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Escherichia coli Genes Whose Products Are Involved in Selenium Metabolism

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Mutants of *Escherichia coli* were isolated which were affected in the formation of both formate dehydrogenase N (phenazine methosulfate reducing) (FDH_N) and formate dehydrogenase H (benzylviologen reducing) (FDH_H). They were analyzed, together with previously characterized pleiotropic *fdh* mutants (*fdhA*, *fdhB*, and *fdhC*), for their ability to incorporate selenium into the selenopolypeptide subunits of FDH_N and FDH_H. Eight of the isolated strains, along with the *fdhA* and *fdhC* mutants, maintained the ability to selenylate tRNA, but were unable to insert selenocysteine into the two selenopolypeptides. The *fdhB* mutant tested had lost the ability to incorporate selenium into both protein and tRNA. *fdhF*, which is the gene coding for the 80-kilodalton selenopolypeptide of FDH_H, was expressed from the T7 promoter-polymerase system in the pleiotropic *fdh* mutants. A truncated polypeptide of 15 kilodaltons was formed; but no full-length (80-kilodalton) gene product was detected, indicating that translation terminates at the UGA codon directing the insertion of selenocysteine. A mutant *fdhF* gene in which the UGA was changed to UCA expressed the 80-kilodalton gene product exclusively. This strongly supports the notion that the pleiotropic *fdh* mutants analyzed possess a lesion in the gene(s) encoding the biosynthesis or the incorporation of selenocysteine. The gene complementing the defect in one of the isolated mutants was cloned from a cosmid library. Subclones were tested for complementation of other pleiotropic *fdh* mutants. The results revealed that the mutations in the eight isolates fell into two complementation groups, one of them containing the *fdhA* mutation. *fdhB*, *fdhC*, and two of the new *fdh* isolates do not belong to these complementation groups. A new nomenclature (*sel*) is proposed for pleiotropic *fdh* mutations affecting selenium metabolism. Four genes have been identified so far: *selA* and *selB* (at the *fdhA* locus), *selC* (previously *fdhC*), and *selD* (previously *fdhB*).

Several enzymes from procaryotic and eucaryotic organisms and a number of tRNA species contain selenium in a covalently bound form (21). For bacteria, the first indication for a biological role of this trace element dates back to 1954, when Pinsent detected that gas production by anaerobic cultures of *Escherichia coli* depended upon the presence of selenium in the medium (20); enzymological studies revealed that it was the formate dehydrogenase component of the formate-hydrogen-lyase complex which specifically required selenium for activity (14, 20). By using [^{75}Se]selenite, it was subsequently demonstrated that the isotope was incorporated into just two proteins in *E. coli*: an 80-kilodalton polypeptide, which is a constituent component of formate dehydrogenase H (FDH_H) and is involved in gas formation, and a 110-kilodalton polypeptide, which is part of formate dehydrogenase N (FDH_N) and delivers the electrons from formate to nitrate reductase (8, 19). FDH_H is formed under anaerobic conditions in the absence of external electron acceptors, whereas synthesis of FDH_N is anaerobically inducible when nitrate is present in the medium.

The gene (*fdhF*) for the 80-kilodalton selenopolypeptide of FDH_H has been cloned and sequenced, and it was found that it contained an in-frame UGA termination codon at amino acid position 140 (25). Gene fusion experiments and the analysis of mutants in which the UGA codon was converted into sense codons demonstrated that translation of the UGA required the presence of selenium in the medium and that it was the opal nonsense codon which cotranslationally directed the incorporation of selenium in the form of selenocysteine (24, 25).

This unique way of incorporating an unusual amino acid raises two main questions: first, which elements permit differentiation of the UGA_{140} within the *fdhF* gene from a normal UGA termination codon, and second, how is selenocysteine synthesized and incorporated at the ribosome? To answer the latter question we have isolated and analyzed mutants which are deficient in selenium incorporation into both FDH_H and FDH_N. Four different genes were characterized whose products have specific, but as yet unknown, functions involved in selenium incorporation into protein or into both protein and tRNA. On the basis of the results obtained, a new nomenclature is proposed for the pleiotropic formate dehydrogenase mutations affecting selenium metabolism. In accordance with the suggestion for *Salmonella typhimurium* (G. F. Kramer and B. N. Ames, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, K-132, p. 215), the gene designation *sel* is proposed.

MATERIALS AND METHODS

Strains, plasmids, media, and growth conditions. Bacterial strains and plasmids used in this study are listed in Table 1. The rich medium used consisted of 1% (wt/vol) tryptone, 0.5% (wt/vol) yeast extract, 1% (wt/vol) NaCl, 5 μM sodium selenite, and 5 μM sodium molybdate. The minimal medium consisted of 100 mM KH_2PO_4, 50 mM Na_2HPO_4, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$, 12 mM $(NH_4)_2SO_4$, 1 μM Na_2SeO_3, and 1 μM Na_2MoO_4. The pH was adjusted to 7.0 with NaOH. When indicated, amino acids were added to a final concentration of 0.01% (wt/vol). Glucose was added as

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TABLE 1. Strains and plasmids used

