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Involvement of EDTP, an Egg-Derived Tyrosine Phosphatase, in the Early Development of *Drosophila melanogaster*

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Previously, we purified a novel protein tyrosine phosphatase from eggs of the flesh fly, *Sarcophaga peregrina*. This protein tyrosine phosphatase, named egg-derived tyrosine phosphatase (EDTP), is expressed during oogenesis and early embryogenesis but is rapidly degraded in middle embryogenesis by lysosomal cathepsin L. Here, we demonstrate the requirement of EDTP in the development of the fruit fly, *Drosophila melanogaster*. Deletion of the *Drosophila EDTP* gene using transposase-catalyzed imprecise excision resulted in homozygous lethals during embryogenesis. Additionally, germline clones generated using the FLP-FRT-*ovo*^{D} system showed severe defects in ovarian development during oogenesis. These results indicate that the *Drosophila EDTP* gene is crucial in oogenesis and embryogenesis.

Key words: cathepsin L, *Drosophila melanogaster*, embryogenesis, oogenesis, protein tyrosine phosphatase.

Abbreviations: FRT, flipase recognition target sequence; FLP, flipase; EST, expression sequence tag; rp49, ribosomal protein 49; GFP, green fluorescence protein; Act, actin; FITC, fluorescein isothiocianate.

Morphogenesis and cell differentiation in multi-cellular organisms require a precise balance between protein phosphorylation and dephosphorylation (*1–3*). Protein tyrosine phosphatase, a member of the protein phosphatase family, has been shown to play important roles in insect oogenesis and embryogenesis. Several soluble protein tyrosine phosphatases involved in these functions have been identified in *Drosophila melanogaster* (*4–9*). For example, Corkscrew is required for normal determination of cell fates at the termini of an embryo. The activation of Torso, a receptor tyrosine kinase essential for the development of terminal structures of an embryo, initiates a signalling cascade that requires the function of the corkscrew phosphatase (*4*). Two other genes, *string* and *twine*, encode for protein tyrosine phosphatases which are structurally similar to the cdc25 phosphatase of *Schizosaccharomyces pombe* (*5–8*). The former gene is required for initiation of mitosis of blastoderm cells, whereas the latter gene is required for proper assembly of the meiotic spindles and the arrest of the developing oocytes at metaphase I. An additional gene, the *tribbles* gene, acts specifically by inducing degradation of the cdc25 mitotic activators, String and Twine, via the proteosome pathway (*9*). By regulating cdc25, Tribbles serves to coordinate entry into mitosis and thus morphogenesis and determination of cell fate. Hence, the appropriate expression of this gene, which is followed by immediate degradation of the protein tyrosine phosphatases by proteases, appears to be important for some aspects of development, including oogenesis and embryogenesis.

Previously, we identified a novel soluble protein tyrosine phosphatase from eggs of the flesh fly, *Sarcophaga peregrina*, which we have named egg-derived tyrosine phosphatase (EDTP) (*10*). EDTP was purified as a substrate protein for cathepsin L, a cysteine proteinase involved in *Sarcophaga* development (*11*, *12*). EDTP is structurally novel and contains the conserved active site of protein tyrosine phosphatases. In addition, EDTP is rich in serine and threonine residues, which account for 145 of 724 residues, and this suggests that it is a phosphorylated protein.

Our work has shown that EDTP is indeed phosphorylated and has protein tyrosine phosphatase activity. Analysis of its expression at various stages showed that the EDTP protein and its mRNA were expressed in both oogenesis and embryogenesis but disappeared during embryonic development. EDTP was rapidly removed from ooplasm by cathepsin L digestion in early embryonic development, and this action may therefore be a prerequisite for the embryonic development of *Sarcophaga* (*10*). These results suggest that EDTP may function during specific events in embryogenesis prior to digestion by cathepsin L.

To clarify the regulation of embryogensis by EDTP using genetic approaches, we cloned *Drosophila* homologous cDNA. Deletion of the *Drosophila EDTP* gene was caused by imprecise excision of a transposable P-element and resulted in homozygous lethals during embryogenesis. In addition, germline clones generated using the FLP-FRT-*ovo*^{D} system showed severe defects in ovarian development

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during oogenesis. These results show that the *Drosophila EDTP* gene is crucial in *Drosophila* oogenesis and embryogenesis.

MATERIALS AND METHODS

Fly Strains—The following P-element insertions were used in the *Drosophila EDTP* locus: l(2)k6310b and EP(2)0922 (provided by the Berkeley *Drosophila* Genome Project, BDGP) (*13–15*). The P-element insertions were mapped to genomic contigs using the inverse PCR data provided on the FlyBase web pages. P-element insertion was mobilized using a Δ2–3 transposase source and excisions were identified by loss of w^+ expression (*16*). Of 200 excisions examined, 2 recessive lethal lines were obtained. The proximal and distal breakpoints of excision were determined by sequencing a Polymerase Chain Reaction (PCR) fragment spanning the deletion.

Germline Clones—All germline clones were generated via the FLP-FRT-ovo^D system (*17*). The null allele $EDTP^{\Delta}$ was recombined into the FRT42B (flipase recognition target sequence 42B) chromosome. Germline clones were generated by crossing FRT-$EDTP^{\Delta}$ into a hsFLP;FRT-P[OvoD] background and inducing mitotic recombination by heat-shocking second instar larvae for 2 h at 37°C for 3 consecutive days. In a control experiment, the egg development was normal when FRT-+ was crossed into a hs-FLP;FRT P[OvoD] (data not shown), showing that the heat-shocking and the mitotic recombination indeed properly worked.

Cloning of cDNA for Drosophila *EDTP*—The BDGP EST clone (clone LD05323), of which 634 bp has been sequenced (*18*) and which shows 56.8% homology with *Sarcophaga* EDTP cDNA, was used to isolate the cDNA of the *Drosophila* EDTP gene. The fragment of this clone was amplified by PCR from a *Drosophila* embryonic cDNA library (Stratagene). The DNA primers used were 5′-CCAGTGTCGTGTGTGTAA-3′ and 5′-AAGAGTCCATTTCCCGGT-3′. The resulting PCR product was purified and used as a probe to obtain *Drosophila* EDTP full-length cDNA. The probe was labelled with [α-32P]-dCTP using a random primer labelling kit (Takara). Two positive clones were isolated from 1×10^5 clones of the *Drosophila* embryonic cDNA library by plaque hybridization. The clone containing the longer insert (3.2 kb) was sequenced using the dideoxy chain-termination method (*19*). The nucleotide sequences of both strands were then determined.

