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SelD Homolog from *Drosophila* Lacking Selenide-dependent Monoselenophosphate Synthetase Activity

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The isolation and molecular characterization of an invertebrate gene that encodes a homolog of the human selenophosphate synthetase 1 is described. This *Drosophila* gene, termed *selD*-like, is located in the cytogenetic interval 50 D/E on the right arm of chromosome 2. It is expressed ubiquitously throughout embryogenesis and found to be highly enriched in the developing gut and in the nervous system of the embryo.

The SelD-like from *Drosophila* was purified after expression in *Escherichia coli*. The purified protein does not catalyze the selenide-dependent ATP hydrolysis reaction and its gene does not complement a *selD* lesion in *E. coli*. These results and the fact that *selD*-like possesses an arginine residue at the position of the essential Cys17 (*E. coli* nomenclature) indicate that the *Drosophila* gene exerts a function different from that of the classical selenophosphate synthetases. Two classes of SelD proteins can therefore be differentiated. The class I proteins contain a cysteine or selenocysteine residue in the active site and display selenide-dependent selenophosphate synthetase activity. Class II proteins, including *Drosophila selD*-like and human *selenophosphate synthetase 1* are devoid of this activity and they possess other amino acids in position 17.

Keywords: selenocysteine; SelD; selenophosphate; selenoprotein synthesis

Introduction

Monoselenophosphate is the key intermediate in selenium metabolism of both prokaryotes and eukaryotes. It serves as the selenium donor molecule in the conversion of seryl-tRNA^{Sec} into selenocysteyl-tRNA^{Sec} and in the replacement of the sulfur in 2-thiouridine in tRNA by selenium yielding 2-selenouridine (for reviews see Baron & Böck, 1995; Stadtman, 1996). Monoselenophosphate (MSP) is the product of the reaction catalyzed by mono-selenophosphate synthetase in which the γ-phosphate group of ATP is transferred to HSe^- resulting in MSP production accompanied by the formation of inorganic phosphate and AMP (Leinfelder *et al.*, 1990; Ehrenreich *et al.*, 1992; Veres *et al.*, 1992; Glass *et al.*, 1993). A detailed kinetic analysis of the reaction mechanism of the enzyme from *Escherichia coli* revealed that a cysteine residue (Cys17) is essential for catalysis (Kim *et al.*, 1992; Veres *et al.*, 1994).

Intriguingly, two other forms of the enzyme related to this active-site-cysteine were discovered. Analysis of the total genome sequence of *Haemophilus influenzae* and *Methanococcus jannaschii* revealed that the *selD* gene that codes for selenophosphate synthetase in those organisms possesses a UGA codon at the position of the *E. coli* Cys17, indicating that the enzyme contains a selenocysteine residue itself (Guimaraes *et al.*, 1996; Wilting *et al.*, 1997). Moreover, a selenocysteine-containing variant was identified in mouse and humans (Guimaraes *et al.*, 1996). A third apparent homolog containing threonine in the respective position was identified by Low *et al.* (1995), also in mammalian cells (SelD1). It was shown to weakly complement a *selD* mutation in *E. coli*. When mammalian cells were transfected with this gene it stimulated Se incorporation into a selenoprotein, indicating a function in selenium metabolism.

During a sequencing project of *Drosophila melanogaster* another apparent *selD* homolog, which we designate *selD*-like, was discovered, representing the first *sel* gene found in an invertebrate. Since

Abbreviations used: MSP, monoselenophosphate; IPTG, isopropyl-β,D-thiogalactopyranoside; FDH, formate dehydrogenase; PEI, polyethyleneimine.

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Drosophila is an excellent model organism to unravel the function of the gene products, we have initiated its genetic and biochemical analysis.

Results

Isolation, structure and expression of a *selD*-like gene of *Drosophila*

The clone carrying the apparent homolog of the gene encoding the human selenophosphate synthetase, SelD, was isolated from a *Drosophila* embryo cDNA library. Cytogenetic localization of the gene by *in situ* hybridization of the cDNA to polytene salivary gland chromosomes showed that *selD*-like is located in region 50D/E on the right arm of the second chromosome (Figure 1a).

In order to determine the structure of the *selD*-like transcription unit, the cDNA was used as a probe to isolate genomic DNA clones from a λ phage library. Restriction enzyme analysis of both the *selD*-like cDNA and its genomic counterpart, cross-hybridization experiments on Southern blots and DNA sequence comparison between cDNA and the corresponding genomic DNA allowed us to establish the structure of the gene (Figure 1b and d). The transcription region of *selD*-like expands over roughly 2 kb. The primary *selD*-like transcript contains a single intron in the 5′ untranslated region and is spliced into a 1.5 kb mature transcript.