Strain or plasmid	Genotype[a]		Reference or source
Strains			
MC4100	F⁻ *araD139* Δ(*argF-lac*)*U169 ptsF25 deoC1 relA1 flbB5301 rpsL150* λ⁻		5
MAL103	F⁻ Δ(*proAB-lac*)*X111 rpsL* Mu cts Mu cts dl(Ap^r *lac*)		5
EMG29	F⁻ *proC23 trp-30 his-51 lac-28 rpsL101* λ⁺		10
GY01	EMG29 *fdhA*		10
MB08	EMG29 *fdhB*		10
MB07	EMG29 *fdhC*		11
K38	HfrC *phoA4 pit-10 tonA22 ompF627 relA1 spoT1* λ⁺		15
AB1157	F⁻ *thi-1 thr-1 leuB6 proA2 his-4 argE3 ara-14 lacY1 galK2 xyl-5 mtl-1 tsx-33 rpsL31 supE44* λ⁻		1
WA321	AB1157 *dam-4*		9
JC10289	AB1157 Δ(*srl-recA*)*306*::Tn*10*		13
WL2	MC4100 *selA2*::Mu cts dl(Ap^r *lac*)		This work
WL5	MC4100 *selA5*::Mu cts dl(Ap^r *lac*)		This work
WL6	MC4100 *selA6*::Mu cts dl(Ap^r *lac*)		This work
WL14	MC4100 *selA14*::Mu cts dl(Ap^r *lac*)	class 1	This work
WL1	MC4100 *selB1*::Mu cts dl(Ap^r *lac*)		This work
WL40	MC4100 *selB40*::Mu cts dl(Ap^r *lac*)		This work
WL98	MC4100 *selB98*::Mu cts dl(Ap^r *lac*)		This work
WL153	MC4100 *selB153*::Mu cts dl(Ap^r *lac*)		This work
WL24	MC4100 *fdh-24*::Mu cts dl(Ap^r *lac*),	class 2	This work
WL25	MC4100 *fdh-25*::Mu cts dl(Ap^r *lac*)		This work
WL131	MC4100 *fdh-131*::Mu cts dl(Ap^r *lac*)	class 3	This work
WL8	MC4100 *fdh-8*::Mu cts dl(Ap^r *lac*)		This work
WL22	MC4100 *fdh-22*::Mu cts dl(Ap^r *lac*)		This work
WL10	MC4100 *fdh-10*::Mu cts dl(Ap^r *lac*)	class 4	This work
WL20	MC4100 *fdh-20*::Mu cts dl(Ap^r *lac*)		This work
WL50153	MC4100 *selB153*::Mu dl ts⁺		WL153 → MC4100; ts⁺ Ap^r
WL30153	MC4100 Δ(*selAB*)*200*		WL153: ts⁺ Ap^s selection
WL31153	MC4100 Δ(*selAB*)*200* Δ(*srl-recA*)*306*::Tn*10*		JC10289 → WL30153; Δ *recA*
Plasmids			
pHC79	Ap^r Tc^r		12
pACYC184	Cm^r Tc^r		6
pKY102	Ap^r *recA*⁺		13
pGP1-2	Km^r T7 gene *1* (RNA polymerase)		22, 23
pT7-6	Ap^r T7 φ10		22, 23

[a] *fdh* denotes an as yet uncharacterized mutation affecting FDH_H and/or FDH_N activity.

carbon source at 0.4% (wt/vol) to the rich medium and to 0.8% (wt/vol) to the minimal medium. When required, antibiotics were added to the following final concentrations: ampicillin, 30 μg/ml for growth of strains harboring an integrated Mu cts dl and 50 μg/ml for selection of plasmids containing a *bla* gene; tetracycline, 12.5 μg/ml; chloramphenicol, 30 μg/ml; kanamycin sulfate, 50 μg/ml, and rifampin, 200 μg/ml. The anaerobic growth technique of Balch and Wolfe (2) was used throughout.

Mutant isolation and screening. Strain MC4100 was mutagenized by infection with phage Mu dl(Ap^r *lac*) (5) at a multiplicity of 1:10. Ampicillin-resistant clones were selected by anaerobic incubation on MacConkey nitrate plates (3) for 48 h at 30°C. Red and dark-red colonies of medium size (3) were picked and purified twice by single-colony isolation on the same plates. They were tested for anaerobic growth on minimal medium plates containing 0.8% (wt/vol) glycerol as carbon source and 1% (wt/vol) potassium nitrate as electron acceptor (4 days at 30°C). Clones exhibiting wild-type growth were then analyzed for ^{75}Se incorporation into macromolecules by the procedure of Cox et al. (8) with the modifications of Pecher et al. (19). The labeling medium contained sodium [^{75}Se]selenite (specific radioactivity, 433 μCi/μmol) at a final concentration of 2.4 μM. Electrophoretic separation of ^{75}Se-labeled cell components and autoradiography were carried out as described previously (19).

Genetic procedures and recombinant DNA techniques. Deletion derivatives of Mu dl(Ap^r *lac*) fusion strains were obtained by selection for growth at 43°C and by screening the temperature-resistant clones obtained for ampicillin sensitivity. The procedure of Blum et al. (4) was used for the transduction of stabilized and immobilized Mu dl(Ap^r *lac*) insertions. Transductions with phage P1 *kc* were carried out by the method of Miller (18). *recA* deletions were introduced into strains by the method of Ihara et al. (13) with JC 10289(pKY102) as the donor organism.

Standard procedures were used for plasmid preparations, restriction enzyme digestions, ligations, transformations, *Bal* 31 exonucleolytic shortening, and gel electrophoresis (17).