RT-PCR Analysis—mRNA was extracted from *Drosophila* embryos using an mRNA purification kit (American Pharmacia Biotech). Reverse transcription was then performed using this mRNA as a template and oligo(dT) primer as a primer. The primers were used to detect EDTP mRNA were 5′-CCATTGTGGACCTCATGGTCG-3′ and 5′-TTGGTGCGCTTGCGGTGCTCC-3′, located between nucleotides +920 to +940 and +1535 to +1515, respectively. As an internal control, rp49 (ribosomal protein 49) mRNA was detected, using oligonucleotide primers (5′-GATCGATATGCTAAGCTGTCGCAC-3′ and 5′-CTCCTTGCTTCTTGGAGGAGACGC-3′) (*20*).

Whole-Mount In Situ *Hybridization to Ovaries and Embryos of* Drosophila *and Polytene Chromosome Hybridization—In situ* hybridization with digoxigenin-labelled probes (Boehringer Mannheim) was carried out according to the procedure developed by Tautz and Pfeifle (*21*), and modified by Ephrussi (*22*). Polytene chromosome was prepared from salivary glands of *Drosophila* Canton-S and processed for *in situ* hybridization via standard methods using a FITC-labelled DNA probe (fluorescein isothiocianate) (*23*).

Northern Blotting—Northern blotting was performed as described previously (*10, 24*) using a 616 bp-DNA fragment (nucleotide numbers +920 to +1535) of the coding region of the *Drosophila EDTP* as a probe. As an internal control, a fragment of the ribosomal protein 49 gene (rp49) transcript was amplified.

RESULTS

Identification of the Drosophila *Orthologue of the* Sarcophaga EDTP *Gene*—To obtain information about the *Drosophila* orthologue of the *Sarcophaga EDTP* gene, we searched for candidate *Drosophila EDTP* genes in the database. We found that the EST cDNA clone LD05323, which has an open reading frame of 163 amino acids, showed 50.9% homology with the partial amino acid sequence of *Sarcophaga* EDTP. Using this sequence information, we isolated two hybridization-positive clones via plaque hybridization. The clone containing the longer insert was then sequenced. This insert contained an open reading frame of 2,241 nucleotides corresponding to 747 amino acid residues. The deduced amino acid sequence was rich in serine and threonine residues, which accounted for 124 of 747 residues (Fig. 1A). This sequence showed approximately 54.1 % identity over the entire sequence with that of *Sarcophaga* EDTP. Furthermore, the putative enzymatic active site of protein tyrosine phosphatase was conserved between this protein and *Sarcophaga* EDTP, indicating that this cDNA is the *Drosophila EDTP* gene. The amino acid sequence of *Drosophila* EDTP was then compared with those of the flesh fly (*Sarcophaga peregrina*) (*10*), human (*25*), and mouse (*25*) putative homologues (Fig. 1B). These sequences were found to have similarities with each other and conserved those amino acid residues essential for a protein tyrosine phosphatase: Cys at position 427, and Arg at position 433 in the *Drosophila* EDTP amino acid sequence. The lengths of mammalian homologues were shorter than those of the insects by approximately two hundred amino residues. These data suggest that the *EDTP* gene is extensively conserved in diverse species, ranging from insects to mammals.

The Developmental Expression of Drosophila *EDTP mRNA—Sarcophaga* EDTP is only expressed in oogenesis and early embryogenesis, following which it is thoroughly degraded by cathepsin L. We therefore performed Northern blotting to examine the expression pattern of mRNA in the *Drosophila EDTP* gene during embryogenesis. As shown in Fig. 2, the amount of *Drosophila* EDTP mRNA detected during embryogenesis peaked in the early and middle embryonic stages. Conversely, no significant amounts of EDTP mRNA were detected in larvae or pupae. We also could not detect the mRNA in newly-emerged flies (mixture of male and female), probably because the EDTP mRNA expression level is lower than the limit of detection or we used immature young adult flies. This expression pattern resembled that of the

Sarcophaga EDTP gene (*10*). The results were confirmed by the following experiment of whole mount *in situ* hybridization using embryos at various developmental stages. As shown in Fig. 3, significant amounts of the mRNA signals were detected uniformly in the cytoplasm of eggs, and embryos at stages 1, 5 and 11, respectively, but they had mostly disappeared in the embryos at late embryonic stage 15. Next, we performed whole mount *in situ* hybridization using ovaries to obtain further information about the expression of *Drosophila EDTP* during oogenesis. The positive signals were detected in the cytoplasm of nurse cells at stage 5 and later stages. In addition, weak signals were detected in the cytoplasm of oocytes (stages 9 and 10) during the maturation stages of ovarian development (*26*) (Fig.4). These data suggest that EDTP is involved in the process of oocyte maturation during oogenesis.

Establishment of the Drosophila *Line Deficient in* EDTP *Gene*—We performed *in situ* hybridization of the *Drosophila* salivary gland polytene chromosome in order to identify the chromosomal region containing *Drosophila EDTP*. We found that this gene is mapped at position 54B, consistent with information from the Berkeley *Drosophila* Genome Project (BDGP) on the FlyBase web page (Fig. 5, A and B). There was no other sequence as similar to the *Sarcophaga EDTP* in the *Drosophila* genome, indicating that the EDTP gene is a single copy gene. Thus, the locus at 54B appears to be the only region to encode the gene for *Drosophila* EDTP in the entire *Drosophila* genome.

