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A Gene Related to the Proto-Oncogene *fps/fes* Is Expressed at Diverse Times during the Life Cycle of *Drosophila melanogaster*

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The proto-oncogene *fps/fes* encodes a distinctive type of protein-tyrosine kinase. We identified a *Drosophila* gene (*dfps85D*) whose product resembles the proteins encoded by vertebrate *fps/fes* and the closely related gene *fer*. *dfps85D* is located at chromosomal position 85D10-13 and is unlikely to correspond to any previously defined genetic locus in *Drosophila melanogaster*. Expression of the gene is entirely zygotic in origin and occurs throughout the life cycle. But hybridization in situ revealed that the pattern of expression is specialized and evolves in a provocative manner. The most notable feature of expression is the diversity of developmental periods, tissues, and cells in which it occurs. In some tissues, expression is transient; in others, it is continuous. Expression occurs in both mitotic and terminally differentiated tissue and, at various times in development, is prominent in imaginal disks, gut, muscle, testes, ovaries, retina, and other neural tissues. It appears that the use of *dfps85D* is more diversified than that of other *Drosophila* protein-tyrosine kinases reported to date and contrasts sharply with the restricted expression of *fps* itself in vertebrates. The detailed description of expression provided here will help guide the search for mutants in *dfps85D*.

Protein-tyrosine kinases (PTKs) provide diverse functions in the governance of cellular phenotype (20). These enzymes fall mainly into two varieties: those that span the plasma membrane and serve as cell surface receptors for growth factors, and those that are located in the cytoplasm, often in association with membranes. With the exception of cell surface receptors that bind known ligands, the physiological purposes of PTKs remain enigmatic. One approach to this enigma is to seek mutations in genes of *Drosophila melanogaster* that encode PTKs. Seven such genes have been described to date: counterparts of the proto-oncogenes *src* (37) and *abl* (18); a previously unidentified PTK gene, designated *Dsrc28C* to denote its kinship to *src* and its chromosomal location (11); and four genes that encode cell surface receptors, including sevenless (12), torso (40), the gene for the insulin receptor (31), and *DER*, which resembles the vertebrate genes *erbB1* and *NEU* (26).

Among the cytoplasmic PTKs is a protein encoded by a gene known as either *fps* (avian isolates) or *fes* (mammalian isolates) (15). Versions of *fps* and *fes* were first encountered as retroviral oncogenes (v-*fps* and v-*fes*) but have since been isolated as proto-oncogenes from several vertebrate species. In addition, a closely related gene designated *fer* has been identified in rodent and human DNA (16, 30).

Here we report the isolation of a *Drosophila* gene that is related to vertebrate *fps/fes* and *fer*, and we describe the use of hybridization in situ to chronicle expression of the gene during the *Drosophila* life cycle. The expression of *dfps85D* is exceptionally diversified and dynamic when compared with that of vertebrate *fps* and the other PTKs of *D. melanogaster* studied to date. The description of expression given here will help guide the search for mutants with mutations in *dfps85D*.

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MATERIALS AND METHODS

Analysis of DNA and molecular cloning. Procedures and sources of most of the reagents have been described previously (22). The probe for analyzing Southern blots and screening genomic libraries was prepared with a 1.3-kbp *Pvu*II-*Sma*I fragment representing the bulk of v-*fps* (14). Hybridization was performed at relatively low stringency as follows. Filters were preincubated at 42°C for 4 to 12 h in hybridization solution (35% formamide [vol/vol], 3× SSC [1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0], 20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.0], 2.5× Denhardt solution [1× Denhardt solution is 0.2 mg of Ficoll per ml, 0.2 mg of polyvinylpyrrolidone per ml, 0.2 mg of bovine serum albumin per ml], 0.2 mg of salmon sperm DNA per ml). Filters were hybridized in fresh hybridization solution containing 2×10^5 to 6×10^5 cpm of radioactively labeled probe per ml at 42°C for 36 to 48 h. The filters were rinsed in 4× SSC–0.1% sodium dodecyl sulfate (SDS) two times for 5 to 10 min each at room temperature to remove excess hybridization solution and probe. Then they were rinsed in 1× SSC–0.2% SDS–0.1% sodium pyrophosphate for 2 to 4 h at 50°C.

Libraries of cDNAs in lambda bacteriophage prepared with polyadenylated RNA from pools of either embryos (2 to 24 h after oviposition) or heads of adult flies were provided by L. Kauvar (32) and G. Rubin, respectively. A genomic clone containing a portion of *dfps85D* (Fig. 1A) was used to screen a cDNA library representing embryonic RNA (2 to 24 h after oviposition). Additional screening was performed with an initial cDNA clone as the probe. Multiple isolates were subjected to restriction mapping and nucleotide sequencing. Clones of various lengths were obtained from the