Cosmid vector pHC79 (12) was used for cloning genomic DNA from *E. coli* MC4100. For this purpose, a partial digest of chromosomal DNA was prepared with restriction endonuclease *Sau*3A, and fragments were ligated into vector pHC79 which had been cut with *Bam*HI and treated with calf intestinal alkaline phosphatase. Recombinant cosmids were packed by using a DNA packaging kit from Boehringer Mannheim GmbH, Mannheim, Federal Republic of Germany.

Cloning of the gene(s) complementing the mutation of WL153. Initially, WL153 was converted into a suitable and stable recipient for cloning experiments by the excision of

Mu cts d1 (strain WL30153) and by introducing a *recA* deletion (strain WL31153) (Table 1). A cosmid bank of genomic DNA from *E. coli* MC4100, partially digested with restriction endonuclease *Sau*3A and cloned into vector pHC79, was then used to infect strain WL31153. Ampicillin-resistant clones were selected and screened for acidification on MacConkey glycerol-nitrate medium (3) and for gas formation under anaerobic conditions. One complementing recombinant cosmid, termed pWL1, was obtained.

DNA was prepared from pWL1 and partially digested with *Sau*3A, and subcloning experiments were carried out with *Bam*HI-linearized pACYC184 as the vector and strain WL50153 as the recipient. Selection was for chloramphenicol resistance; transformants were screened for acquisition of the FDH^+ phenotype by means of the benzylviologen overlay technique (16). Six positive clones were obtained, which, according to the size and the restriction map of the inserts, could be assigned to two groups; plasmids pWL101 and pWL107 (see Fig. 4B) are representatives of each group.

DNA from plasmid pWL107 was digested with endonucleases *Sal*I and *Cla*I, and the 1.7-kilobase (kb) *Sal*I-*Cla*I fragment was cloned into pACYC184, replacing the vector *Sal*I-*Cla*I fragment. The resulting plasmid was pWL171 (see Fig. 4B).

***Bal* 31 exonuclease digestion of plasmid pWL101.** pWL101 was linearized either at the *Sal*I or the *Cla*I site and shortened by *Bal* 31 exonuclease digestion. After recessed ends had been filled in with DNA polymerase (Klenow fragment), *Sal*I or *Cla*I linkers were added, and *Eco*RI-*Sal*I and *Cla*I-*Eco*RI fragments, respectively, were isolated and ligated into appropriately digested vector pACYC184.

Expression of wild-type and mutant *fdhF* genes in the phage T7 promoter-polymerase system. Plasmid pT7-6 contains the T7 ϕ10 promoter upstream of a multiple cloning site (S. Tabor, unpublished data [22]). It was used for the construction of hybrid plasmids pFM70 and pFH703, in which the wild-type *fdhF* gene containing the in-frame UGA_{140} codon or a mutant derivative with a UCA_{140} (serine) codon, respectively, can be expressed from this promoter. The cloning procedure is outlined in Fig. 1 for plasmid pFM70. It was obtained by cloning a *Nsi*I-*Bam*HI fragment of plasmid pFM30 into pT7-6 by using the *Pst*I and *Bam*HI restriction sites in the multilinker. pFM30 was derived from plasmid pFM20 (25) by C-terminal *Bal* 31 shortening. It contains 750 base pairs (from the *Sma*I site) (25) of the *fdhF* upstream region and 70 bp of the downstream flanking region. This fragment then was cloned into plasmid pACYC184 (6), replacing the *Bam*HI-*Hin*cII fragment (Fig. 1) and generating pFM30. Plasmid pFH703 is identical to pFM70, except that the UGA_{140} codon is replaced by UCA (24). Plasmids pFM70 and pFH703 were transformed into strains K38, WL31153, MB07, and MB08, into which the compatible plasmid pGP1-2 (23) had already been introduced by transformation. pGP1-2 contains the gene coding for T7 RNA polymerase under the control of the heat-inducible λ expression system (23).

Proteins expressed from pFM70 and pFH703 were radioactively labeled by the procedure of S. Tabor as given by Streber et al. (22).

Miscellaneous procedures. The activities of FDH_H and FDH_N were determined by the method of Lester and DeMoss (14) with benzylviologen and phenazine methosulfate–dichlorophenol-indophenol as electron acceptors, respectively. Gas formation was visualized as described previously (19). Transformants were screened for an FDH_H-positive phenotype by means of the benzyl-viologen agar overlay technique (16).

Special chemicals. Sodium [^{75}Se]selenite and L-[^{35}S]methionine were purchased as aqueous solutions from Amersham-Buchler, Brunswick, Federal Republic of Germany. Benzylviologen was obtained from Serva, Heidelberg, Federal Republic of Germany, and phenazine methosulfate and dichlorophenol-indophenol were from Sigma, Munich, Federal Republic of Germany. Enzymes used for recombinant DNA procedures were from Boehringer or from Pharmacia, Freiburg, Federal Republic of Germany.

RESULTS

Isolation and characterization of mutants deficient in selenium metabolism. Mutants deficient in selenite uptake and reduction or in the biosynthesis and incorporation of selenocysteine are expected to lack both FDH_H and FDH_N activities. As a consequence, anaerobically produced formate is no longer oxidized but excreted into the medium. The rationale of mutant isolation was therefore to screen for colonies in a Mu cts d1 mutagenized population which acidify the medium (3) and to analyze acidifying clones for ^{75}Se incorporation into macromolecules.

