyme. First, the size of the selenopolypeptide is identical to that of the selenopolypeptide of FDH_N; second, the enzyme with which the selenopolypeptide is associated has a very high catalytic activity with formate as a substrate; and third, synthesis of the polypeptide is *sel* gene dependent. Furthermore, a recent report has indicated that antibodies raised against the FDH_N enzyme of *E. coli* cross-react with a second polypeptide (39). The authors speculate that this protein may be a second FDH_N isoenzyme. It will be of interest to determine whether the third selenopolypeptide and this second FDH_N isoenzyme are one and the same protein.

The reasons why this third selenopolypeptide was not identified in earlier experiments are twofold: first, as mentioned above, the M_r of the polypeptide is 110,000 and therefore is masked by the major selenopolypeptide when cells are grown anaerobically with nitrate; and second, the expression of the gene encoding this polypeptide must be low, since it is synthesized at low levels, and consequently the weak 110-kDa selenopolypeptide previously observed in aerobically grown cells was mistakenly assumed to be the result of low-level expression of the *fdnG* gene encoding the anaerobic 110-kDa selenopolypeptide. It is now clear that *fdnG* gene expression is completely repressed aerobically (5). Furthermore, construction of a mutant deleted for the *fdnG* and *fdhF* genes provided unequivocal proof for the existence of the third selenoprotein. Indeed, this finding also helps to clarify the enigmatic selenoprotein profile of a mutant which we isolated several years ago (29). The mutant produced a weak 110-kDa selenopolypeptide, as well as a truncated selenopolypeptide of approximately 95 kDa (Fig. 8). The truncated selenopolypeptide results from insertion of a Mud1 phage in the *fdnG* structural gene (5).

As mentioned already, a very strong correlation exists between the synthesis of the aerobic selenoprotein and a highly active, $selC^+$-dependent formate oxidase activity which was analyzed in aerobically grown cells. This enzyme activity is likely to be equivalent to that identified previously in aerobically growing cells of *E. coli* (35). Since formate is not normally generated in the aerobic *E. coli* cell, assignment of a physiological function to the enzyme must remain speculative until more studies have been undertaken. If formate indeed proves to be the physiological substrate of the enzyme, then a possible dual function in the aerobic cell is that of detoxification of, and energy generation from, formate which could be transported into the cell from the immediate environment. Examination of the gene sequence should prove to be very interesting, particularly if it exhibits similarity to that of the other 110-kDa selenopolypeptide. This, together with a detailed kinetic study of the purified enzyme, will be required to either verify or refute these proposals.

Significantly, it is now apparent that this third selenopolypeptide, along with the two anaerobically inducible ones, is also widespread among members of the family *Enterobacteriaceae* (21a, 22). Indeed, it is now likely that the initially perplexing observation that *Enterobacter aerogenes*, *Serratia marcescens*, and *Serratia liquefaciens* possess no nitrate-inducible 110-kDa selenoprotein of FDH_N but rather yielded only a weak signal in this region may be an indication that these organisms only have the gene encoding the third selenoprotein (22).

Transcription studies on the *sel* genes demonstrate that *selC*, which encodes a tRNA, is monocistronic, whereas the three *sel* genes which encode proteins are organized in operons (Fig. 9). *selA* and *selB* form one operon, which was anticipated from the fact that the termination codon of the *selA* gene overlaps the translational initiation codon of the *selB* gene (19). These findings are strongly suggestive of tight translational coupling between the two genes and coordinate control of synthesis of the proteins. Immunological studies support this, since it could be estimated that both proteins are present in approximately 1,200 to 2,000 copies in the aerobic cell (8a, 20).

Somewhat more surprising, however, was the discovery that the *selD* gene is also part of an operon. This was all the more surprising when it was realized that one of the other genes encodes topoisomerase III, which was sequenced recently by DiGate and Marians (15). The other gene, which we have provisionally termed *orf183*, encodes a 20-kDa protein which shows no similarity at the amino acid sequence level with any currently known protein. Interestingly, neither gene product is necessary for selenium incorporation into macromolecules, a finding which is also consistent with the fact that the genes have never been identified in a search for further mutant *sel* genes (29; unpublished data).