A

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GTTTTTTTAGTCGCGGCTGTTTGACGTTTGTCGCTTTCCTCCGTTGCGAATATAATATATTACGTAGCTCATTTTTATACAAACGGAATTACGAGCGCAACGACGACAGCAACACTAGTA -361
GCACTAATCGTAAGCGCAGGGGCCAAAAATTAAATTGCGTTTGCGGCCGCAAAGATTTGATGACGTCGCATACGCCGTCTTCTAGGGCGTAAAAAGCAAAGCAAAGCAAACAAACGCGAA -241
AGCGAAACGTGTAAACGGCGTAGAAGCGATAAACGCGACTCAAATACGCAGCAGATAAAATACAATACGCGAGAAGAGAAAAGTCACGGGAAATATTGTTCATATTCGGCGTCTTTCTGC -121
GAGCGTAAACGTGTGTGCGTGGGCTTGTGCTTTTGCCAGTGTCGTGTGTGTTAAGTGCCTGTGTGTGTGTGATTAAGAAGATATAAAGGAATATAACGGTAAATGCAGCGCCGCAAAA   -1
                                                                                                                        *
ATGTGCAGCCTGACGCCAAACCACATGGTAAACGTAACGCAGCAGCACCTACACGATCTATTGGAAACTTTCGAAAAAAAGTCCTTCGAGGCGGCGGCCTTTGAGGAAGGGACGGCGGAG  120
M  C  S  L  T  P  N  H  M  V  N  V  T  Q  Q  H  L  H  D  L  L  E  T  F  E  K  K  S  F  E  A  A  A  F  E  E  G  T  A  E    40
TACGACATCTCCAAAAAATGCGAATACCTGTTTAAGCTCGACTACAGCCTAATTGAGCTGGATAATACGAACGGATTGCTCAGTCCGCGATATCCTGGCCGAATACTCATCCCGGAATAT  240
Y  D  I  S  K  K  C  E  Y  L  F  K  L  D  Y  S  L  I  E  L  D  N  T  N  G  L  L  S  P  R  Y  P  G  R  I  L  I  P  E  Y    80
GAGCACGGGCACATGGCCAAGACGCTGGTACCGGGAAATGGACTCTTCGGGCAAGTTGGTGGGGGAGTGGGAGGTGGAGGCTCCTCGGGAACAACCGCCACTGCCACGCCTCTGAACAGC  360
E  H  G  H  M  A  K  T  L  V  P  G  N  G  L  F  G  Q  V  G  G  G  V  G  G  G  G  S  S  G  T  T  A  T  A  T  P  L  N  S   120
AGTGCAGGAAGCACCGGAAGTGAGGGTGTGGGCATCCAAGCCTTTGTGACCTTTGCCAATCCCCTGCAGACGCAACAACAGCATCCGCTCCAGCAACAATATCCCTCGCAGCAGATGCAT  480
S  A  G  S  T  G  S  E  G  V  G  I  Q  A  F  V  T  F  A  N  P  L  Q  T  Q  Q  Q  H  P  L  Q  Q  Q  Y  P  S  Q  Q  M  H   160
CCCCTCCACGCGCAATATCCCTCCCAGCAGCCACATCCACTCCAGCAGCAGCAGCAGCAGCCATCGCAACAGCAACCACAAAATACGATATACGAGGATCAGTATGATATCCAGCGAATG  600
P  L  H  A  Q  Y  P  S  Q  Q  P  H  P  L  Q  Q  Q  Q  Q  Q  P  S  Q  Q  Q  P  Q  N  T  I  Y  E  D  Q  Y  D  I  Q  R  M   200
CGGGAATTGGTAACGATGGCCAAATATGCGAGATGCCGTCAAAGATTCGCCGTGCCTGTGATTATGTATCGCGGAAAGTACATATGCCGCTCTGCCACGCTATCCGTCATGCCAGAAACC  720
R  E  L  V  T  M  A  K  Y  A  R  C  R  Q  R  F  A  V  P  V  I  M  Y  R  G  K  Y  I  C  R  S  A  T  L  S  V  M  P  E  T   240
TACGGCCGAAAAGTGGTGGACTATGCCTACGACTGCCTGAGTGGCGGCAATTACACCGCGCCAAACGGAGAAGAGAACGATGCTGACTCCACGGACGAGTCGCTGATCACCCACATGCAC  840
Y  G  R  K  V  V  D  Y  A  Y  D  C  L  S  G  G  N  Y  T  A  P  N  G  E  E  N  D  A  D  S  T  D  E  S  L  I  T  H  M  H   280
GACCAGGCGCAGTCGCAGTTCAGCTACGACGAAGTCATCAAGAGTGACATCCAGCTGCTGCATACGCTCAATGTCTCAACCATTGTGGACCTCATGGTCGAAAACCGCAAAATCAAATAC  960
D  Q  A  Q  S  Q  F  S  Y  D  E  V  I  K  S  D  I  Q  L  L  H  T  L  N  V  S  T  I  V  D  L  M  V  E  N  R  K  I  K  Y   320
TTCATGGCCGTTTCCTCGTCAGAGAAAGCGGATCCCAACAAGCACTATAAGAGCTTTAACCTTCTATCCCTGCCGTATCCGGGCTGTGAGTTCTTCAAAAAGTTCCGGGACAATAATTAC 1080
F  M  A  V  S  S  S  E  K  A  D  P  N  K  H  Y  K  S  F  N  L  L  S  L  P  Y  P  G  C  E  F  F  K  K  F  R  D  N  N  Y   360
ATGGCTCGCAACCTGCACTACAACTGGAAGCAAACGTTCAACGATGCGAATATCAACATTCCCAACATGGGACCCGCTGCGGATATCGATGTGGCGTGGTCGGAGTACCGGGATTGGGAT 1200
M  A  R  N  L  H  Y  N  W  K  Q  T  F  N  D  A  N  I  N  I  P  N  M  G  P  A  A  D  I  D  V  A  W  S  E  Y  R  D  W  D   400
CTGGTGGCAATCACCCAAAACTATTTGAGAGCTACACTGAAATACGTGCAAGAGGAAAACTCCGGCCTGCTGATTCACTGCATCAGCGGTTGGGATCGCACGCCACTGTTTGTCTCCTTG 1320
L  V  A  I  T  Q  N  Y  L  R  A  T  L  K  Y  V  Q  E  E  N  S  G  L  L  I  H  C  I  S  G  W  D  R  T  P  L  F  V  S  L   440
GTCAGGCTGTCTCTGTGGGCAGATGGACTCATCCATCAGTCGCTGAACGCCATGCAAATGGCCTATTTCACACTGGCCTACGACTGGTACCTGTTTGGCCATCAACTTCCCGATCGCCTG 1440
V  R  L  S  L  W  A  D  G  L  I  H  Q  S  L  N  A  M  Q  M  A  Y  F  T  L  A  Y  D  W  Y  L  F  G  H  Q  L  P  D  R  L   480
AAACGAGGCGAAGACATCATGTTCTTCTGCTTCCACGTGCTGAAGTTTATCACGGACGAGGAGTTCAGCATTGTGGAGCACCGCAAGCGCACCAAGACATCCAGCAGCAGCGGCAGTAGT 1560
K  R  G  E  D  I  M  F  F  C  F  H  V  L  K  F  I  T  D  E  E  F  S  I  V  E  H  R  K  R  T  K  T  S  S  S  S  G  S  S   520
GTAATAGTAATCAAATCCGATTGCTGCGACGATGAACCGCTCAAGGAAGACTACATCCTTTCGTTCGATCAAGATAGCAACGACAGCTACTCAAACTGTTCCAACTGTGATATGTCCATA 1680
V  I  V  I  K  S  D  C  C  D  D  E  P  L  K  E  D  Y  I  L  S  F  D  Q  D  S  N  D  S  Y  S  N  C  S  N  C  D  M  S  I   560
ACAGATAACTTCTATGCCACGACGCCGGCGCAAGTCAATCCGTTGACCAGCAGGTCGCCAAATCCGAAGAGATCTAGAACCAGCCCCATTTCAGTGCCCGGATCAAATGCGCGGCAAAGA 1800
T  D  N  F  Y  A  T  T  P  A  Q  V  N  P  L  T  S  R  S  P  N  P  K  R  S  R  T  S  P  I  S  V  P  G  S  N  A  R  Q  R   600
CAGGAGTCTACATCGTCCAATGGTAGCTGGCAGGTGGTTACCGACACGGGTTCAATTGACTCCATGATGAACGGCAGCTACATGATGCGCTTTGTGGCGCAACAGGCAGCCGATGGTGGC 1920
Q  E  S  T  S  S  N  G  S  W  Q  V  V  T  D  T  G  S  I  D  S  M  M  N  G  S  Y  M  M  R  F  V  A  Q  Q  A  A  D  G  G   640
GGCTCCTCAAACATTCCTTTATGCAATGGCGGCAATGGTTACCACTGCAGCATCAATGCAGCATCGAGTGGCAGTGGGAGCGGAAGTGGTAGCAGTATCAGTAACGGCAGCTCGACGCAC 2040
G  S  S  N  I  P  L  C  N  G  G  N  G  Y  H  C  S  I  N  A  A  S  S  G  S  G  S  G  S  G  S  S  I  S  N  G  S  S  T  H   680
GGTTTCGCAAACGGTTCCTCCAAAGACGTAGGCGGCAGCACTATGGCCAGCAAGCAATGCATCAACTTACGAAAGCAACGCCTGAATGCAGTGCGCGCCATTTTTATACAAGCCTACGGC 2160
G  F  A  N  G  S  S  K  D  V  G  G  S  T  M  A  S  K  Q  C  I  N  L  R  K  Q  R  L  N  A  V  R  A  I  F  I  Q  A  Y  G   720
AAGACGATTGGACTGAAATTCAAGGAGGGCTCATCCATGAACCTGGCCACGTTCATTGGGAACCTGGCGGACCAACTGTTTTGAG AAAGGATTAGCAGCTATGACCGTTTGTTAGATTT 2280
K  T  I  G  L  K  F  K  E  G  S  S  M  N  L  A  T  F  I  G  N  L  A  D  Q  L  F  *                                          747
CATCAACATTGTTTGTTTCTTGTTAAGTGCTACGCTTTCAACCAACGACTGTATTATAGACCTGTTAAATTGCGTTCTTTAGTATCTGTTGACGATTGTGTAGTGTAACGGATTACTGAT 2400
TACCGATAAGCATCGAAAGAACCAGGCGATCGTAGGCAACGAGCAAACAAAATCCTGTCGTCCTTCTGCCCCGGCTAAACCGCTCTCCTGTTCTCTAGGTTCTCCCGCTCCTTGTTTGTT 2520
TAGTTATTAGTTCTTCGAGAATGCAAAGCAAGTGAAATATAAAATGCTATTTGGATTATACAATAGTTTTAAGTTGTAATTTATA GTAATGTATTTTTTAATAAACTTATGTGCATTTT 2640
ATACG-polyA
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Fig. 1. Continued.