A total of 60 acid-producing strains were tested for ^{75}Se incorporation in a nitrate-containing medium, and 21 of them displayed an altered labeling pattern; they could be differentiated into essentially four classes (Fig. 2). Class 1 mutants (Table 1) incorporated radioactivity neither into the 110-kilodalton selenopolypeptide of FDH_N (Fig. 2B) nor into the 80-kilodalton selenopolypeptide of FDH_H (not shown). Members of classes 2 to 4 incorporated selenium into the

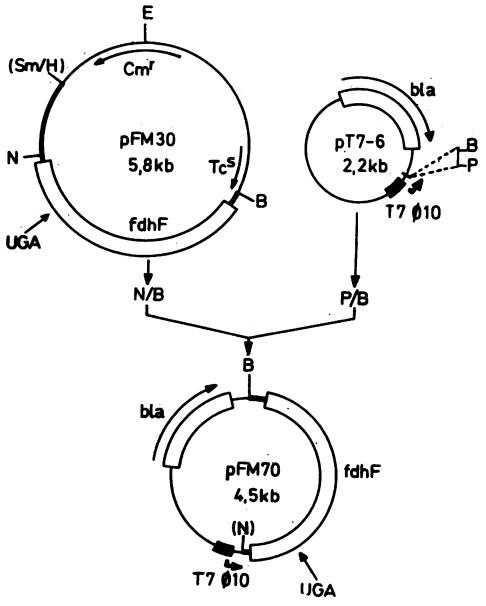

FIG. 1. Construction of plasmids pFM70 and pFH703, in which the *fdhF*(UGA) gene (25) and the *fdhF*(UCA) gene (24), respectively, are expressed from the T7 ϕ10 promoter. Abbreviations: E, *Eco*RI; N, *Nsi*I; P, *Pst*I; B, *Bam*HI; S, *Sma*I; H, *Hin*cII. The direction of transcription from the T7 ϕ10 promoter is indicated by an arrow. *bla* denotes the position of the gene encoding β-lactamase, which is not expressed from pT7-6 (opposite orientation). The position of the UGA codon within the *fdhF* gene is indicated. Plasmid pFH703 is identical to pFM70, except that the UGA_{140} codon was changed to a UCA (serine) codon.

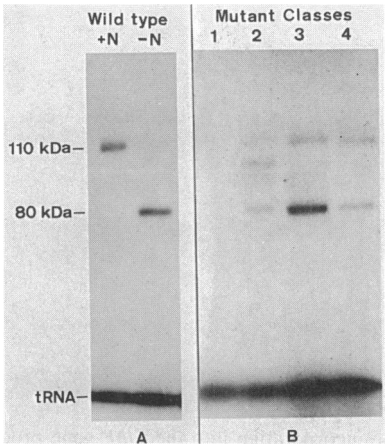

FIG. 2. ^{75}Se incorporation by wild-type *E. coli* MC4100 (A) and by representatives of mutant classes 1 to 4 (B). An autoradiogram of a sodium dodecyl sulfate-gel in which sodium dodecyl sulfate lysates of labeled cells were electrophoretically separated is shown. The migration positions of the 80- and 110-kilodalton selenopolypeptides of FDH_H and FDH_N and of selenylated tRNA are indicated. Abbreviations: −N, growth of cells in the absence of nitrate; +N, growth in the presence of nitrate. Mutants were grown in the presence of nitrate. The representative mutants analyzed in panel B are WL153 (class 1), WL24 (class 2), WL25 (class 3), and WL22 (class 4).

80-kilodalton *fdhF* gene product in the absence of nitrate (not shown). They were, however, unable to induce the synthesis of the 110-kilodalton selenopolypeptide (Fig. 2B). The class 2 mutant (strain WL24) also produced an apparently truncated form of this protein, and class 3 strains (WL25 and WL131) were no longer able to repress the synthesis of the *fdhF* gene product in the presence of nitrate. Members of the last three classes were not further characterized, since they appear to be either structural gene (class 2) or regulatory gene (classes 3 and 4) mutants.

FDH_H and FDH_N enzyme activities of the class 1 mutants were determined. Extracts of these strains contained no detectable enzyme activity (results not shown).

Mutants similar to those in class 1, in which the activities of both FDH_H and FDH_N are pleiotropically affected, have previously been isolated and characterized by several groups (3, 7, 10, 11, 16). According to the mapping position of the respective mutations on the *E. coli* chromosome, they were assigned to three genetic classes, namely *fdhA*, *fdhB*, and *fdhC* (11). To investigate whether the mutants with these mutations exhibit a defect in selenium metabolism, we also analyzed them for the incorporation of ^{75}Se upon anaerobic growth in the absence and presence of nitrate (Fig. 3). The results show that *fdhA* and *fdhC* mutants are indeed unable to incorporate ^{75}Se into the 80- and 110-kilodalton polypeptides of FDH_H and FDH_N; however, they still can form selenylated tRNA. The *fdhB* mutant MB08, however, is blocked in the selenylation of both protein and tRNA.

The *fdhA* locus contains two genes with a putative function in selenium incorporation. To resolve the relationship between the class 1 mutants isolated and the *fdhA*, *fdhB*, and *fdhC* strains, we cloned the gene(s) complementing the mutation of one of the original class 1 isolates, strain WL153 (Fig. 4) (see Materials and Methods).