Sequence analysis of the *selD*-like gene and comparison of the *Drosophila* SelD to other known SelD proteins

Sequence analysis of the *selD*-like cDNA (Figure 1d) revealed that the 1540 bp transcript contains 123 bp of non-translated 5′ sequence and a single polyadenylation signal followed by a poly(A) tail extending the 193 bp 3′ untranslated region. The putative translation start site (AGCAAGATG) corresponds to the *Drosophila* consensus sequence C/AAAA/CATG (Cavener, 1987). The coding sequence extends over a 1194 bp open reading frame that codes for a protein of 398 amino acid residues. This putative protein can be aligned with other SelD proteins from all evolutionary domains (Figure 2). Interestingly, the amino acid residue in the catalytic site, corresponding to cysteine 17 in the *E. coli* sequence (Leinfelder *et al.*, 1990), is arginine. With one exception, namely the human SelD1, all other known SelD proteins have cysteine or a selenocysteine in this position.

Expression in *E. coli* and purification of the *Drosophila selD*-like gene product (Dm-SelD)

In the plasmid isolated from the original λZAP clone (pDmD-SelD) the *selD*-like gene is already under the control of the *lac* promoter. However, no band corresponding to the product of *selD*-like, Dm-SelD, could be detected in Coomassie brilliant blue-stained gels when cells harboring this plasmid were induced with 0.5 mM IPTG. This was not unexpected, since the *selD*-like gene has no ribosome binding site of *E. coli* type and, hence, will probably not be translated. In λZAP there is a possibility for a fusion between *lacZ* and the cloned gene, in pDMD-SelD, however, the reading frame of *lacZ* is in the −1 frame compared to that of *selD*. To overcome this problem and to increase the production of Dm-SelD, two plasmids were constructed. In one of them, pBP46, the expression of the *selD*-like gene was set under the control of both the *lac* promoter and the *lacZ* Shine-Dalgarno motif. In the other, pT7-DmD, selD-like expression was controlled by the phage T7 ϕ10 promoter and a synthetic Shine-Dalgarno sequence. For details on the plasmid construction, see Experimental Procedures.

The strain BL21/pT7-DmD produced large amounts of Dm-SelD at 37°C. However, when S30 extracts were made from these cells, the Dm-SelD protein was found almost exclusively in an insoluble form. This problem was overcome by cultivation of strain BL21/pT7-DmD at 37°C to an A_{600} of 0.5, whereafter the culture was shifted to +14°C. After one hour at this temperature, production of Dm-SelD was induced by the addition of 0.1 mM IPTG and the culture was left at +14°C for approximately 30 hours. Generally about 50% of the protein produced at +14°C turned out to be in a soluble form (Figure 3). Dm-SelD was purified from cells grown in LB medium at +14°C. Cells were broken and an S100 extract was made. After ammonium sulfate-precipitation the fractions containing Dm-SelD were used for further purification by ion-exchange chromatography and gel filtration (see Experimental Procedures). After the gel-filtration chromatography the Dm-SelD protein was in an apparently homogeneous state (Figure 3). N-terminal sequencing proved its authenticity with the predicted product of selD-like and confirmed the translational start site deduced from the nucleotide sequence (see Figure 1), with the N-terminal methionine residue being cleaved off (data not shown).

In vitro activity of Dm-SelD

Selenophosphate synthetase catalyzes the reaction:

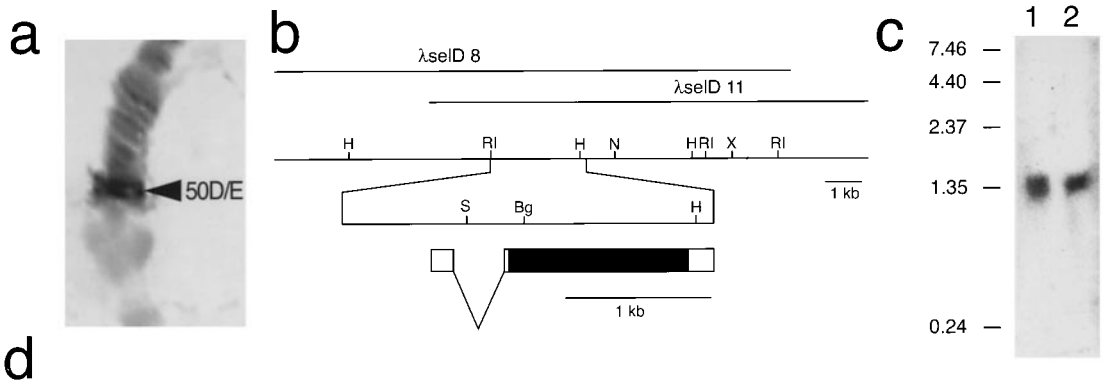
$$\mathrm{ATP} + \mathrm{HSe}^- \rightarrow \text{selenophosphate} + \mathrm{AMP} + \mathrm{P_i}$$