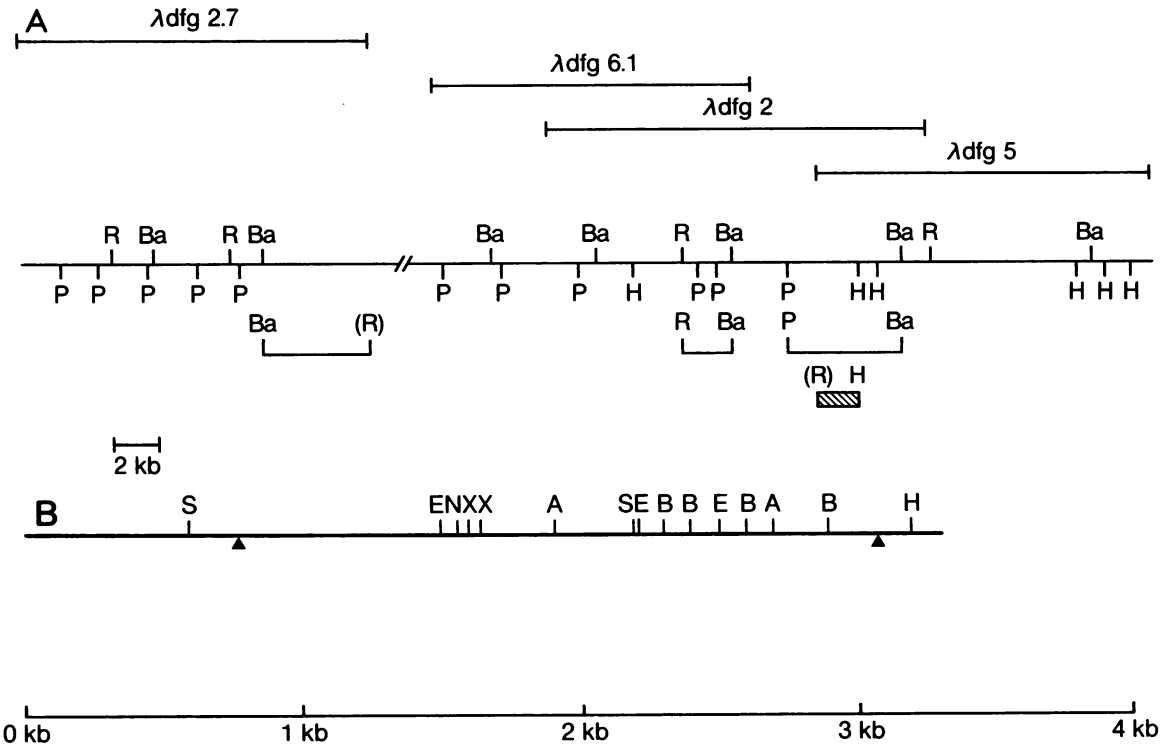

FIG. 1. Molecular clones representing *dfps85D*. (A) Genomic clones. The diagram illustrates the topographies of four clones representing portions of *dfps85D*. Restriction sites are designated as follows: Ba, *Bam*HI; H, *Hin*dIII; P, *Pst*I; and R, *Eco*RI [*Eco*RI sites shown in parentheses (R) were generated by cloning]. Sites not yet mapped include *Hin*dIII in λdfg2.7 and λdfg6.1 and *Pst*I in λdfg5. The hatched box designates a restriction fragment used as probe on Northern blots and to isolate the first batch of cDNAs. Bars below the restriction map designate genomic fragments that hybridized with cDNA clones. (B) cDNA clone. The diagram summarizes the topography of the longest cDNA representing *dfps85D* in embryonic RNA. Restriction sites are designated as follows: A, *Acc*I; B, *Bgl*II; E, *Eco*RV; H, *Hin*dIII; N, *Nco*I; S, *Sph*I; X, *Xmn*I. The triangles demarcate a fragment used as a probe for hybridizations in situ to tissue sections and spreads of chromosomes.

embryonic library, but all overlapped. We report here only on the longest of the clones (Fig. 1B).

Nucleotide sequencing of DNA was performed according to published protocols (34). All sequence was confirmed by analyzing both strands of DNA.

Cytogenetic analysis. A probe was prepared with the DNA fragment indicated in Fig. 1B by using nick translation with biotinylated-dUTP (Bio16-dUTP; Enzo Biochem) and then hybridized to squashed preparations of salivary chromosomes from the Canton-S strain of *D. melanogaster*. Hybridization was detected with a conjugate of strepavidin and horseradish peroxidase. Details of these procedures have been reported previously (47).

Analysis of RNA by Northern (RNA) blotting and hybridization in situ. RNA was extracted at various stages of development and analyzed by gel electrophoresis and Northern blotting as described previously (22). The probe was prepared with the fragment indicated in Fig. 1A.

Expression of *dfps85D* at various times during the life cycle of *D. melanogaster* was analyzed by hybridization in situ, using a modification of the technique of Hafen et al. (13) as described by Kornberg et al. (25) with one addition. To reduce nonspecific binding of probe to cuticle, especially in late pupae and adults, we acetylated sections from postembryonic stages immediately after they had been treated with pronase and fixed by the method of Hayashi et al. (17). The probe was prepared with the cDNA fragment indicated in Fig. 1B, using nick translation with [^{35}S]dATP. Autoradiographic exposures were from 2 to 4 weeks, after which the sections were stained with Giemsa and mounted with Permount (Fisher). The analysis employed serial sections of samples from various periods in development. Controls for nonspecific hybridization were performed in two ways: by pretreating the sections with pancreatic RNase A before hybridization, and by using probe prepared with plasmid vector alone. Photomicroscopy was performed with a Zeiss Axiophot microscope. Selected sections were chosen to illustrate the principal conclusions.

Nucleotide sequence accession number. The nucleotide sequence reported here has been entered in the EMBL Nucleotide Sequence Data Library under accession number X52844.

RESULTS

Isolation of *Drosophila* gene related to *fps/fes*. Hybridization at relatively low stringency with a radioactive probe representing avian v-*fps/fes* detected a number of restriction fragments in Southern blots of DNA from *D. melanogaster* (data not shown). We then used the same probe and conditions of hybridization to screen a genomic library of *Drosophila* DNA in lambda phage and isolated multiple clones that represented genes encoding PTKs. Among these were *Dsrc28C* (11) and *DER*, a gene related to *erbB1* (26). In addition, we identified a gene that is the subject of this report, that eventually proved to be related to *fps/fes*, and that for convenience is designated here as *dfps85D*.