Plasmids pWL107, pWL101, and pWL171 were transformed into all the class 1 mutants and into strain WL31153. The transformants were tested for gas formation, i.e., for a FDH^+ phenotype. (For the new gene designation, see Discussion.) Only plasmid pWL107 was able to complement the FDH defects of all strains (Fig. 4B). Plasmid pWL171, in contrast, was found to exclusively confer the ability to produce gas to just one group of mutants, namely to WL2, WL5, WL6, and WL14 but not to WL1, WL40, WL98, or WL153. Plasmid pWL101 showed the opposite complementation specificity. Neither pWL101 nor pWL171 could complement strain WL31153 (Fig. 4B). This complementation pattern strongly indicates that the class 1 mutants are genetically heterogeneous and possess lesions in different but linked genes. It also indicates that Mu *cts* d1 excision from strain WL153, resulting in strain WL31153, has created a deletion of both of these genes. Hybridization experiments with pWL101 as probe and *Mlu*I- and *Sal*I-digested genomic DNA from MC4100 and WL31153 revealed that this is indeed the case (results not shown).

Strain GY01 (*fdhA*) was also tested for complementation. It was complemented by pWL171 and pWL107, but not by pWL101.

To narrow the coding area of the 4.0-kb insert of plasmid pWL101 required for complementation, we prepared a series of *Bal* 31 deletion clones. The strategy is given in Fig. 4A. This resulted in the generation of a series of plasmids in which the insert of pWL101 was progressively deleted from one or the other end. Only the border deletion clones are given in their complementation pattern in Fig. 4B. The results show that about 2 kb (pWL144) of the original 4.0 kb-insert of pWL101 is required to confer the FDH^+ phenotype on mutants WL1, WL40, WL98 and WL153, whereas 3.5 kb of contiguous DNA suffices to complement both groups of class 1 mutants.

To confirm that complementation of the ability to form gas is paralleled by the capacity to incorporate selenium into protein, the labeling experiments depicted in Fig. 5 were carried out. A full correlation was obtained; plasmids which promoted the appearance of overall formate hydrogen lyase activity, as measured by gas formation, also restored the capacity to synthesize a selenylated *fdhF* gene product. Introduction of pWL171 into Δ*selAB* mutant WL31153 gave a pattern similar to that found with pWL101 (Fig. 5, lane 6; data not shown).

Class 1 and *fdhA*, *fdhB*, and *fdhC* mutants have a defect in selenium metabolism. Lack of incorporation of selenium into

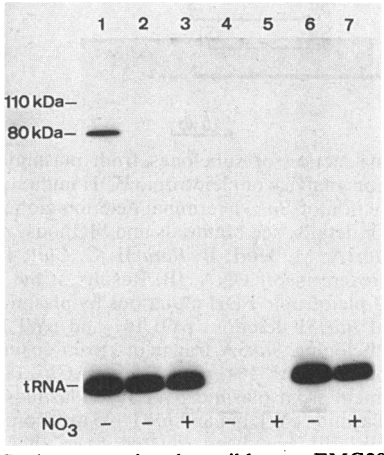

FIG. 3. ^{75}Se incorporation by wild-type EMG29 (lane 1) and mutants GY01 (*fdhA*; lanes 2 and 3), MB08 (*fdhB*; lanes 4 and 5), and MB07 (*fdhC*; lanes 6 and 7) grown in the absence (−) and presence (+) of nitrate under anaerobic conditions. For other details, see the legend to Fig. 2.

protein does not necessarily indicate that the respective strain possesses a defect in selenium metabolism. The possibility exists that the mutation is in a regulatory gene controlling the synthesis of both formate dehydrogenases or in some common subunit of both FDH_H and FDH_N which is required for the formation of stable 80- and 110-kilodalton selenopolypeptides.

The availability of mutant *fdhF* genes in which the UGA_{140} codon directing the incorporation of selenocysteine has been converted into sense codons (24) offers an experimental approach to the resolution of these questions. Translation of UGA_{140} requires the presence of selenium (25), whereas translation of UCA_{140} (24) should be independent of the presence of selenium or of a functional selenium incorporation pathway. What would be expected, therefore, when *fdhF*(UGA) and *fdhF*(UCA) genes are transferred into a genetic background deficient in the biosynthesis or incorporation of selenocysteine is (i) possible termination of protein synthesis at the position of the UGA_{140} codon in the *fdhF* (UGA) mRNA and (ii) synthesis of a full-sized 80-kilodalton gene product from the *fdhF*(UCA) mRNA.