Topoisomerase III was originally identified as a DNA-relaxing activity in *topA* mutants (14) and was subsequently shown to possess a potent decatanase activity (14). This has led to speculation about the physiological function of the protein being involved in partitioning of chromosomal DNA during cell division (15). It is perhaps noteworthy that the protein is present in as few as 1 to 10 copies per cell (14). Several factors are probably involved in maintaining this low abundance of protein: (i) the ribosome binding site of the gene is very poor, (ii) transcription of the operon is relatively weak, and (iii) it appears that the *topB* mRNA transcript is subject to some form of processing, or rapid turnover, which is influenced by growth conditions. The *selD* portion of the transcript, in contrast, is not affected. It should be emphasized that both the *selD* and *orf183* genes have very good Shine-Dalgarno sequences and consequently are probably translated with high efficiencies.

The promoters of the *selAB* and *selD* operons are rather weak as determined on the basis of the low levels of mRNA which were identified by S1 mapping and primer extension analyses. This is most probably a reflection of the poor sequence similarity to consensus -10 and -35 promoters of *E. coli*. Furthermore, the operon transcripts are very unstable, and consequently the full-length transcripts are difficult to distinguish from the degradation products by Northern

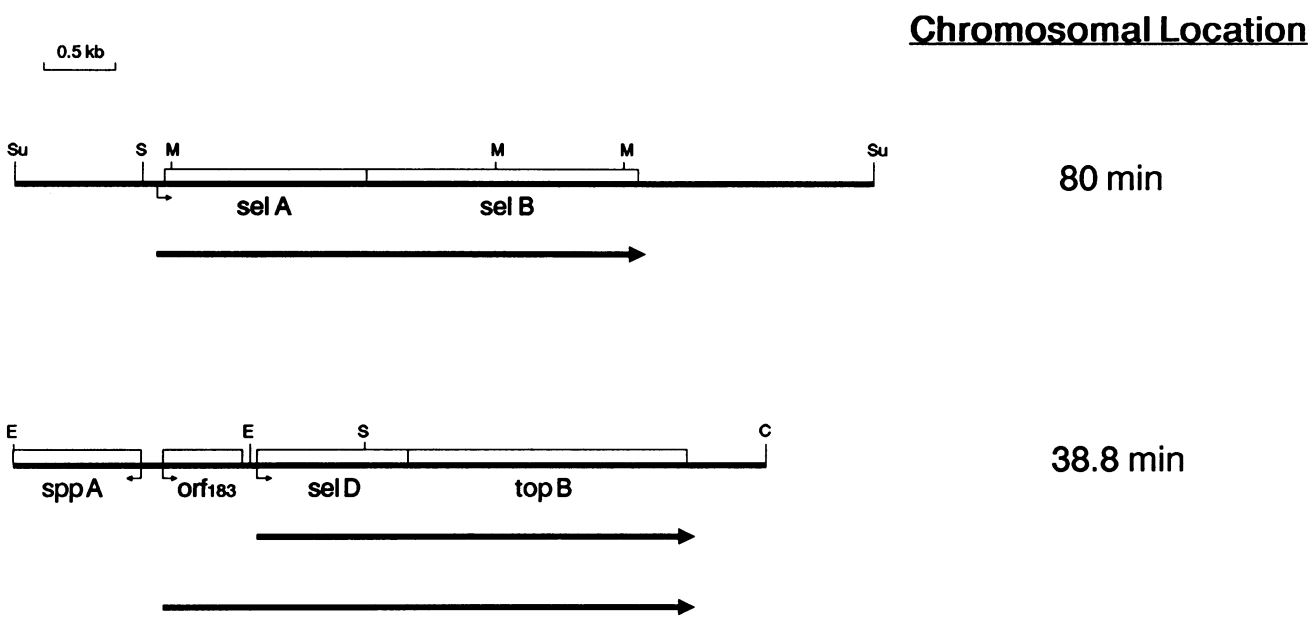

FIG. 9. Summary of *selAB* and *selD* operon transcription. The thick arrows indicate the direction of transcription and length of the respective transcripts. The transcript terminating behind the *selD* gene (Fig. 3) is not included. The positions of potential promoters of the respective genes are signified by ↳.

hybridization analysis (data not shown). These two features of *sel* gene transcription probably obviate the requirement for regulation, for example, in response to intracellular levels of selenium. Both operons have potential rho-independent termination signals in their 3′ nontranslated regions (15; data not shown). The transcription signals of the *selC* gene have been discussed previously (9).