B

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D  MCSLTPNHMVNVTQQHLHDLLETFEKKSFEAAAFEEGTAEYDISKKCEYLFKLDYSLIELDNTNGLLSPRYPGRILIPEYEHGHMAKTLV  90
S  -----MSTTIEVTQKDLHDLLEIFEKKPFDAGSCEKGSLEEDITNRCEFLFKKDYTLIELDNSNGVLSPRYPGRIFIPEYEHGHMTSTIT  85
H  ----------------------------------------------------------------------------------------
M  ----------------------------------------------------------------------------------------

D  PGNGLFGQVGGGVGGGGSSGTTATATPLNSSAGSTGSEGVGIQAFVTFANPLQTQQQHPLQQQYPSQQMHPLHAQ-YPSQQPHPLQQQQQ 179
S  PNNTNLG-TQATNHASPQSGKNGNGLPHNPPSPSNSIASDSTTP-ST-SSGIQSFVTFANSATNDQQTSNPLYNQNYFPQLQHPIASTSN 172
H  ----------------------------------------------------------------------------------------
M  ----------------------------------------------------------------------------------------

D  QPSQQQPQNTIYEDQYDIQRMRELVTMAKYARCRQRFAVPVIMYRGKYICRSATLSVMPETYGRKVVDYAYDCLSGGNYT--APN-GEEN 269
S  SISNNTANDTIYEDLYDVNKVRELITMAKYARCRQRFAVPVIMYRGKYICRSATISINAETYGRKAVDYAYDCINGTNYSERANHVADDE 262
H  ----------------------------MARCRGRFVCPVILFKGKHICRSATLAGWGELYGR-S-GYNYFFSGGAD-DAWA-DVEDVT  57
M  ----------------------------MARCRGRFVCPVILFKGKHICRSATLAGWGELYGR-S-GYNYLFSGGAD-DTWA-STEDVT  57

D  DADSTDESLITHMHDQAQSQFSYDEVIKSDIQLLHTLNVSTIVDLMVENRKIKYFMAVSSSEKADPNKHYKSFNLLSLPYPGCEFFKKFR 356
S  NNDNSEDSLMNPPLN-GQSQFSYEEVIKSDMQLLNSLNVTTIIDLMVENRKIKYFMAVSSSEKADPENHYESFNLVSLPYPGCEFFKKFR 351
H  EEDCALRS-GDTHLFD-KVR-GYD--IK----LLRYLSVKYICDLMVENKKVKFGMNVTSSEKVDKAQRYADFTLLSIPYPGCEFFKEYK 138
M  EEDFVLRS-GDTHLFD-KVR-GYD--IK----LLQYLSVKYICDLMVENKKVKFGMNVTSSEKVDKAQRYANFTLLSIPYPGCEFFKEYK 138

D  DNNYMARNLHYNWKQTFNDANININPNMGPAADIDVAWSEYRDWDLVAITQNYLRATL-KYVQEENSGLLIHCISGWDRTPLFVSLVRLSL 445
S  DNNYMAEGLHYNWKQSFNDATLSVPESGPARELNIDWSDYKNWDLVCITQNYVKTCL-KYIKDEKSGLLIHCISGWDRTPLFISLIRLSL 440
H  DRDYMAEGLIFNWKQDYVDAPLSIPDF-LTHSLNIDWSQYQCWDLVQQTQNYLKLLLSLVNSDDDSGLLVHCISGWDRTPLFISLLRLSL 227
M  DRDYMAEGLIFNWKQDYVDAPLNIPNF-LTQSLNIDWSQYQSWDLVQQTQNYLKLLLFIMNRDDDSGLLVHCISGWDRTPLFISLLRLSL 227