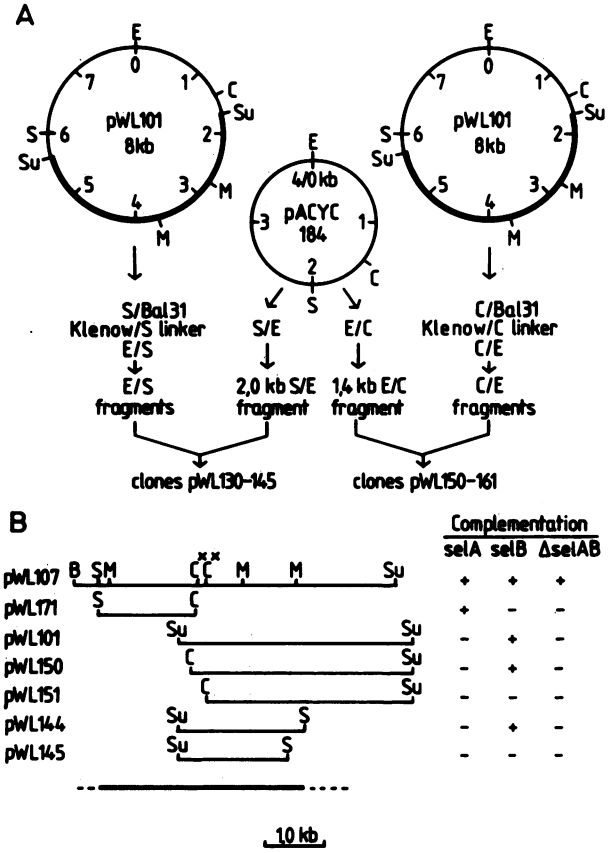

FIG. 4. Construction of subclones from plasmid pWL101 and complementation analysis of pleiotropic FDH mutants. (A) Strategy for the construction of *Bal* 31 terminal deletion clones of the insert of pWL101. For details, see Materials and Methods. Abbreviations: S, *Sal*I; Su, *Sau*3A; M, *Mlu*I; B, *Bam*HI; C, *Cla*I; E, *Eco*RI. The thick lines represent insert DNA. (B) Results of the complementation analysis of pleiotropic FDH mutations by plasmids obtained by subcloning and *Bal* 31 deletion. pWL101 and pWL107 were constructed by subcloning *Sau*3A fragments from cosmid pWL1 into the *Bam*HI site of pACYC184. pWL171 is pACYC184 carrying the *Sal*I-*Cla*I fragment from plasmid pWL107. Plasmids pWL150 and pWL151 and plasmids pWL144 and pWL145 are clones in which the insert from pWL101 has been deleted from the left-hand and right-hand side, respectively. Complementation was assessed by the ability of strains to produce gas. *selA* mutants: WL2, WL5, WL6, WL14 and GY01; *selB* mutants: WL1, WL40, WL98, WL153; Δ*selAB* mutant: WL31153 (see Discussion). ×, Restriction is possible only in strain WA321 (*dam-4*) (Table 1).

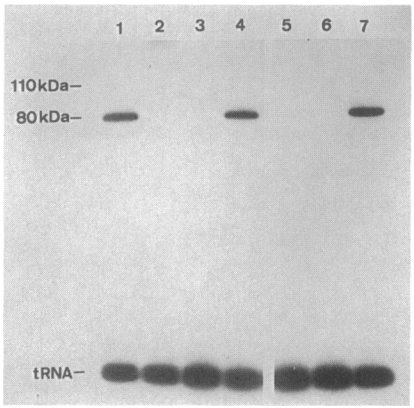

FIG. 5. ^{75}Se incorporation into the *fdhF* gene product by strain MC4100 (lane 1) and by the Δ*selAB* mutant WL31153 (lane 2) transformed with vector pHC79 (lane 3), cosmid pWL1 (lane 4), pACYC184 (lane 5), pWL101 (lane 6), and pWL107 (lane 7). Strains were grown under anaerobic conditions in the absence of nitrate. For other details, see the legend to Fig. 2.

The T7 ϕ10 promoter-polymerase (23) system provides an elegant means of testing these predictions. To this end, plasmids pFM70 and pFH703, in which the *fdhF*(UGA) and *fdhF*(UCA) genes, respectively, are under the control of the T7 ϕ10 promoter, were transferred into strains K38 and WL31153 which had already been transformed with the compatible plasmid pGP1-2 (23). The synthesis of plasmid-encoded gene products was followed by the incorporation of L-[^{35}S]methionine and by autoradiography of cell extracts electrophoretically separated in sodium dodecyl sulfate-gels (Fig. 6). A small amount of the 80-kilodalton gene product was expressed from the wild-type *fdhF*(UGA) gene (lane 2); it migrated to the same position as the ^{75}Se-labeled gene product (data not shown). The amount of this polypeptide was greatly increased upon expression from pFH703, which carried the *fdhF*(UCA) gene (lane 3). Furthermore, pFM70 directed the massive synthesis of a 15-kilodalton polypeptide (lane 2) which was absent from the pFH703 expression system (lane 3). In the genetic background of strain WL31153, only the 15-kilodalton and no 80-kilodalton polypeptide was expressed from pFM70 (lane 5), whereas pFH703 again promoted the synthesis of a full-sized *fdhF* gene product (lane 6). The intensity of the 80-kilodalton polypeptide expressed in WL31153 (lane 6) was reduced relative to that in background K38 (lane 3). This effect was found to be reproducible, and we attribute it to differences in the strains.

The formation of pFM70- and pFH703-encoded gene products was also analyzed in the genetic background of strains MB08 (*fdhB*) and MB07 (*fdhC*). An identical result was obtained as with strain WL31153, namely exclusive formation of a 15-kilodalton polypeptide from plasmid pFM70 and of an 80-kilodalton product from pFH703 (Fig. 7).