The selenide-dependent hydrolysis of ATP to AMP was used as an assay for the selenophosphate synthetase activity of Dm-SelD (Ehrenreich *et al.*, 1992). The experiments were performed under anaerobic conditions. Controls without selenide were included as well as controls with *E. coli* SelD instead of Dm-SelD. As shown in Figure 4, no selenophosphate was found with Dm-SelD, whereas a strong selenide-dependent activity was obtained with *E. coli* SelD.

In vivo activity of Dm-SelD

One of the selenocysteine-containing proteins in *E. coli* is the formate dehydrogenase H (FDH-H). The *in vivo* activity of this enzyme can be measured by providing cells with formate as the natural substrate and benzyl viologen as an artificial electron acceptor (Mandrand-Berthelot *et al.*, 1978).

The plasmid pBP46 was used to transform the selenophosphate synthetase-deficient strain BP005



d

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1    ACA ACC CTG TTG TGA CAT CTC GCT CGT TGG AAA AGC AAA AAC CAG GCG CAA AAT AAA TAT
61   AAT TTG CTA ACG TTA CTC TAC AAT TAT CTT TGT ACA ATC GCA CAA GCA AAC▼CTA TAA AGC
         M   S   Y   A   A   D   V   L   N   S   A   H   L   E   L   H   G   G   G  19
121  AAG ATG AGC TAC GCC GCT GAT GTG CTG AAC TCG GCC CAT TTG GAG CTC CAT GGT GGT GGC
     D   A   E   L   R   R   P   F   D   P   T   A   H   D   L   D   A   S   F   R  39
181  GAC GCC GAG TTG CGT CGT CCA TTC GAT CCC ACG GCC CAT GAT TTG GAT GCA TCC TTC CGC
     L   T   R   F   A   D   L   K   G   R   G   C   K   V   P   Q   D   V   L   S  59
241  CTT ACA CGC TTC GCA GAT CTA AAG GGG CGC GGC TGC AAA GTG CCG CAG GAT GTG CTC TCC
     K   L   V   S   A   L   Q   Q   D   Y   S   A   Q   D   Q   E   P   Q   F   L  79
301  AAG CTG GTG TCC GCC CTG CAG CAG GAC TAC TCT GCC CAG GAC CAG GAA CCG CAG TTT CTC
     N   V   A   I   P   R   I   G   I   G   L   D   C   S   V   I   P   L   R   H  99
361  AAC GTG GCC ATT CCC CGC ATC GGT ATT GGC CTG GAC TGC TCC GTC ATT CCG CTG CGA CAC
     G   G   L   C   L   V   Q   T   T   D   F   F   Y   P   I   V   D   D   P   Y 119
421  GGC GGA CTC TGC CTG GTG CAG ACC ACC GAC TTC TTC TAC CCC ATT GTC GAT GAT CCC TAC
     M   M   G   K   I   A   C   A   N   V   L   S   D   L   Y   A   M   G   V   T 139
481  ATG ATG GGC AAA ATT GCG TGC GCC AAT GTG CTC AGC GAT TTG TAT GCC ATG GGA GTC ACA
     D   C   D   N   M   L   M   L   L   A   V   S   T   K   M   T   E   K   E   R 159
541  GAT TGC GAT AAC ATG CTG ATG CTG TTG GCG GTC AGT ACC AAG ATG ACA GAG AAG GAG CGT
     D   V   V   I   P   L   I   M   R   G   F   K   D   S   A   L   E   A   G   T 179
601  GAC GTG GTC ATT CCG CTG ATA ATG CGA GGC TTT AAG GAC TCC GCT TTG GAA GCA GGC ACC
     T   V   T   G   G   Q   S   V   V   N   P   W   C   T   I   G   G   V   A   S 199
661  ACT GTG ACT GGA GGC CAG AGC GTG GTC AAT CCG TGG TGC ACC ATT GGT GGG GTT GCC TCG