Four genomic clones representing *dfps85D* were isolated,

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   1  TAA CCA ATT CGA GAT ACT CGG CGC TGA GCT CCA TTC GCG ATT TTT GAG TTC TGT GGC CAT GTG AAG TGC ACG CAT CTC GAA GTG CCA AAA
  91  TAA GTT CTG ATA AAG CCT TCA AAA CGA TAA CCA GCC TTA TTC AAT TAT GTT TGT GCC AAG AAC TGT GCT CTA GTT ACG CGA TTT GCA AGT
 181  GAA ATC CGA CGG CAA AGA AAT TCT GCG AGA GCC GCG ACC CAA ATA GGG AGT AAC GAG AGC ATC

       Met Gly Phe Ser Ser Ala Leu Gln Ser Arg Ala Ala Ala His Glu Ala Leu Ile Val Arg Gln Asp Ala Glu Leu Arg Leu Glu Leu Met Glu Thr Met
 244   ATG GGC TTC TCA TCA GCC CTC CAA AGT CGC GCC GCC CAC GAG GCG CTG ATC GTC CGC CAG GAT GCC GAA CTC CGG CTA ATG GAG ACA ATG

       Lys Arg Ser Ile Gln Met Lys Ala Lys Cys Asp Lys Glu Tyr Ala Ile Ser Leu Thr Ala Val Ala Gln Gln Gly Leu Lys Thr Asp Arg
 334   AAG CGA TCC ATC CAG ATG AAG GCC AAG TGC GAC AAG GAG TAC GCC ATC AGC CTG ACC GCT GTG GCC CAG CAG GGT CTC AAG ACT GAT CGG

       Ala Asp Glu Met Gln Gly Ser Leu Ile Ser Lys Ser Trp Arg Ser Tyr Met Asp Glu Leu Asp His Gln Ala Lys Gln Phe Lys Phe Asn
 424   GCT GAC GAA ATG CAA GGC AGC CTG ATC TCC AAA TCC TGG CGA TCC TAC ATG GAT GAA CTG GAC CAC CAG GCC AAA CAG TTC AAG TTC AAC

       Ala Glu Gln Leu Glu Val Val Cys Asp Lys Leu Thr His Leu Ser Gln Asp Lys Arg Lys Ala Arg Lys Ala Tyr Gln Glu Glu His Ala
 514   GCG GAG CAG CTG GAG GTC GTC TGC GAT AAG TTG ACC CAC CTC TCG CAG GAC AAA CGG AAG GCC AGA AAG GCC TAC CAG GAG GAG CAT GCC

       Lys Ile Ala Ala Arg Leu Asn His Leu Thr Asp Glu Val Val Arg Lys Lys Ser Glu Tyr Gln Lys His Leu Glu Gly Tyr Lys Ala Leu
 604   AAG ATC GCA GCA CGC CTG AAT CAT CTC ACC GAC GAG GTG GTG CGC AAG AAA TCT GAA TAC CAA AAA CAT TTG GAG GGC TAC AAG GCG CTG

       Arg Thr Arg Phe Glu Glu Asn Tyr Ile Lys Ala Pro Ser Arg Ser Gly Arg Lys Leu Asp Asp Val Arg Asp Lys Tyr Gln Lys Ala Cys
 694   CGC ACG CGC TTC GAG GAG AAC TAC ATA AAG GCA CCC AGT CGC AGT GGC CGC AAG CTG GAC GAT GTG CGT GAC AAG TAT CAG AAG GCC TGC

       Arg Lys Leu His Leu Thr His Asn Glu Tyr Val Leu Ser Ile Thr Glu Ala Ile Glu Val Glu Lys Asp Phe Arg Asn Val Leu Leu Pro
 784   CGC AAA TTG CAT CTC ACA CAC AAC GAG TAC GTG CTG TCC ATC ACG GAA GCC ATC GAA GTG GAG AAG GAC TTT CGC AAC GTC TTG CTG CCG

       Gly Leu Leu Glu His Gln Gln Ser Val Gln Glu Ser Phe Ile Leu Leu Trp Arg Asn Thr Cys Arg Arg Arg Pro Ser Met Ala Thr Ser
 874   GGA CTG CTG GAG CAT CAG CAG TCC GTT CAG GAG AGC TTC ATC CTG CTG TGG CGC AAC ACC TGC AGG AGG CGG CCC AGT ATG GCG ACC TCA

       Arg Pro Thr Ser Thr Arg Arg Phe Gln Lys Arg Ile Asp Thr Val Ile Gly Ser Ile Asn Pro Thr Glu Glu Tyr Gly Glu Phe Thr Glu
 964   CGG CCG ACA AGT ACA AGG AGA TTC CAG AAG CGC ATT GAC ACT GTG ATA GGG AGC ATC AAT CCG ACC GAG GAG TAC GGC GAG TTC ACC GAG

       Lys Tyr Lys Thr Ser Pro Thr Pro Leu Leu Phe Gln Phe Asp Glu Thr Leu Ile Gln Asp Ile Pro Gly Lys Leu Gln Ser Ser Thr
1054   AAA TAC AAA ACC TCT CCC ACA ACA CCG CTG CTC TTC CAG TTC GAT GAG ACG CTC ATC CAG GAT ATT CCC GGC AAA TTG CAG TCG AGC ACA

       Leu Thr Val Asp Asn Leu Thr Val Asp Trp Leu Arg Asn Arg Leu Gln Glu Leu Glu Glu Pro Ser Gly Leu Pro Gly Glu Ala Asp Glu
1144   TTG ACA GTG GAC AAC CTC ACG GTG GAC TGG CTG CGT AAT CGT CTC CAG GAA CTG GAG GAG CCG TCA GGA TTG CCA GGA GAA GCA GAT GAA

       Asp Asp Arg Ala Cys Glu Trp Leu Ala Val Ala Asn Gly Ser Ile Ile Ser Asn Gly Ser Asn Thr Ser Asn Gly Ile Gln Ser Asn
1234   GAT GAT CGA GCA TGT GAA TGG CTC GCG GTG GCC AAT GGC AGC ATT ATA TCC AAC GGC AGC AAC ACA TCC AAC GGC ATT CAG TCC AAC

       Lys Asp Asp Leu Cys Arg Gln Ser Lys Asp Leu Asn Ala Leu Arg Cys Gln Glu Lys Gln Lys Gln Lys Leu Val Asp Met Ile Lys Cys
1324   AAG GAT GAC CTA TGC CGC CAG TCG AAG GAC CTT AAT GCA CTG CGC TGC CAG GAG AAG CAG AAA CAG AAG CTG GTG GAC ATG ATC AAG TGC

       Ala Leu Asn Glu Val Gly Cys Glu Glu Leu Pro Ser Gly Cys Asp Asp Leu Thr Leu Glu Gln Asn Phe Ile Glu Asn Gly Tyr Asn
1414   GCC CTG AAC GAG GTG GGA TGC GAG CTG CCC TCG GGT TGC GAC GAT GAC CTC ACC CTC GAG CAG AAC TTC ATC GAG AAT GGC TAT AAC