D  WADGLIHQSLNAMQMAYFTLAYDWYLFGHQLPDRLKRGEDIMFFCFHVLKFITDEEFS-IVEHRKRTKTSSSSGSSVIVIKSDCC-DD-E 532
S  WADGLIHQSLNPYQMTYFTIAYDWYLFGHQLPDRIKRGEDIMFFCFHVLKYIMGEEFS-IAEQRRRTKTSSSSGSSIVVLKCDSG-DESA 528
H  WADGLIHTSLKPTEILYLTVAYDWFLFGHMLVDRLSKGEEIFFFCFNFLKHITSEEFSALKTQRRKSLPARDGGFTLEDICMLRRKDRGS 317
M  WADGLIHTSLKPAEILYLTVAYDWFLFGHMLVDRLSKGEEIFFFCFNFLKHITSEEFC-LKTQRRKSLPTRDAGFTVEDICMLRHKDRGS 316

D  PLKEDYILSFDQDSNDSYSNCSNCD-MSITDNFYA-TTP---------AQ--V----NPL----------------------TSRSPNPK 583
S  SLKE-SLL-FDQDSNESFSNSSNCDNLPVTDGYYAPVTG----------------------PSTSTTAT-AQGSSVA---GNNPLTNRSPNLK 583
H  TTSLGSDFSLVMESSPGATGSFTYEAVELVPAG-APTQAAWRKSHSSSPQSVLWNRPQPSEDRLPS-QQGLAEARS-SSSSSSNHSDNFF 404
M  TTSLGSDFSLVLEHSPGAVGSFSYETVELAPAG-APTQAAWRKSHSSSPQSMLWSRPQPSEERLPS-HHGLTEAKS-SSSSSSNHSDNFF 403

D  RSRTSPISVPG--SNARQR-QESTSSN-GSWQVVTDTGSIDSMMNGSYMMRFVAQQAADGGGSSNIPLCNGGNGYHCSINAASSGSGSGS 665
S  RSKTSPISVPG-ASAARQR-HESYSSTGGSWQVVTVTGSIDST-NGSLTKRMTEQRDD----------------SDNRDTSDNGENS 661
H  RMGSSPLEVPKPRSVDHPLPGSSLSTDYGSWQMVTGCGSIQ--------ERAVLHTDSSLPFSFPDELPN-----SCLLAALSDRET--- 478
M  RMGSSPLEVPKPRSVDHPLPGSSLSTDFGSWQLVSGCGSIQ--------DRPVLHTDSSLPFSFQDELPN-----SCLLTALSDRET--- 477

D  GSSISNGSSTHGFANGSSKDVGGSTMA-SKQCI-NLRKQRLNAVRAIFIQAYGKTIGLKFKEGSSMNLATFIGNLADQLF---------- 743
S  GNNSSNTASTSKEEESSSKPPDTPRM----------QRLNAVRAIFIQSYGKQIGLKFKEGSSTNLAT-LIGLR-------------- 724
H  ---------------------------------------RLQEVRSAFLAAYSSTVGLRAVAPS---PSGAIGGLLEQFARGVGLRSISS 526
M  ---------------------------------------RLQEVRSAFLAAYSSTVGLRAATPS---PSGAIGGLLEQFARGVGLRGTST 525

D  ---                                                                                        743
S  ---                                                                                        724
H  NAL                                                                                        529
M  STL                                                                                        528
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Fig. 1. **Cloning of cDNA for the *Drosophila EDTP*.** (A) Nucleotide and deduced amino acid sequence of the cDNA encoding *Drosophila* EDTP. The deduced amino acid sequence of *Drosophila* EDTP is shown below the nucleotide sequence. Nucleotide and amino acid numbers starting from the first methionine codon are shown to the right of each line. A putative active site of protein tyrosine phosphatase is shown in the open box. Serine and threonine residues are shown in shaded boxes. The poly(A) addition signal is double underlined. The asterisks show the termination codons. (B) Comparison of the amino acid sequence of *Drosophila* EDTP with EDTPs from other organisms. The amino acid sequence of the *Drosophila* EDTP (AB036800, first row) is compared with those of *Sarcophaga* (AC013617, second row), putative *Homo sapiens* (BC018294, third row) (*25*), and putative *Mus musculus* EDTP (BC018294, fourth row) (*25*). The numbers on the right refer to positions in each sequence. Amino acid residues are numbered starting from the first methionine residue. Gaps have been added to indicate maximal sequence similarity. Residues conserved in more than three sequences are shown in boldface type. The putative active site of protein tyrosine phosphatases is shown in the shaded box. Arrowheads indicate cysteine and arginine residues essential for protein tyrosine phosphatase activity.

We identified several P-element insertions within the *Drosophila EDTP* locus in the fly stocks of BDGP, namely EP(2)0922, EP(2)2075, EP(2)2389, and l(2)k6310b, which are inserted in the region corresponding to 5′-UTR of the *EDTP* transcript. All of these strains are homozygous viable. However, RT-PCR experiments showed that *EDTP* transcripts were clearly detected in these homozygous adult flies (data not shown). In order to investigate

the function of the *Drosophila EDTP* gene during development, we took advantage of the fact that transposase-catalyzed excision of the P-element is often imprecise, resulting in the deletion of flanking sequences (*13*). The *EDTP* allele, EDTP$^{\Delta}$, was isolated by imprecise excision of a P-element from line EP(2)0922, which was inserted in the genomic region corresponding to the 5′-UTR of the *Drosophila EDTP* gene (indicated as an arrow in Fig. 6A). Introduction of transposase into the EP(2)0922 line led to the isolation of one homozygous lethal mutation. The deletion junction was cloned by PCR, and sequencing of the resulting genomic fragment revealed that this excision deleted a 757 bp fragment containing most of the first exon, and part of the first intron (Fig. 6, A and B). Nucleotides corresponding to –472–+118 of the mRNA were thus deleted, resulting in the loss of 5.2% (codons 1–39) of the predicted coding amino acid sequence of *Drosophila* EDTP. Additionally, introduction of transposase into l(2)k6310b carrying one P-element led to one homozygous lethal mutation (data not shown). These two mutants, showing homozygous lethals, fell into a single complementation group. As expected, the RT-PCR experiments showed that *EDTP* transcripts were not detected in homozygous embryos derived from EDTP$^{\Delta}$ (Fig. 6C). It is unlikely that any of the small EDTP protein derived from truncated small mRNA would remain enzymatically active because the primer pairs were designed to amplify the region corresponding to the conserved amino acid sequences of the active site of protein tyrosine phosphatase. We therefore concluded that EDTP$^{\Delta}$ is a null allele of the *Drosophila EDTP* locus.