     T   I   C   Q   P   N   E   Y   I   V   P   D   N   A   V   V   G   D   V   L 219
721  ACC ATT TGC CAG CCA AAT GAG TAT ATT GTG CCA GAT AAT GCC GTG GTA GGG GAT GTA CTG
     V   L   T   K   P   L   G   T   Q   V   A   V   N   A   H   Q   W   I   D   Q 239
781  GTG CTA ACC AAG CCA CTG GGC ACC CAA GTG GCC GTG AAT GCC CAC CAG TGG ATT GAC CAG
     P   E   R   W   N   R   I   K   L   V   V   S   E   E   D   V   R   K   A   Y 259
841  CCG GAG CGC TGG AAC CGC ATT AAG CTG GTG GTG TCC GAA GAA GAT GTA CGC AAG GCC TAC
     H   R   A   M   N   S   M   A   R   L   N   R   V   A   A   R   L   M   H   K 279
901  CAC CGG GCG ATG AAC TCT ATG GCT CGC CTC AAC CGC GTA GCC GCT CGC CTC ATG CAC AAG
     Y   N   A   H   G   A   T   D   I   T   G   F   G   L   L   G   H   A   Q   T 299
961  TAC AAT GCC CAC GGA GCC ACC GAT ATC ACT GGA TTC GGA CTA CTA GGC CAC GCT CAA ACC
     L   A   A   H   Q   K   K   D   V   S   F   V   I   H   N   L   P   V   I   A 319
1021 CTG GCT GCA CAT CAG AAA AAG GAT GTC TCC TTT GTT ATC CAC AAT CTG CCG GTG ATC GCC
     K   M   A   A   V   A   K   A   C   G   N   M   F   Q   L   L   Q   G   H   S 339
1081 AAG ATG GCT GCA GTG GCC AAA GCC TGT GGC AAC ATG TTC CAG CTG CTG CAG GGC CAC TCG
     A   E   T   S   G   G   L   L   I   C   L   P   R   E   Q   A   A   A   Y   C 359
1141 GCG GAG ACC TCG GGA GGA TTA CTG ATC TGC CTG CCG CGA GAG CAG GCC GCT GCT TAT TGC
     K   D   I   E   K   Q   E   G   Y   Q   A   W   I   I   G   I   V   E   K   G 379
1201 AAG GAC ATC GAG AAG CAG GAG GGC TAC CAG GCC TGG ATC ATC GGC ATC GTT GAG AAG GGC
     N   K   T   A   R   I   I   D   K   P   R   V   I   E   V   P   A   K   D     398
1261 AAT AAG ACC GCA CGC ATC ATC GAC AAG CCC CGC GTC ATT GAG GTG CCC GCC AAG GAC TAG
1321 GTC TCC ATT GCC TGA CCA TCT GCT ATT GTC TAC CTT AAG TAT CTT AAT ATT AAC GTA ACG
1381 GCT GCT ACA TTC AGT TGT AAC CGG CAT GAT CGG TCT TTG TTT TTT ATA AAA AAG CTT ATC
1441 AAC TAC AGA TTC ACT GAT CAT TAT AAT CTT GGA CGT CTC TAC TGC AAT ACG AGT GAA TAA
1501 AAT GAA AAC ACT TGA AAA AAA AAA AAA AAA AAA AAA AAA
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Figure 1. Molecular characterization of the *Dm-selD* gene locus. a, *In situ* hybridization of *selD* cDNA to polytene chromosome showing that the gene is localized within the position 50D/E at the right arm of chromosome 2. b, Position fragment, intron and exon boundaries of the transcribed region within a 16.5 kb fragment of genomic DNA. Bg, *Bgl*II; H, *Hin*dIII; RI, *Eco*RI; S, *Sal*I; X, *Xba*I. c, Northern blot of total (lane 1) and poly(A)$^+$ fraction (lane 2) of embryo RNA indicates a single *selD*-like transcript of 1.5 kb. d, Nucleotide and protein sequence of the full-size *selD*-like cDNA is shown. Start and stop codons are underlined and the position of the 365 bp intron is marked by an arrowhead; numbers of nucleotides (left) and amino acid residues (right) are shown.