       Asn Glu Gln Gln Ile Ser Leu Ser Thr Asn Arg Pro Leu Tyr Glu Glu Glu Trp Phe His Gly Val Leu Pro Arg Glu Glu Val Val Arg
1504   AAC GAA CAG CAG ATA TCG CTC TCC ACA AAT CGT CCG CTT TAC GAG GAG GAA TGG TTC CAT GGC GTT CTG CCG CGC GAG GAA GTG GTT CGA

       Leu Leu Asn Asn Asp Gly Asp Phe Leu Val Arg Glu Thr Ile Arg Asn Glu Glu Ser Gln Ile Val Leu Ser Val Cys Trp Asn Gly His
1594   TTG CTG AAT AAC GAT GGT GAC TTC CTG GTC CGC GAA ACG ATT CGA AAC GAG GAG AGC CAG ATT GTG CTC AGT GTC TGT TGG AAT GGC CAT
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      Lys His Phe Ile Val Gln Thr Thr Gly Glu Gly Asn Phe Arg Phe Glu Gly Pro Pro Phe Ala Ser Ile Gln Glu Leu Ile Met His Gln
1684  AAG CAC TTC ATT GTC CAG ACC ACC GGA GAG GGT AAT TTC CGG TTC GAG GGA CCG CCA TTT GCC AGC ATC CAG GAG CTG ATC ATG CAT CAG

      Tyr His Ser Glu Leu Pro Val Thr Val Lys Ser Gly Ala Ile Leu Arg Arg Thr Val Cys Arg Glu Arg Trp Glu Leu Ser Asn Asp Asp
1774  TAT CAC TCG GAA TTG CCA GTG ACC GTG AAA TCG GGA GCC ATA CTC CGA CGA ACC GTT TGC CGG GAG CGC TGG GAG CTG AGC AAC GAT GAT

      Val Val Leu Leu Glu Arg Ile Gly Arg Gly Asn Phe Gly Asp Val Tyr Lys Ala Lys Leu Lys Ser Thr Lys Leu Asp Val Ala Val Lys
1864  GTG GTA CTT CTG GAG AGG ATT GGT CGG GGA AAC TTT GGG GAT GTC TAC AAG GCC AAA CTG AAG TCC ACC AAA CTG GAT GTG GCT GTC AAA

      Thr Cys Arg Met Thr Leu Pro Asp Glu Gln Lys Arg Lys Phe Leu Gln Glu Gly Arg Ile Leu Lys Gln Tyr Asp His Pro Asn Ile Val
1954  ACC TGT CGA ATG ACC CTG CCC GAC GAA CAG AAA CGT AAA TTC CTG CAG GAA GGG CGC ATC CTC AAG CAA TAC GAT CAT CCA AAT ATC GTA

      Lys Leu Ile Gly Ile Cys Val Gln Lys Gln Pro Ile Met Ile Val Met Glu Leu Val Leu Gly Gly Ser Leu Leu Thr Tyr Leu Arg Lys
2044  AAA TTG ATT GGC ATT TGT GTG CAG AAG CAG CCC ATC ATG ATT GTC ATG GAA TTG GTG CTC GGT GGT TCG CTT TTA ACT TAT TTG CGC AAG

      Asn Ser Asn Gly Leu Thr Thr Arg Glu Gln Met Gly Met Cys Arg Asp Ala Ala Ala Gly Met Arg Tyr Leu Glu Ser Lys Asn Cys Ile
2134  AAC TCC AAT GGC CTC ACC ACT CGC GAA CAA ATG GGC ATG TGC AGA GAT GCG GCG GCA GGC ATG CGA TAT CTG GAG TCC AAA AAC TGC ATT

      His Arg Asp Leu Ala Ala Arg Asn Cys Leu Val Asp Leu Glu His Ser Val Lys Ile Ser Asp Phe Gly Met Ser Arg Glu Glu Glu Glu
2224  CAT CGC GAT CTG GCG GCG CGT AAT TGT CTC GTT GAC TTG GAG CAC AGT GTG AAG ATC TCC GAT TTC GGA ATG TCT CGC GAG GAA GAG GAA

      Tyr Ile Val Ser Asp Gly Met Lys Gln Ile Pro Val Lys Trp Thr Ala Pro Glu Ala Leu Asn Phe Gly Lys Tyr Thr Ser Leu Cys Asp
2314  TAT ATA GTT TCC GAT GGC ATG AAA CAA ATA CCT GTG AAG TGG ACA GCT CCC GAG GCC TTG AAT TTC GGC AAG TAC ACT TCG TTG TGC GAT

      Val Trp Ser Tyr Gly Ile Leu Met Trp Glu Ile Phe Ser Lys Gly Asp Thr Pro Tyr Ser Gly Met Thr Asn Ser Arg Ala Arg Glu Arg
2404  GTG TGG TCC TAT GGC ATA CTG ATG TGG GAG ATC TTC TCC AAG GGC GAC ACA CCC TAC TCC GGC ATG ACC AAC TCC AGA GCC AGA GAG CGC

      Ile Asp Thr Gly Tyr Arg Met Pro Thr Pro Lys Ser Thr Pro Glu Glu Met Tyr Arg Leu Met Leu Gln Cys Trp Ala Ala Asp Ala Glu
2494  ATC GAT ACG GGA TAT CGT ATG CCA ACG CCG AAG AGC ACG CCC GAG GAG ATG TAC CGA CTG ATG CTC CAG TGC TGG GCA GCC GAC GCC GAA

      Ser Arg Pro His Phe Asp Glu Ile Tyr Asn Val Val Asp Ala Leu Ile Leu Arg Leu Asp Asn Ser His ---
2584  TCC CGA CCG CAT TTC GAT GAG ATC TAC AAT GTG GTG GAT GCA CTG ATT CTG CGC CTG GAC AAC AGC CAC TAA GAG CGG CTC CAT GCG GAA