DISCUSSION

E. coli has two formate dehydrogenases, FDH_H and FDH_N. Both enzymes contain iron-sulfur clusters, a molyb-

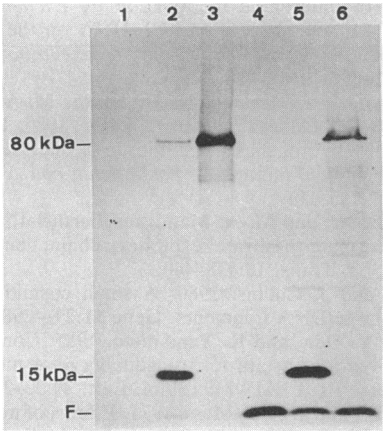

FIG. 6. Synthesis of *fdhF*(UGA) and *fdhF*(UCA) gene products from plasmids pFM70 and pFH703, respectively, in strains K38 and WL31153. An autoradiograph of a 7.5 to 15% polyacrylamide gradient gel is shown in which sodium dodecyl sulfate lysates from equal numbers of cells were separated. Cells were labeled as follows. Induction of the T7 promoter-polymerase system (23) was achieved by incubating cells at 42°C for 15 min, adding rifampin (200 μg/ml), and incubating the mixture for a further 10 min at 42°C. The temperature was shifted down to 30°C for 20 min, and then the cells were pulse-labeled with L-[^{35}S]methionine for 5 min (22). The size of the gene products is indicated; they were taken from the migration of Coomassie-stained standard proteins. F, Migration front. Lanes contained lysates from K38(pT7-6) (lane 1), K38(pFM70) (lane 2), K38(pFH703) (lane 3), WL31153(pT7-6) (lane 4), WL31153(pFM70) (lane 5), WL31153(pFH703) (lane 6). All strains carried plasmid pGP1-2.

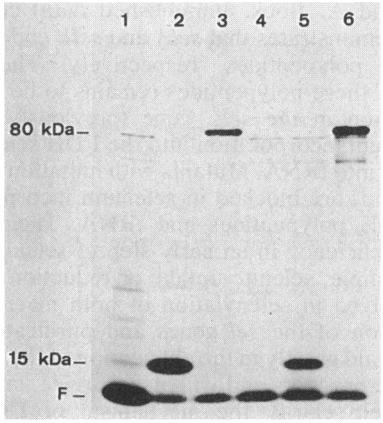

FIG. 7. Synthesis of *fdhF*(UCA) and *fdhF* (UCA) gene products from plasmids pFM70 and pFH703, respectively, in the *fdhB* mutant MB08 and the *fdhC* mutant MB07. For details, see the legend to Fig. 6. Lanes: 1, MB08(pT7-6); 2, MB08(pFM70); 3, MB08(pFH703); 4, MB07(pT7-6); 5, MB07(pFM70); 6, MB07(pFH703).

denum cofactor (14), and covalently bound selenocysteine (8, 25). Possible mutations which pleiotropically affect the formation of these enzymes could be located (i) in genes involved in the biosynthesis and incorporation of these compounds, (ii) in some regulatory gene governing the synthesis of both enzymes, or (iii) in the gene for a common subunit. The fact that the class 1 mutants isolated in this study and those described in the literature (3, 11, 16) are chlorate sensitive and able to grow anaerobically on glycerol-nitrate medium precludes a lesion in the molybdenum cofactor pathway. Rather, the results presented show that these mutants are defective in selenium metabolism.

Several lines of evidence support this conclusion. First, the class 1 strains isolated and the *fdhA*, *fdhB*, and *fdhC* mutants are unable to incorporate selenium into the 80- and 110-kilodalton polypeptides of FDH_N and FDH_H, respectively. Although the class 1 mutants and the *fdhA* and *fdhC* strains are still able to form selenylated tRNA, the *fdhB* strain lacks the ability to incorporate selenium both into protein and into tRNA. Second, when the gene for the 80-kilodalton selenopolypeptide (*fdhF*) is expressed in the genetic background of these mutants, no protein of this size is synthesized. This observation is in accordance with the result that translation of the UGA codon of the *fdhF*(UGA) mRNA requires the presence of selenium in the medium or of a functional selenocysteine incorporation pathway (24, 25). Third, when the UGA codon is converted into a sense codon such as UCA, the dependence on selenium for translation of *fdhF*(UGA) mRNA is relieved. A nonselenylated 80-kilodalton gene product is synthesized in an amount greatly exceeding that expressed from the UGA containing *fdhF* mRNA. Therefore, functional products of those genes which are altered in the pleiotropic *fdh* mutants investigated are required for the synthesis or the insertion of selenocysteine at the site of the UGA_{140} codon.

This conclusion is further corroborated by the finding that a 15-kilodalton polypeptide is formed when the *fdhF*(UGA) gene is expressed in the genetic background of the pleiotropic *fdh* mutants described above. Its size is in accordance with that of the presumptive product of premature termination of translation at the UGA_{140} codon. This 15-kilodalton polypeptide is not formed when the *fdhF*(UCA) gene is expressed, which supports the notion that it is a truncated polypeptide. It is, however, present in wild-type cells (Fig. 6) in addition to the full-length 80-kilodalton gene product. This indicates that the biochemical capacity for selenocysteine biosynthesis and incorporation is limiting, especially under the condition of massive mRNA overproduction. The same conclusion has been drawn previously (25).

On the basis of these results, the gene designation *sel* is proposed. Table 2 gives a correlation of the previous and new gene nomenclature.

fdhA and the class 1 mutants analyzed above fall into two complementation groups. Recent sequence analysis (W.