(*selD::cat*). To examine whether the transformants contain FDH activity they were first grown on plates in an anaerobic vial filled with N_2 at 30°C or 37°C, and than shifted to +14°C, as it was shown that the Dm-SelD is produced in a soluble form only at 14°C. It was found that at none of these temperatures could the pBP46 or similar *selD*-like containing plasmids complement the FDH-H deficiency of the *E. coli selD* mutant. Thus, both *in vitro* and *in vivo* experiments consistently show that the SelD-like protein of *Drosophila* lacks selenide-dependent selenophosphate synthetase activity.

Expression of *selD*-like in the *Drosophila* embryo

The expression pattern of the *selD*-like gene during embryogenesis was examined by *in situ* hybridization with the digoxigenin-labeled *selD*-like cDNA probe to whole-mount preparations of embryos. Figure 5c to f indicates that *selD*-like transcripts are ubiquitously distributed, at low levels, throughout the developing embryo. From early gastrulation onwards (Figure 5c), *selD*-like transcripts became highly enriched in the endodermal anlagen of the midgut especially in the gastric caecae and in the developing nervous system (Figure 5d and e). In both locations they remain at high levels until the end of embryogenesis (Figure 5f).

Discussion

Genes coding for components required for selenocysteine biosynthesis and insertion into polypeptides have been sequenced in the last few years from a number of organisms. A comparison of the sequences revealed that SelD, the monoselenophosphate synthetase, was much more conserved during evolution than the primary structures of $tRNA^{Sec}$ (Tormay *et al*., 1994), SelB (Kromayer *et al*., 1996) or selenocysteine synthase (unpublished results). This is surprising, since SelD was thought to exclusively catalyze the transfer of a phosphate moiety from ATP to the HSe^- anion, a reaction that appeared *a priori* not to be subject to such strong sequence constraints as those for the other *sel* gene products that undergo macromolecular interactions.

The SelD homologs characterized in the past few years and in this work can be divided into two major classes. Class I is characterized by the existence of a cysteine or selenocysteine residue in the active site and by catalyzing the selenide-dependent formation of MSP (Leinfelder *et al*., 1990; Guimaraes *et al*., 1996; Wilting *et al*., 1997). Replacement of the selenocysteine residue by cysteine decreased but did not abolish enzyme activity