2674  CAC TTA GCG TAT ACT CAA CGT AGA TTT AGT CAG CGC TTT TAA AGT ACG TTA CGT CCG AGG ACT TGA CAA CGA GTT GCC TGT ATT TAT GAG
2764  ATA GCA CAT ACC TAC TAT ACA TTT TAC CAT ATA AAC CAT ATT TAT ATA GAG ACA TAT ATA TTT TCT ATC CAT GTA AAT GTA AAT TTC GAT
2854  GTG TAC TGT AGT GTG TGT TTG CCT GAC TGA GCG TAT GTT TAA AAT CCT TAA TGT GCC AAA AAC CGC AAG AGA TCT TAA AAC AAA AAA AAA
2944  AGT GAA ACG AAA AAA GAT AAG AGA AAA TAA AAT AAA ACT GAT ATT GTG TGT CTG CTT GAA CAA GGC GCT TGC TAA CCT AAG CTC ATG AAT
3034  TTA TAT ATT TTT GAT TGT TTT GTG TTT TGA TTT TTA GGC AGT GAT TCA TAA TTT GTA AAT TAT GCA ATC CCT ACG TTT GTC AAT TTG TTT
3124  CTA GAC TTT GTA TTC TAT GTA TAT TTA TTT ACA CCT AAT TTA CAT TTC TAC TTC GAC TAC GAT TAT GTT TAC TCT AAC ACA ACT AAG CTC
3214  TAA GTT AAA GCT TTA AGT TTT GCT GCG CTG CTG AAG TGA ATA AAT AAT ATG TGT ACT ATA TAT ATA TAT ATA AAA AAA AAA AAA
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FIG. 2. Nucleotide sequence of cDNA for *dfps85D*. The illustrated data were obtained with cDNAs derived from the embryonic cDNA library and appear to represent virtually all the principal mRNA (3.3 kb) for *dfps85D* (see text). The beginning of the translated reading frame was deduced as described in the text.

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   85D  MGFSSALQSRAAHEALIVRQDAELRLMETMKRSIQMKAKCDKEYAISLTAVAQQ      GLKTDRADEMQGSLI  69
Ck fps  ...GP-.WCPKG.--.-RL..-....-.L..-W--Q-..S.-...GM.-H-F-.LEKQE..GHL..T D-S.Q.  73
Mu fes  .....-.C.PQG.G.-QQM.-.....-.G.--W-AQ-V.S.-...GL.-H--L.DSGGQS     W-S GPD.P-  69
   FES  .....-.C.PQG.GV.QQM.-.....-.G.--W-AQ--.S.-...GL.-H--L.DSGGQS     ..I SPD.P.  69
   FER  ...G.D.  -N-...--KL..W....-..-.-F-A--I.S.....S-.QN-C-.VDKEST     VQM NYV.N-  67


   85D  SKSWRSYMDELDHQAKQFKFNAEQLEVVCD KLTHLSQDKRKARKAYQEEHAKIAARLNHLTDEVVRKKSEYQK 142
Ck fps  G-..WVLAS-T--L--T--R-..-.-AGPLA..-I.I-..--L...--.-WQQ--QEYA-T.--E-E.LK-QY- 147
Mu fes  .-..A-ITS-T--L--V--Q-..-.-SGPLS..-V.I----SL..T.-.-WQQ-QQE.T-T-S-D-E.LK-QY- 143
   FES  .-..A-ITS-T-GL--L--Q-..-.-SGPLS..-L.I-----L..T.-.-WQQ-QQE.T-T-S-D-E.LK-QY- 143
   FER  ....LLM---T--L--I-.T-..-.-SGPLH-..M-I-..--V-.-.IGV.Q-.-.E-I--.K-E-E.LKCSY- 141

   85D  HLEGYKALRTRFEENY IKAPSRSGRKLDDVRDKYQKACRKLHLTHNEYVLSITEAIEVEKDFRNVLLPGLLEH 215
Ck fps  S.VRDSTQAK.KY--A S.DK-..-KA.      -..V--LW..-.L..-...--R-.ALHH---Y-RA..T.H.S 215
Mu fes  -.VRDSTQAR.KY--A S.DKD.DKA.       ...V--LW..-A-..R...G-R-.QLHH----RF-.....-S 211
   FES  A.-RDS.QAK.KY--A S.DKD.DKA.       ...V--LW..-A-..R...G-R-.QLHH-----.....RS 211
   FER  -.IKEMNSAKEKY--ALA.GK-T-KA.       --.-..TM...-L..-...--KG.QLH----Y--T..L..-S 210

   85D  QQSVQESFILLWRNTCRRRPSMATSRPTSTRRFQKRIDTVIGSINPTEEYGEFTEKYKTSPTTPLLFQFDETLI 289
Ck fps  LY.-.----.-L--ILGEYC.---LVQ-DVLAI--E-A-A-EM.-.A-..S-.V-C--YDS-V.PAVT...-.- 289
Mu fes  L.D--.--AG-L--IL-EYL----LVQADVA-I--E-AAAAA-.-.-F..LG.LR-.G--.DV.PCVT...-.- 285
   FES  L.D--.--AC-L--IL-EYL----LVQD-VVAI--E-AAAAA-.-.---..QG.LR-.G--.DV.PCVT...-.- 285
   FER  L.K-..--.KAL-GIFDEYS--T-LVT--IVNV-.E.-MS-E-.-.---..N-.I-V--.--AK-QEI-..--.- 284

   85D  QDIP    GKLQSSTLTVDN    LT V DWLR             NRL QEL  EEPSGLPG EADEDDRACE 336
Ck fps  --T-SLEP.-..L--.---VQHS..S-E-E.LASREAVSSKEQRVWE.QV..RG..L-LS..  .RVHLLGKR- 362
Mu fes  -.G-QLEP.-..L--.---VQHT..S.T.E.AVATKEVLSRQEMVS-.Q-..QS..QNTH.R .RM-LLGKR- 358
   FES  --G-PLEP.-..L--.---VQHT..S.T.E.AVATEMVFRRQEMVT-.Q...RN..--TH.R .RV-LLGKR- 358
   FER  --N-NLQAN--MW-N..A--LQVM.KT-A-E.MQTQQMLLNKEEAVLE.E-R-EE----CEKKS-IVLLL-QK- 358