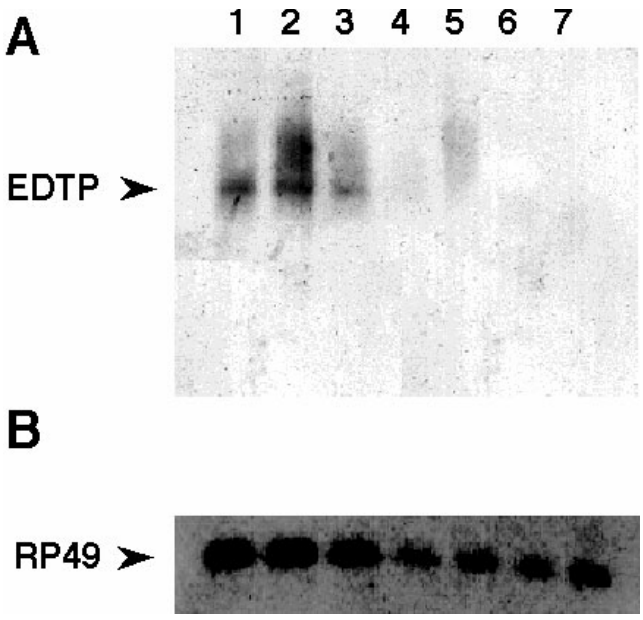

Fig. 2. **Northern blot hybridization of *Drosophila* EDTP.** Total RNA was extracted from various sources, and 20 μg of each extract was analyzed by RNA blotting. The probes used were a fragment of *Drosophila* EDTP cDNA (nucleotides +920 to +1535) (A) and a cDNA fragment of *Drosophila* ribosomal protein 49(RP 49) (a PCR product) (B). Source of RNA: lane 1, embryos at an early developmental stage; lane 2, embryos at an intermediate developmental stage; lane 3, embryos at a late developmental stage; lane 4, larvae; lane 5, pupae at an early developmental stage; lane 6, pupae at a late developmental stage; lane 7, adult flies (mixture of male and female).

To determine the lethal period of EDTP$^{\Delta}$, embryos from CyO-pAct-GFP/EDTP$^{\Delta}$ carrying GFP-balancer were collected and scored by counting the number of hatchings. Since GFP gene expression driven by the Actin 5C promoter can be seen under UV light in first instar larvae, we can distinguish between homozygotes and heterozygotes. We found that 24% of the progeny failed to hatch, matching the homozygous percentage for EDTP$^{\Delta}$. 76% of the progeny hatched as first instar lavae. Among the first instar larvae, however, 6.5% of the progeny which did not show GFP fluorescence died soon after hatching (homozygotes, EDTP$^{\Delta}$/EDTP$^{\Delta}$). These results indicate that homozygous deletion of the *EDTP* gene is semi-lethal embryonically. Cuticle patterings from these unhatched embryos were not indistinguishable from wild type, suggesting that the EDTP$^{\Delta}$ mutation in the *Drosophila EDTP* gene does not have a significant detectable effect on embryo segment polarity (*24*). Another independently established mutant line from l(2)k6310b exhibited similar phenotypes (data not shown).

Since *EDTP* was strongly expressed during oogenesis (Fig. 4), we expected that deletion of the *EDTP* gene might also affect oogenesis. To investigate the function of *EDTP* in oogenesis, we generated germline clones of a null allele of *EDTP* using the FLP-FRT-*ovo*^{D} system (Fig. 7).

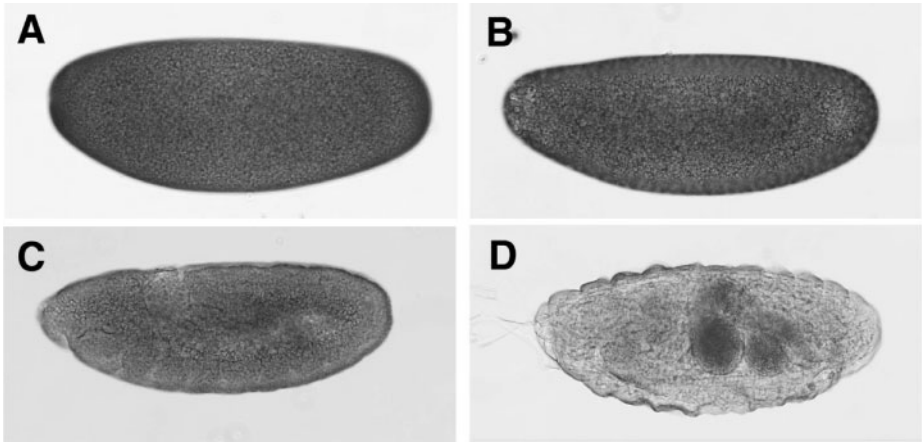

Fig. 3. **mRNA *in situ* hybrididation pattern of *Drosophila* EDTP in embryos.** A 616 bp fragment of *Drosophila* EDTP cDNA (nucleotides +920 to +1535) was used to make antisense RNA. (A) Freshly laid egg (stage 1). (B) Embryo at cellular blastdermal stage (stage 5). (C) Embryo at an intermediate developmental stage (stage 11). (D) Embryo at a late developmental stage (stage 15). The bar indicates 50 μm.