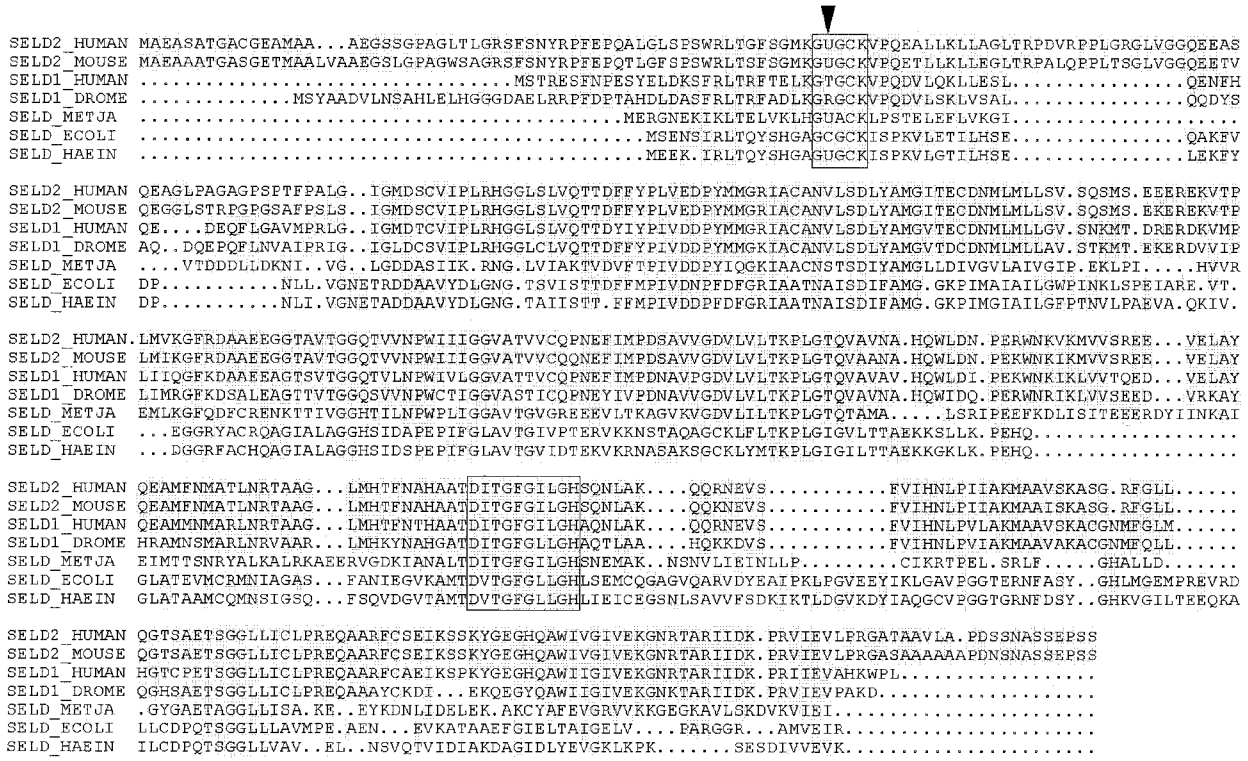

Figure 2. Alignment of SelD proteins from *Homo sapiens* (HUMAN), *Drosophila melanogaster* (DROME), *Haemophilus influenzae* (HAEIN), *Escherichia coli* (ECOLI) and *Methanococcus jannaschii* (METJA). The residue corresponding to the active-site cysteine residue (*E. coli* enzyme) is indicated by an arrow. The sequence motifs putatively involved in ATP binding are boxed. U denotes selenocysteine.

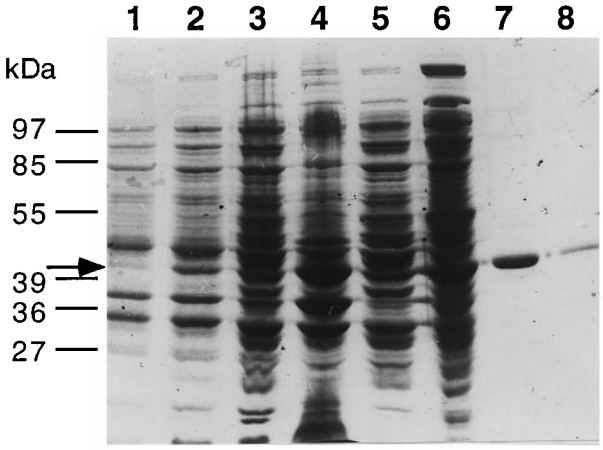

Figure 3. Purification of Dm-SelD. Lane 1, whole-cell extract before induction. Lane 2, whole-cell extract after 20 hours of induction with 0.1 mM IPTG at +14°C. Lane 3, S30 supernatant. Lane 4, S30 pellet. Lane 5, S100 supernatant. Lane 6, Proteins precipitated in the 35 to 50% saturated ammonium sulfate fraction. Lane 7, Dm-SelD after the Q-Sepharose anion-exchange chromatography. Lane 8, Dm-SelD after the Superdex 75 gel-filtration.

(Kim *et al.*, 1997), whereas exchange of the Cys17 residue of the *E. coli* enzyme against serine destroyed activity (Kim *et al.*, 1992).

Class II SelD variants have now been demonstrated for man (Low *et al.*, 1995) and for *Drosophila* in the present work. This class, although sharing a high level of sequence similarity with class I enzymes, does not have the active-site cysteine or selenocysteine residue in position 17 of the *E. coli* enzyme. Our results show that the SelD-like protein from *Drosophila* does not catalyze the selenide-dependent selenophosphate formation *in vitro* and that its gene is unable to complete a *selD* deletion in an *E. coli* mutant *in vivo*. This finding correlates well with the fact that targeted replacement of

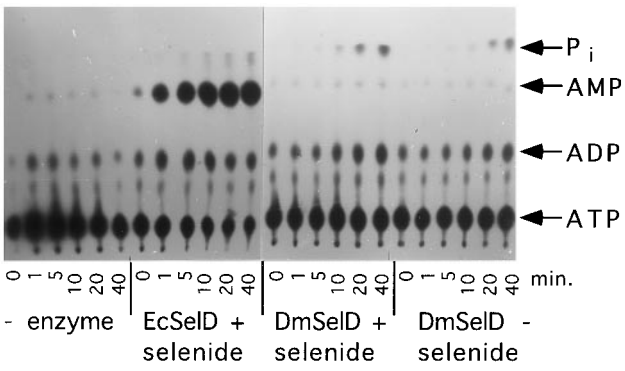

Figure 4. The selenide-dependent hydrolysis of [α-^{32}P]ATP by Dm-SelD from *Drosophila* and SelD from *E. coli*. Samples were taken at the time-points indicated and separated by PEI-cellulose thin-layer chromatography. The selenite added is chemically reduced by DTT in the reaction assay.