   85D  WWLAVANGSIISNGSNTSNGIQSNKDDLCRQSKDLNALRCQEKQKQKLVDMIKCALNEVGCEELPSGCDDDLTL 410
Ck fps  GLQ-AQ-QLQGLVC--AKLQA.--MLANKLA-LGS--PPPALPL--DRQS-CSTDQ-RS.V - -LETIK-HIS 434
Mu fes  MLQ-AI-.LQ.-LC.-DKLQA.--LL-SKM--LGTG-PPAVPLLQDDRHSTSST  -RE.GR-PTLEILKSHFS 430
   FES  VLQ-AL-.LQ--LC.-AKLQA.--LL-TKL-HLGPG-PPPVLLL--DRHSTSSS-Q-RE.GR-PTLEILKSHIS 432
   FER  ALE--K-SVQQLRC--AKFSA.K-LL-QKV.--.G--PPPVV-Y--DARS-TSM-  RK ERLSKFESIR-SIA 429

                        >SH2
   85D  EQNF IENGY NNEQ QISLSTNRPLYEEEWFHGVLPREEVVRLLNNDGDFLVRETIRNE ESQIVLSVCWNGH 480
Ck fps  GIFS PRFSL PP-V P-IP-V--..C---.-..A-..-..QE..-CS........-QG-- .Y  ....L.-.- 502
Mu fes  GIFR P-FSI PP-L .-VP-V--.....-L.-..A-.W-..AE..T-T........-QG-- .Y  ....M.-.. 498
   FES  GIFR P-FSL PP-L .-IP-V--..-.-L.-..A-..-..AE..V-S........-QG-- .Y  ....L.-.L 500
   FER  GIIRSP--AVG---LS--ISI---..A.--.-..A-..I.AQE..---........-HG--G.Y  ....YS-.- 501

                                          SH2<          >KINASE
   85D   KHFIVQTTGEGNF RFEGPPFASIQELIMHQYHSELPVTVKSGAILRRTVCRE RWELSNDDVVLLERIGRGN 551
Ck fps  P-...-.AA  D.-Y.-..DG.--.PL..D.LL-.-Q.-.R...I-.T.A.L-- -.V.---..-.G..... 573
Mu fes  P-...-.-L  D.-Y.-..DG.-..PL..T.LLS.-Q.-.K...V-.F.A.P-- -.V.K--.-..G.-.... 569
   FES  P-...-.-L  D.-Y.-..G.-..PL..D.LLS--Q.-.K...V-.-.A.P-- -.V.---.-..G.-.... 571
   FER  R-...-.YV  D.-Y....TG.--.P-..D.-.---QV-.K...V-.LNP-P--K-.I...-..-.G.L-.-.. 573

   85D  FGDVYKAKLKSTKLDVAVKTCRMTLPDEQKRKFLQEGRILKQYDHPNIVKLIGICVQKQPIMIVMELVLGGSLL 625
Ck fps  ..-.-SG-.--D-TP....-..E...P.L.A.....A.....-.....-...-.T.....Y......Q..D-. 647
Mu fes  ..-.-SG-.--D-TP....-..E...P-L.A.....A.....M.....-...-.T.....Y......Q..D-. 643
   FES  ..-.-SG-.--D-TL....-..E...P-L.A.....A.....S.....-...-.T.....Y......Q..D-. 645
   FER  ..-...GT.. D.TS.....-ED..-.L.I.....A-.............-.T.-..-Y.-....S..D-. 646

   85D  TYLRKNSNGLTTREQMGMCRDAAAGMRYLESKNCIHRDLAARNCLVDLEHSVKISDFGMSREEEE  YIVSDGM 697
Ck fps  --..S-GPH.KM-.L-K.ME-.....E.....-.............TE----.........-..-GI.AS-G.. 721
Mu fes  .-..T-GAR.RV--L-Q.MG......E.....C.............TE--V-.............----GI.AACS.- 717
   FES  .-..T-GAR.RV--L-Q.VG......E.....C.............TE--V-.............--GV.AA.G.- 719
   FER  .-..--K-E.KL--L-K-SL......L.................GE--V-..........-.-GGV. S.S.- 719

   85D  KQIPVKWTAPEALNFGKYTSLCDVWSYGILMWEIFSKGDTPYSGMTNSRARERIDTGYRMPTPKSTPEEMYRLM 771
Ck fps  .............-.-.-.ES....-...-..A..L.AV..-N--.--T..A.-Q.V.--P.--C..-..... 795
Mu fes  -.-...........-.-.-.ES....-...-..T..L.A-..PN-...--T..F--K.-.-.C.-LC.----... 791
   FES  -.-...........-.-.-.ES....-...-..T..L.A-..PN--.--T..F--K.G.-.C.-LC.----... 793
   FER  ....-.........-.-.-.ES....-...-..T..L.VC..P....--....---R....SA.-HC..--S--. 793

   85D  LQCWAADAESRPHFDEIYNVVDALILRLDNSH                                          803
Ck fps  Q-...-Y.-R-..S.G----D-I.-RK.HR                                           824
Mu fes  E....Y--G-..S.SI.C-E-H--RK.HR                                            820
   FES  E....Y--G-..S.S-..-E----RK.HR                                            822
   FER  --..DYK-.-...-.S.-Q-E-TI-KR-.T                                           822
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FIG. 3. Comparison of the proteins encoded by *dfps85D* and vertebrate *fps/fes* and *fer*. Amino acid sequences are given with the conventional single-letter code. Dots denote identities; dashes indicate chemically conservative substitutions as defined by McLachlan (27); blank spaces represent arbitrary gaps created to achieve maximum alignment of the several sequences. The approximate boundaries of the SH2 and kinase domains are marked.