TABLE 2. *E. coli* mutations affecting selenium metabolism

Mutant	Gene designation		Map position (min)	Product[a]	Reference
	Old	New			
WL2, WL5, WL6, WL14, GY01	*fdhA*	*selA*	80	50-kDa protein	11; this work
WL1, WL40, WL98, WL153		*selB*	80	70-kDa protein	This work
MB07	*fdhC*	*selC*	82	Unknown	11
MB08	*fdhB*	*selD*	38	Unknown	11

[a] kDa, kilodalton.

Leinfelder and A. Böck, unpublished data) confirms this notion and demonstrates that *selA* and *selB* code for 50- and 70-kilodalton polypeptides, respectively. The biological function(s) of these polypeptides remains to be elucidated.

The mutation in the *selC* gene (previously *fdhC*) also affects selenium incorporation into the FDH selenopolypeptides, but not into tRNA. Mutants with mutations in *selD*, on the other hand, are blocked in selenium incorporation into both the FDH polypeptides and tRNA. Their phenotype indicates a deficiency in an early step of selenium metabolism, for example, selenite uptake or reduction, or in some enzyme involved in selenylation of both macromolecules. Overexpression of the *sel* genes and purification of their products will aid greatly in the elucidation of the biochemical roles of these products and will also provide valuable information to help clarify the mechanism of UGA-directed selenocysteine incorporation into proteins.

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LITERATURE CITED

1. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
2. **Balch, W. E., and R. S. Wolfe.** 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethane sulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. **32**:781–791.
3. **Barrett, E. L., C. E. Jackson, H. T. Fukumoto, and G. W. Chang.** 1979. Formate dehydrogenase mutants of *Salmonella typhimurium*: a new medium for their isolation and new mutant classes. Mol. Gen. Genet. **177**:95–101.
4. **Blum, P., L. Blaha, and S. Artz.** 1986. Reversion and immobilization of phage Mu*dl cts* (Ap^r *lac*) insertion mutations in *Salmonella typhimurium*. Mol. Gen. Genet. **202**:327–330.
5. **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.
6. **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.
7. **Chippaux, M., F. Casse, and M.-C. Pascal.** 1972. Isolation and phenotypes of mutants from *Salmonella typhimurium* defective in formate hydrogenlyase activity. J. Bacteriol. **110**:766–768.
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. **Dreiseikelmann, B., R. Eichenlaub, and W. Wackernagel.** 1979. The effect of differential methylation by *Escherichia coli* of plasmid DNA and phage T7 and λ DNA on the cleavage by restriction endonuclease *Mbo*I from *Moraxella bovis*. Biochim. Biophys. Acta **562**:418–428.
10. **Graham, A., H. E. Jenkins, N. H. Smith, M.-A. Mandrand-Berthelot, B. A. Haddock, and D. H. Boxer.** 1980. The synthesis of formate dehydrogenase and nitrate reductase proteins in various *fdh* and *chl* mutants of *Escherichia coli*. FEMS Microbiol. Lett. **7**:145–151.
11. **Haddock, B. A., and M.-A. Mandrand-Berthelot.** 1982. *Escherichia coli* formate-to-nitrate respiratory chain: genetic analysis. Biochem. Soc. Trans. **10**:478–480.
12. **Hohn, B., and J. Collins.** 1980. A small cosmid for efficient cloning of large DNA fragments. Gene **11**:291–298.
13. **Ihara, M., Y. Oda, and K. Yamamoto.** 1985. Convenient construction of strains useful for transducing *recA* mutations with bacteriophage P1. FEMS Microbiol. Lett. **30**:33–35.
14. **Lester, R. L., and J. A. DeMoss.** 1971. Effects of molybdate and selenite on formate and nitrate metabolism in *Escherichia coli*. J. Bacteriol. **105**:1006–1014.
15. **Lyons, L. B., and N. D. Zinder.** 1972. The genetic map of the filamentous bacteriophage f1. Virology **49**:45–60.
16. **Mandrand-Berthelot, M.-A., M. Y. K. Wee, and B. A. Haddock.** 1978. An improved method for identification and characterization of mutants of *Escherichia coli* deficient in formate dehydrogenase activity. FEMS Microbiol. Lett. **4**:37–40.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Pecher, A., F. Zinoni, and A. Böck.** 1985. The selenopolypeptide of formic dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*: genetic analysis. Arch. Microbiol. **141**:359–363.
20. **Pinsent, J.** 1954. The need for selenite and molybdate in the formation of formic dehydrogenase by members of the coli-aerogenes group of bacteria. Biochem. J. **57**:10–16.
21. **Stadtman, T. C.** 1980. Selenium-dependent enzymes. Annu. Rev. Biochem. **49**:93–110.
22. **Streber, W. R., K. N. Timmis, and M. H. Zenk.** 1987. Analysis, cloning and high-level expression of 2,4-dichlorophenoxy acetate monooxygenase gene *tfdA* of *Alcaligenes eutrophus* JMP134. J. Bacteriol. **169**:2950–2955.
23. **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.
24. **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.
25. **Zinoni, F., A. Birkmann, T. C. Stadtman, and A. Böck.** 1986. Nucleotide sequence and expression of the selenocysteine-containing polypeptide of formate dehydrogenase (formate-hydrogen-lyase-linked) from *Escherichia coli*. Proc. Natl. Acad. Sci. USA **83**:4650–4654.