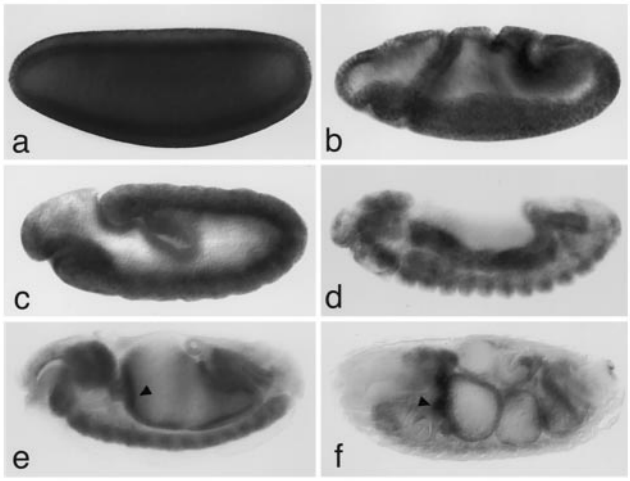

Figure 5. Expression of *selD*-like as revealed by *in situ* hybridization to whole-mount embryos preparations of embryos at various stages of *Drosophila* embryogenesis. a, Embryo at cellular blastoderm stage; note the strong maternal contribution. b, Embryo during gastrulation shows ubiquitous expression. c, Embryo at maximum elongated germband (stage 11) shows weak overall expression and enriched levels of transcripts in the invaginating gut anlagen and in the mesoderm. d, At stage 13, transcripts are strongly expressed in the midgut and in the nervous system. e, At stage 14 transcripts continue to persist in the central nervous system and the midgut. Note the concentration of the transcript in the anlagen of the gastric caecae (arrowhead). f, In the differentiated embryo (stage 17) *selD*-like is expressed in the central nervous system, brain and midgut with strong enhancement in the gastric caecae (arrowhead). Stages are according to Campos-Ortega & Hartenstein (1985).

Cys17 from *E. coli* SelD inactivates the enzyme completely (Kim *et al.*, 1992) and that the human and *Drosophila* variants with threonine and arginine possess chemically diverse amino acids in the equivalent position. Finally, recent experiments by Kim *et al.* (1997) with the human class I enzyme showed that the replacement of selenocysteine by cysteine in position 17 (*E. coli* nomenclature) drastically reduced enzyme activity, indicating that this amino acid residue, and not some other cysteine residue is essential also for eukaryotic selenophosphate synthetase activities.

Our opinion, that class II SelD proteins are unable to catalyze the selenide-dependent selenophosphate synthesis, at first sight appears to be at variance with a report in the literature that the gene for human SelD1 complements an *E. coli selD* lesion (albeit weakly) and also stimulates ^{75}Se incorporation into a selenoprotein in mammalian cells (Low *et al.*, 1995). In view of the fact that organisms that possess class II enzymes also contain a class I protein, it may well be that the SelD class II proteins have some function in selenium metabolism different from catalyzing selenide-dependent selenophosphate formation. From the

strong conservation of the ATP-binding sequence motif (Figure 2) and from the fact that mutagenesis of residues within this motif affect the stimulation of ^{75}Se incorporation into protein in transfected mammalian cells (Low *et al*., 1995), it appears likely that this unidentified reaction requires ATP binding and/or hydrolysis. The sequence identity of 67% between human SelD1 and *Drosophila* SelD supports the notion that this function is homologous in invertebrates and vertebrates. Since *Drosophila* genes are easily accessible to mutagenesis we shall perform targeted inactivation of *selD*-like to elucidate its role *in vivo*.

Experimental Procedures

Cloning and characterization of *selD*-like DNA

The *selD*-like cDNA clone was obtained during a low stringency screening of a λ ZAP cDNA phage library prepared from 0 to 18 hours old embryos (Stratagene). The corresponding genomic DNA of the *selD*-like transcription unit (see Figure 1b) was isolated from a λ Fix genomic *Drosophila* DNA library (Canton S; Stratagene). Screening of DNA libraries, the structural analysis of the *selD*-like gene and its transcripts as well as cytogenetic analysis by *in situ* hybridization to polytene chromosomes obtained from third instar salivary glands were done as described (Hartmann & Jäckle, 1995).