TABLE 1. Comparison of *fps/fes* and *fer* with *dfps85D*

Gene	Similarities with *dfps85D*[a]			
	Amino acids 1–120	SH2	Kinase	Overall
Chicken *fps*	35 (60)	46 (73)	57 (78)	35 (60)
Mouse *fes*	32 (59)	48 (71)	56 (77)	36 (58)
Human *FES*	34 (60)	47 (72)	55 (77)	36 (60)
Human *FER*	34 (62)	45 (73)	57 (77)	34 (60)

[a] Expressed as percentage of identities and identities plus similarities (latter in parentheses).

representing ca. 48 kilobase pairs (kbp) from the locus, with a gap of uncertain size between clones λdfg2.7 and λdfg6.1 (Fig. 1A). A portion of one of these clones (λdfg5, Fig. 1A) was then used as a probe to isolate multiple cDNA clones from a library prepared with the polyadenylated RNA of embryos (2 to 24 h after oviposition). All these cDNA clones overlapped with one another. We report here on only the longest (3.2 kbp), which is likely to represent virtually the entire length of the principal mRNA for *dfps85D* (see Discussion) (Fig. 1B). The cDNA extends into poly(A) at the 3′ end of the mRNA but falls short of the 5′ end by 90 to 100 nucleotides (20b).

Nucleotide sequence of *dfps85D*. The complete nucleotide sequence for the 3.2-kbp cDNA included an open reading frame that could encode a protein of 803 amino acids with a calculated molecular weight of 92,505 (Fig. 2). We believe that the methionine codon with which this reading frame opens is the authentic site of initiation for translation from *dfps85D* for the following reasons. The 5′ end of the cDNA falls within 100 nucleotides of the end of the mRNA when mapped by primer-initiated reverse transcription (20a); the first methionine codon lies in a context that is favorable for initiation of translation in *D. melanogaster* (5) and is downstream of termination codons in all three reading frames; and the length of the encoded protein is akin to that of *fps/fes* proteins in vertebrates (15).

The protein encoded by *dfps85D* bears hallmarks of PTKs (Fig. 2 and 3), including a 30-kDa catalytic domain that composes the carboxy-terminal third of the protein, an amino acid sequence characteristic of ATP-binding sites (residues 548 to 553 and lysine at 570), motifs of sequence that serve as signatures of tyrosine-specific kinases (20), a tyrosine (residue 691) whose phosphorylation appears to be involved in enzymatic activation of the *fps* protein (28, 45), and a domain known as SH2 that is conserved in the cytoplasmic PTKs and that is thought to serve regulatory functions for the enzymes (29).

We compared the amino acid sequence of the protein encoded by *dfps85D* with the sequences of all known PTKs. Greatest resemblance was found with the products of vertebrate *fps* and a related gene known as *fer* (Fig. 3). The extents of the resemblances to *fps* and *fer* were virtually identical (Fig. 3 and Table 1).

Cytogenetic localization of *dfps85D*. We used a portion of the embryonic cDNA to prepare a biotinylated probe and then hybridized the probe with spreads of polytene chromosomes from salivary glands. Histochemical analysis was used to locate the site of hybridization, which proved to be position 85D10-13 (39) on the right arm of chromosome 3 (data not shown), in the vicinity of the gene for a testicular form of beta-tubulin (23). None of the previously identified genes for PTKs in *D. melanogaster* map at or near this position.

Expression of *dfps85D*. We first analyzed polyadenylated RNA extracted at various stages of development (data not shown). A single form of RNA with an estimated size of 3.3 kb predominated at all points. This RNA was detectable but scarce in pools of either 0- to 2-h-old embryos or first- and second-instar larvae, but it was relatively abundant at all the other stages examined. Several smaller RNAs were also detected in relatively scant quantities at one or more stages. At present, we are not certain whether any of these are authentic transcripts from *dfps85D*.

To obtain greater resolution in our analysis of expression, we turned to hybridization in situ. By this means, expression was first detectable at several positions in the late cellular blastoderm, including the yolk nuclei (vitellophages), where expression was especially strong and maintained into early gastrulation (Fig. 4B). Expression was also observed in dorsomedial, dorsolateral, and posterior positions (Fig. 4A and B). These regions encompass the anlage for the amnioserosa, dorsal epidermis, and proctodeum.

During germ band extension, expression continued in the amnioserosa (Fig. 4B and C) and the dorsal epidermis (Fig. 4B) and became pronounced in the proctodeum (Fig. 4C). In the fully extended embryo, expression also appeared in the ventral ectoderm, clypeolabrum, invaginating stomadeum, and mesoderm (data not shown, but see below). Subsequent to germ band shortening, expression became more general but was still not universal (Fig. 4D). Sites of expression included the clypeolabrum, foregut, visceral mesoderm, somatic mesoderm, ventral epidermis, procephalic lobe, amnioserosa, and the dorsal ridge. Expression was notably absent from most of the developing nervous system (Fig. 4D and E), including the supraesophageal ganglia, the subesophageal ganglia, and the majority of lateral cell bodies of the ventral nerve cord. Expression was detected, however, in cells located at the midline of the ventral nervous system (Fig. 4E).

In the final stages of embryogenesis, expression appeared transiently in somatic muscle (Fig. 4E), pharyngeal muscles, tracheal epithelium (Fig. 4F), and spiracles (data not shown) and persistently in the frontal sac (Fig. 4F), esophagus, and proventriculus (data not shown).

In third-instar larvae, expression was detected in all imaginal disks. Transcripts were distributed unevenly within the disks and were especially prominent in the adepithelium (Fig. 5A). In the eye portion of the eye-antennal disks, expression was weak anterior to the morphogenetic furrow but became strong in both the apical and basal levels immediately posterior to the furrow; in more posterior positions, expression was predominantly in the basal portion of the tissue (Fig. 5C). Expression was also apparent in neural tissue, specifically the cellular cortices of the midbrain (Fig. 5B and C and data not shown) and ventral ganglia (Fig. 5B). Expression levels in the optic lobe were much lower and more discrete. Other tissues expressing *dfps85D* were the testes (data not shown), immature blood cells of the lymph glands (Fig. 5D), and the polyploid epithelial cells of the midgut (Fig. 5D). There was no detectable expression in the ovaries (data not shown) or in the polyploid tissue of salivary glands, fat body, and larval muscles (Fig. 5B and D).