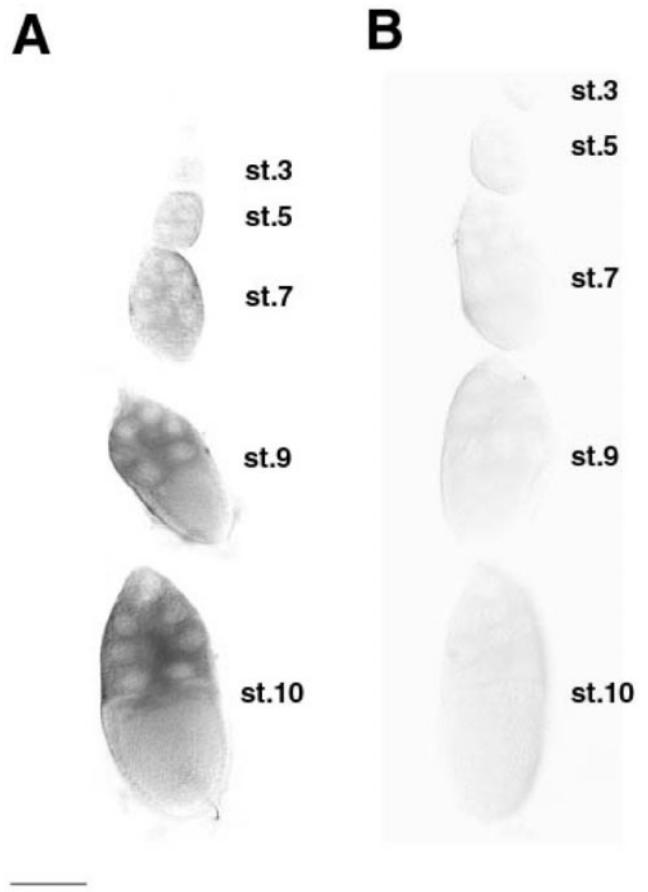

Fig. 4. **mRNA *in situ* hybridization pattern of *Drosophila* EDTP in the ovary.** Ovaries were dissected and the 616 bp fragment of *Drosophila* EDTP cDNA (nucleotides +920 to +1535) was used to make antisense RNA (A). The corresponding sense strand specific probe was used in (A). (B) The figure shows a picture of a single ovariole containing stage 3 (st3) stage 5 (st5), stage 7 (st7), stage 9 (st9), and stage 10 (st10) egg chambers, respectively. The bar indicates 50 μm in both A and B.

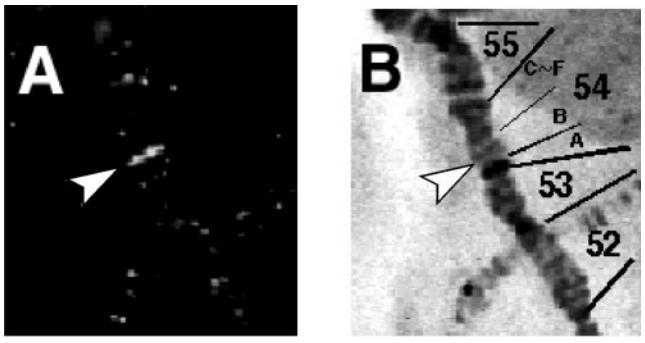

Fig. 5. **Chromosome mapping of the *Drosophila EDTP* gene.** Salivary gland polytene chromosomes were hybridized with digoxigenin-labeled *Drosophila* EDTP cDNA (nucleotides +920 to +1535). The hybridized regions were detected using a FITC-conjugated anti-digoxigenin antibody. The arrowheads indicate a signal detected at the 54B locus in dark field (A) and bright field (B) and visualized by Giemza staining.

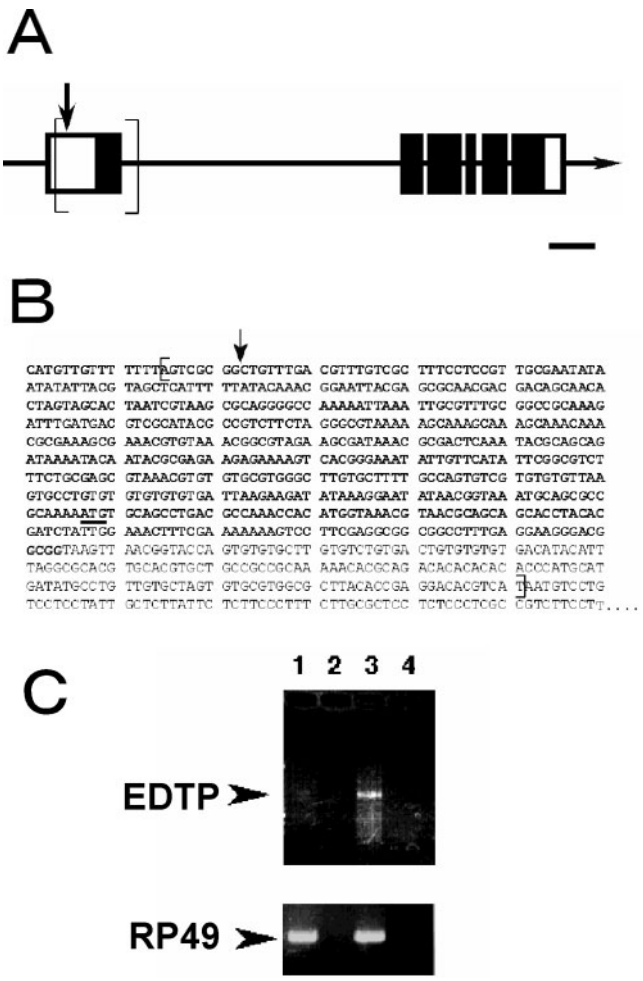

Fig. 6. **Generation of a null allele of the *Drosophila EDTP* gene.** (A) Organization of the *Drosophila EDTP* gene. Exons are indicated by boxes in which coding regions are shown in black and UTRs are shown in white. The arrow indicates the EP(2)0922 insertion, and the region deleted (Δ) in $EDTP^{\Delta}$ is indicated in brackets. The bar indicates 1 kb (B). Wild-type genomic DNA sequence at the junctions of the deletion. Exon sequences are shown in bold and intron sequences are shown in non-bold lettering. The P-element is inserted between base pairs corresponding to nucleotides –464/–463 of the mRNA. The imprecise P-element excision in $EDTP^{\Delta}$ resulting in the deletion of 757 bp of genomic DNA is shown in brackets and corresponds to nucleotides –472–+118 and including the initiation codon of the mRNA. (C) CyO, an actin-GFP balancer, was used to identify homozygous $EDTP^{\Delta}$ embryos. RT-PCR was performed using total RNA from $EDTP^{\Delta}$ embryos (lane 1, RT+; lane 2, RT–) and wild-type embryos (lane 3, RT+, lane 4, RT–).

When compared to wild-type controls (Fig. 7B), the sizes of the mutant ovaries were smaller. In the ovaries, we observed egg chambers from stage 1 to 4, but not from stage 5 and later stages. Furthermore, no mature oocyte was observed (Fig. 7E). These results suggest that *EDTP* is an essential gene in ovariole development and plays a role in stages later than stage 5. Judging from *in situ* hybridization data on ovaries, EDTP transcripts were expressed from stage 5 and later stages (Fig. 4), which coincides with the defects detected in the mutant ovaries. These data indicate that EDTP is involved in the process of oogenesis including oocyte maturation.