Whole-mount *in situ* hybridizations to wild-type embryos were done with digoxigenin-labeled RNA probes as described (Tautz & Pfeifle, 1989). All DNA fragments isolated from phages or cDNA clones were subcloned into Bluescript vectors (Stratagene). cDNA and corresponding regions in genomic DNA were sequenced by the dideoxynucleotide procedure (Sanger *et al*., 1977) using an automated sequencing device. The sequence has been deposited in the EMBL Data Base under the accession number A-J000672.

Plasmids and strains used for purification and complementation with Dm-SelD in *E. coli*

The bacterial strains used were the *E. coli* B strain BL21(DE3) *hsd*S, *gal*, which carries the gene for the phage T7 RNA polymerase under the control of the *lac* promoter on the λDE3 prophage (Studier *et al*., 1990), and BP005 Hfr P067, *selD::cat*, a derivative of KL19 (Low, 1968). For expression, two plasmids were constructed, pBP46 and pT7-DmD. The *selD*-like gene was put under the control of the *lac* promoter and *lacZ* Shine-Dalgarno sequence, by the aid of reverse PCR. The primers Dm1-rev (binding from the −1 position upstream of the ATG start codon of *lacZ* and reading towards the *lac* promoter) and Dm2-rev (binding from the ATG of *selD*-like and reading towards the end of the gene) were used to make a PCR product using the original pBluescript-KS + -DmD plasmid as template. The product was ligated and used to transform *E. coli* strain JM109. Ampicillin-resistant clones were tested for their ability to express Dm-SelD when induced with 0.1 mM IPTG. Plasmids from positive clones were sequenced from the *lac* promoter to the *Bgl*II site in *selD*-like. The sequence downstream of the *Bgl*II site was exchanged for the same sequence from the original plasmid to make certain that the final construct pBP46 contained no sequence error caused by the PCR amplification.

In order to put the *selD*-like gene under the control of the phage T7 ϕ10 promoter, it was PCR amplified using one primer that alters the sequence at the ATG of *sel*D-like to CATATG (*Nde*I) and one primer downstream of the *Hin*dIII site which is located downstream of *selD*-like. This PCR product was cut with *Nde*I and *Hin*dIII, and ligated to the pT7-7 vector (Tabor, 1994) cut with the same enzymes. As with the *lac* promoter construct, the region from the ϕ10 promoter to the *Bgl*II site in *selD*-like was sequenced, and the sequence from the *Bgl*II to the *Hin*dIII site was exchanged for that of the original plasmid. This places the *selD*-like under the control of the T7 ϕ10 promoter and of an artificial Shine-Dalgarno sequence (plasmid pT7-DmD).

Purification of Dm-SelD

The Dm-SelD protein was purified as follows. Cells were harvested and resuspended in buffer A (50 mM Tris-HCl (pH 7.5), 50 mM KCl, 1 mM DTT), and broken by three passages through a French press cell at 16,000 psi (1 psi ≃ 6.9 kPa). The lysate was centrifuged at 30,000 *g* for 30 minutes to remove cell debris, and the supernatant was further centrifuged at 100,000 *g* for two hours to obtain an S100 extract. The proteins precipitating between 35% and 50% ammonium sulfate saturation were dialyzed and used for further purification. The first chromatographic step was a Q-Sepharose anion-exchange chromatography. The proteins were eluted with an 0.1 M to 1 M KCl gradient in buffer A. Dm-SelD elutes very early in this gradient. The second chromatography step was Superdex 75 gel-filtration in buffer A containing 100 mM KCl. After the gel filtration chromatography the Dm-SelD protein contained no detectable contaminating protein (Figure 3).

Selenide-dependent ATPase assay of SelD

As test for the selenophosphate synthetase activity of SelD the selenide- dependent hydrolysis of ATP to AMP was used (Ehrenreich *et al*., 1992). These experiments were performed under strictly anaerobic conditions. The reaction mixture contained 50 mM Tris-HCl (pH 7.5), 50 mM KCl, 20 mM ATP, 5 mM $MgCl_2$, 2 mM DTT, 100 μM sodium selenite and 2000 cpm/ml [α-^{32}P]ATP in a total volume of 100 μl. Controls without selenite were included as well as controls with *E. coli* SelD instead of *Drosophila* Dm-SelD. The enzyme reaction was started by the addition of 600 pmol of SelD. At the time-points given in Figure 4 samples (10 μl) were withdrawn and the reaction stopped by addition of an equal volume of 2 M formic acid. The samples were lyophilized and resuspended in 2 μl of water; 1 μl of each sample was chromatographed on polyethylenimine-cellulose thin-layer plates with non-radioactive ATP, ADP and AMP as markers. The plates were dried and autoradiographed for four hours.

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