These studies leave many questions unanswered, such as the following. What are the functions of the Orf183 and topoisomerase III proteins? Does SelD have a function secondary to its role in selenium metabolism? Why the necessity for the relatively long (116-bp) intergenic region between the *orf183* and *selD* genes and the requirement that both genes have their own promoters? Perhaps most intriguing of all, what is the physiological function of the third selenoprotein in *E. coli*? The analysis of specific deletion mutants should aid greatly in making headway in answering these questions.

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REFERENCES

1. **Aiba, H., S. Adhya, and B. de Crombrugghe.** 1981. Evidence for two functional *gal* promoters in intact *Escherichia coli* cells. J. Biol. Chem. **256**:11905–11910.
2. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **32**:781–791.
3. **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.
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. **Berg, B. L., and V. Stewart.** 1990. Structural genes for nitrate-inducible formate dehydrogenase in *Escherichia coli* K12. Genetics **125**:691–702.
6. **Berk, A. J., and P. A. Sharp.** 1977. Sizing and mapping of early adenovirus mRNAs by gel electrophoresis of S1 endonuclease-digested hybrids. Cell **12**:721–732.
7. **Birkmann, A., F. Zinoni, G. Sawers, and A. Böck.** 1987. Factors affecting transcriptional regulation of the formate-hydrogenlyase pathway of *Escherichia coli*. Arch. Microbiol. **148**:44–51.
8. **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.

8a. **Boesmiller, K., K. Forchhammer, and A. Böck.** Unpublished data.

9. **Burkard, U., and D. Söll.** 1988. The unusually long amino acid acceptor stem of *Escherichia coli* selenocysteine tRNA results from abnormal cleavage by RNase P. Nucleic Acids Res. **16**:11617–11624.
10. **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.
11. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**: 1141–1156.
12. **Chen, E. Y., and P. W. Seeburg.** 1985. Supercoil sequencing: a fast and simple method for sequencing plasmid DNA. DNA **4**:165–179.
13. **Crosa, J. H.** 1989. Genetic and molecular biology of siderophore-mediated iron transport in bacteria. Microbiol. Rev. **53**:517–530.
14. **DiGate, R. J., and K. J. Marians.** 1988. Identification of a potent decatenating enzyme from *Escherichia coli*. J. Biol. Chem. **263**:13366–13373.