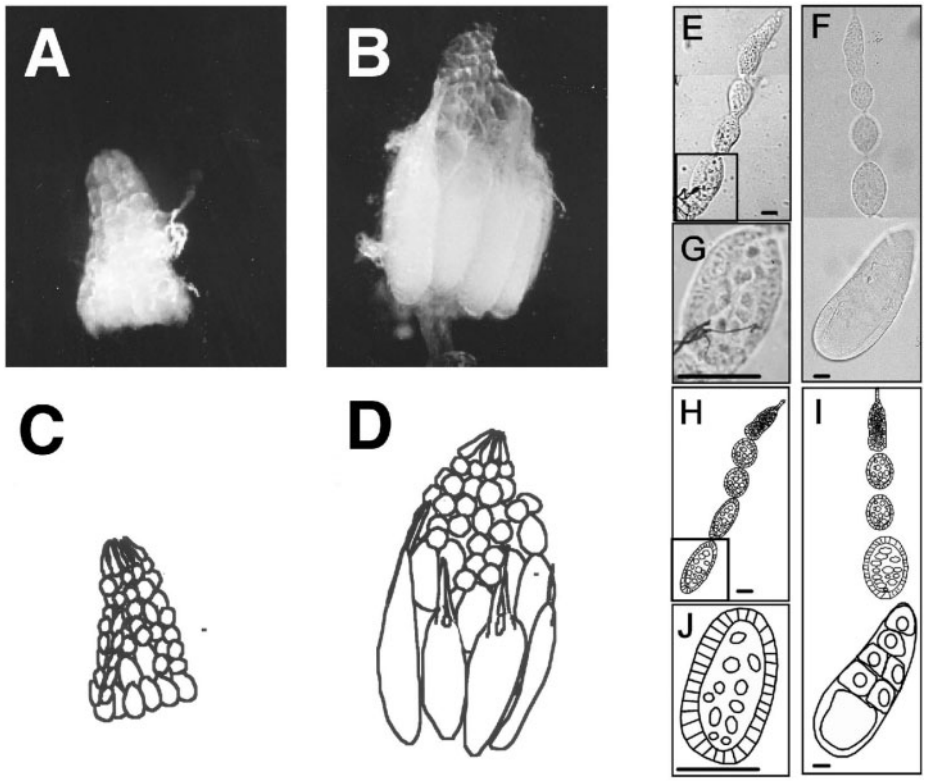

Fig. 7. **Ovarian effect of *Drosophila EDTP* gene mutation.** Mutant ovaries were consistently smaller in size (A and C; C is a schematic drawing of A) compared to those from wild-type flies (B and D; D is a schematic drawing of B). Ovaries were fixed and stained with Hoechst in order to analyze egg-chamber development within individual ovarioles. (E) In the mutant ovary, egg chambers from stage 1 to 4, but not from stage 5 and later stages were observed. No mature oocyte was observed. (F) Wild-type egg chambers show one oocyte and a regular array of large polyploid nurse cell nuclei developing within each follicle. (G) A magnified view of (E). (H) is a schematic drawing of (E). (I) is a schematic drawing of (F). (J) is a schematic drawing of (G). The bar indicates 50 μm.

DISCUSSION

EDTP is a novel member of the soluble protein tyrosine phosphatase family, and is rich in serine and threonine residues. In this paper, we have identified the *Drosophila* orthologue cDNA of EDTP. We showed that *Drosophila EDTP* transcripts were detected in oogenesis and embryogenesis and that the *Drosophila EDTP* gene is essential in early development.

Some gene expressions are shown to be repressed during oogenesis in *Drosophila*. Thus, the accumulation of mRNA for *EDTP* gene does not necessarily ensure its translation. However, the severe defects of *EDTP* deletion mutants in oogenesis strongly suggest translation into EDTP protein and its requirement in this stage (*10*, *27*). The expression of EDTP at the protein level should be further examined, but it is highly probable that the EDTP protein is present in oogenesis, considering the fact the *Sarcophaga* EDTP protein was purified from an egg extract (*10*).

In two independently established mutant lines from EP(2)0922 and l(2)k6310b deficient in *EDTP* gene, a small percentage of homozygous larvae with deleted *EDTP* genes survived through embryogenesis, although *EDTP* transcripts were only detected during oogenesis and embryogenesis and not in subsequent developmental stages. One possibility is that the function of the *EDTP* gene in embryogenesis may be partially compensated for by other protein phosphatases, because it is known that protein phosphatases generally have relatively broad substrate specificity (*28*, *29*). The substrate protein of EDTP can also be the substrate of other protein phosphatases. The future identification of substrate proteins of EDTP using, for example, a two-hybrid system, could help to determine the various molecular interactions and signaling cascades involving EDTP. Recently, EDTP has been identified in association with 3 ribosomal proteins including RpS9 and a metabolic protein, Cyp28d1 (*30*). EDTP may therefore be involved in protein synthesis or the metabolic processes required for the dramatic changes which take place during oogenesis and early embryogenesis. Another possible strategy is to determine the genetic interactions of EDTP with mutants in the genes known to play roles in oocyte development for understanding signaling cascades involving EDTP.

An interesting feature of EDTP is the rapid degradation after fertilization; *Sarcophaga* EDTP is removed rapidly from ooplasm by cathepsin L digestion at an early stage of embryonic development, and is probably a prerequisite for the subsequent embryonic development of *Sarcophaga* (*10*). The *cathepsin L* gene expression in *Drosophila* and *Sarcophaga* have been shown to be similar in oogenesis and embryogenesis (*11*, *31*), arousing our interest in a genetic linkage between *EDTP* and *cathepsin L* in *Drosophila*.

So what is the possible function of mammalian EDTP? While this remains to be fully established, a number of interesting findings should be noted; first, the lengths of the mammalian EDTP homologues were shorter than those of the insects by approximately two hundred amino acid residues. This suggests that some function based on the deleted amino acid residues might have been lost during evolution. Second, the deduced mammalian amino acid sequences showed significant homology throughout the entire range of sequences with those of insect EDTPs; third, the putative enzymatic active sites of protein tyrosine phosphatases were conserved in the diverse organisms. Taken together with these data, it is suggested

that the enzymatic function of the *EDTP* gene has been conserved during evolution, even though the mechanisms of oogenesis and embryogenesis in insects and mammals have developed differently. Future investigation of the roles of EDTP in protein biosynthesis and metabolism may elucidate the common molecular cascades conserved during the early development of divergent animal species.

The nucleotide sequences reported in this paper have been submitted to the GenBankTM/EBI Data Bank with accession number AB036800.We thank K. Nakahara for critically reading the manuscript. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (K. J. H.), Sankyo Foundation of Life Science (K. J. H.), Core Research for Evolutional Science and Technology of the Japan Science and Technology Corporation (S. N.), Japan Science for the Promotion of Science (S. Y.), Uehara Memorial Foundation (S. Y. and K. J. H.). All fly stocks were provided by Bloomington Stock Center at Indiana University.

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