15. **DiGate, R. J., and K. J. Marians.** 1989. Molecular cloning and DNA sequence analysis of *Escherichia coli* topB, the gene encoding topoisomerase III. J. Biol. Chem. **264**:17924–17930.
16. **Engelberg-Kulka, H., and R. Schoulaker-Schwarz.** 1988. A flexible genetic code, or why does selenocysteine have no unique codon? Trends Biochem. Sci. **13**:419–421.
17. **Forchhammer, K., and A. Böck.** 1991. Selenocysteine synthase from *Escherichia coli*: analysis of the reaction sequence. J. Biol. Chem. **266**:6324–6328.
18. **Forchhammer, K., W. Leinfelder, and A. Böck.** 1989. Identification of a novel translation factor necessary for the incorporation of selenocysteine into protein. Nature (London) **342**:453–456.
19. **Forchhammer, K., W. Leinfelder, K. Boesmiller, B. Veprek, and A. Böck.** 1991. Selenocysteine synthase from *Escherichia coli*: nucleotide sequence of the gene (*selA*) and purification of the protein. J. Biol. Chem. **266**:6318–6323.
20. **Forchhammer, K., K.-P. Rücknagel, and A. Böck.** 1990. Purification and biochemical characterisation of SELB, a translation factor involved in selenoprotein synthesis. J. Biol. Chem. **265**:9346–9350.
21. **Hanahan, D.** 1986. Techniques for transformation of *E. coli*, p. 109–136. *In* D. M. Glover (ed.), DNA cloning: a practical approach. IRL Press, Oxford.
21a. **Heider, J., and A. Böck.** Unpublished data.
22. **Heider, J., K. Forchhammer, G. Sawers, and A. Böck.** 1991. Interspecies compatibility of selenoprotein biosynthesis in *Enterobacteriaceae*. Arch. Microbiol. **155**:221–228.
23. **Helling, R. B.** 1990. The glutamate dehydrogenase structural gene of *Escherichia coli*. Mol. Gen. Genet. **223**:508–512.
24. **Henikoff, S.** 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene **28**:351–359.
25. **Ichihara, S., T. Suzuki, M. Suzuki, and S. Mizushima.** 1986. Molecular cloning and sequencing of the *sppA* gene and characterisation of the encoded protease IV, a signal peptide peptidase, of *Escherichia coli*. J. Biol. Chem. **261**:9405–9411.
26. **Kim, H., and R. J. Maier.** 1990. Transcriptional regulation of hydrogenase synthesis by nickel in *Bradyrhizobium japonicum*. J. Biol. Chem. **265**:18729–18732.
27. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
28. **Leinfelder, W., K. Forchhammer, B. Veprek, E. Zehelein, and A. Böck.** 1990. *In vitro* synthesis of selenocysteinyl-$tRNA_{UCA}$ from seryl-$tRNA_{UCA}$: involvement and characterization of the *selD* gene product. Proc. Natl. Acad. Sci. USA **87**:543–547.
29. **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.
30. **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 (London) **331**:723–725.
31. **Lyons, L. B., and N. D. Zinder.** 1972. The genetic map of the filamentous bacteriophage f1. Virology **49**:45–60.
32. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
33. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
34. **Neidhardt, F. C., P. L. Bloch, and D. F. Smith.** 1974. Culture medium for *Enterobacteriaceae*. J. Bacteriol. **119**:736–747.
35. **Pinsent, J.** 1954. The need for selenite and molybdate in the formation of formic dehydrogenase by members of the *Coliaerogenes* group of bacteria. Biochem. J. **57**:10–16.
35a. **Rossman, R., G. Sawers, and A. Böck.** Submitted for publication.
36. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
37. **Sawers, G., and A. Böck.** 1989. Novel transcriptional control of the pyruvate formate-lyase gene: upstream regulatory sequences and multiple promoters regulate anaerobic expression. J. Bacteriol. **171**:2485–2498.
38. **Sawers, G., A. F. V. Wagner, and A. Böck.** 1989. Transcription initiation at multiple promoters of the *pfl* gene by $E\sigma^{70}$-dependent transcription in vitro and heterologous expression in *Pseudomonas putida* in vivo. J. Bacteriol. **171**:4930–4937.
39. **Schlindwein, C., G. Giordano, C.-L. Santini, and M.-A. Mandrand.** 1990. Identification and expression of the *Escherichia coli fdhD* and *fdhE* genes, which are involved in the formation of respiratory formate dehydrogenase. J. Bacteriol. **172**:6112–6121.
40. **Shapira, S. K., J. Chou, F. V. Richardson, 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 β-galactosidase. Gene **25**:71–82.
41. **Shine, J., and L. Dalgarno.** 1974. 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71**:1342–1346.
42. **Silhavy, T. J., M. L. Berman, and L. W. Enquist.** 1984. Experiments with gene fusions. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
43. **Silver, S., and T. K. Misra.** 1988. Plasmid-mediated heavy metal resistances. Annu. Rev. Microbiol. **42**:717–743.
44. **Stadtman, T. C.** 1990. Selenium biochemistry. Annu. Rev. Biochem. **59**:111–127.
45. **Tabor, S., and C. C. Richardson.** 1985. A bacteriophage T7 RNA polymerase/promoter system for controlled exclusive expression of specific genes. Proc. Natl. Acad. Sci. USA **82**:1074–1078.
46. **Tabor, S., and C. C. Richardson.** 1987. DNA sequence analysis with a modified bacteriophage T7 DNA polymerase. Proc. Natl. Acad. Sci. USA **84**:4767–4771.
47. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33**:103–119.
48. **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.