The pattern of expression established in larvae persisted into the early pupal stage but was supplemented by the appearance of expression in the tracheal epithelium and abdominal histoblasts (Fig. 6A) and in precursors for visceral muscle (Fig. 6B). Later in pupal development, expression in most epithelial tissues diminished appreciably but was prominent in developing skeletal muscle (7) (Fig. 6C).

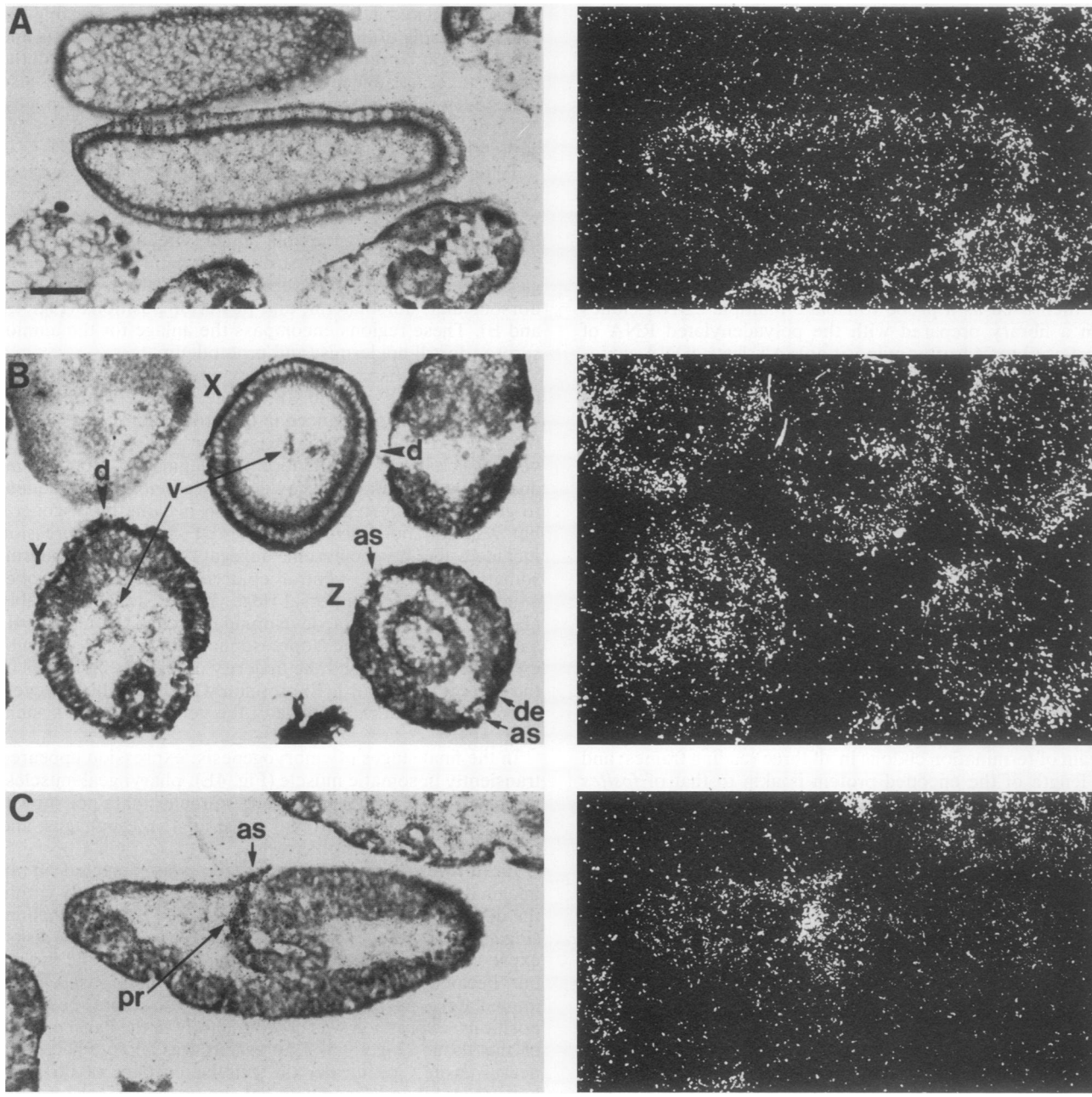


FIG. 4. Expression of *dfps85D* during embryogenesis. Each panel includes a bright-field (left) and corresponding dark-field (right) photomicrograph taken after autoradiography. Orientation of all longitudinal sections (A, C, D, E, and F) is anterior to the left. Stages referred to are those described by Campos-Ortega and Hartenstein (4). The horizontal bar in panel A corresponds to 0.05 mm. All embryos are shown at the same magnification. Abbreviations: as, amnioserosa; cl, clypeolabrum; de, dorsal epidermis; dr, dorsal ridge; fg, foregut; fs, frontal sac; phm, pharyngeal muscles; pl, procephalic lobe; pr, proctodeum; sbg, subesophageal ganglia; sm, developing somatic muscles; spg, supraesophageal ganglia (brain); tr, developing trachea; v, vitellophages (yolk nuclei); vm, visceral mesoderm. (A) Lateral sagittal section (dorsal up) of a late cellular blastoderm embryo, stage 5, is shown in the center. Above is a section of a preblastoderm embryo, at which stage no *dfps85D* transcripts were detected. (B) Cross-sections of three embryos at different stages of development: x, a late cellular blastoderm embryo; y, a gastrulating embryo, stage 6; and z, a germ band-extended embryo. The dorsal surface (d) indicated in x and y includes the anlage of the amnioserosa and the dorsal epidermis. (C) Parasagittal section (dorsal up) of a late stage 8 germ band-extended embryo. (D) Parasagittal section (dorsal up) of a stage 13 germ band-shortened embryo. (E) Horizontal section of a stage 14 or 15 embryo showing the developing ventral nerve cord. Transcripts were detected over cells residing at the midline (m) of the nerve cord but not over the lateral cell bodies (l). (F) Parasagittal section (dorsal up) of a stage 16 embryo.

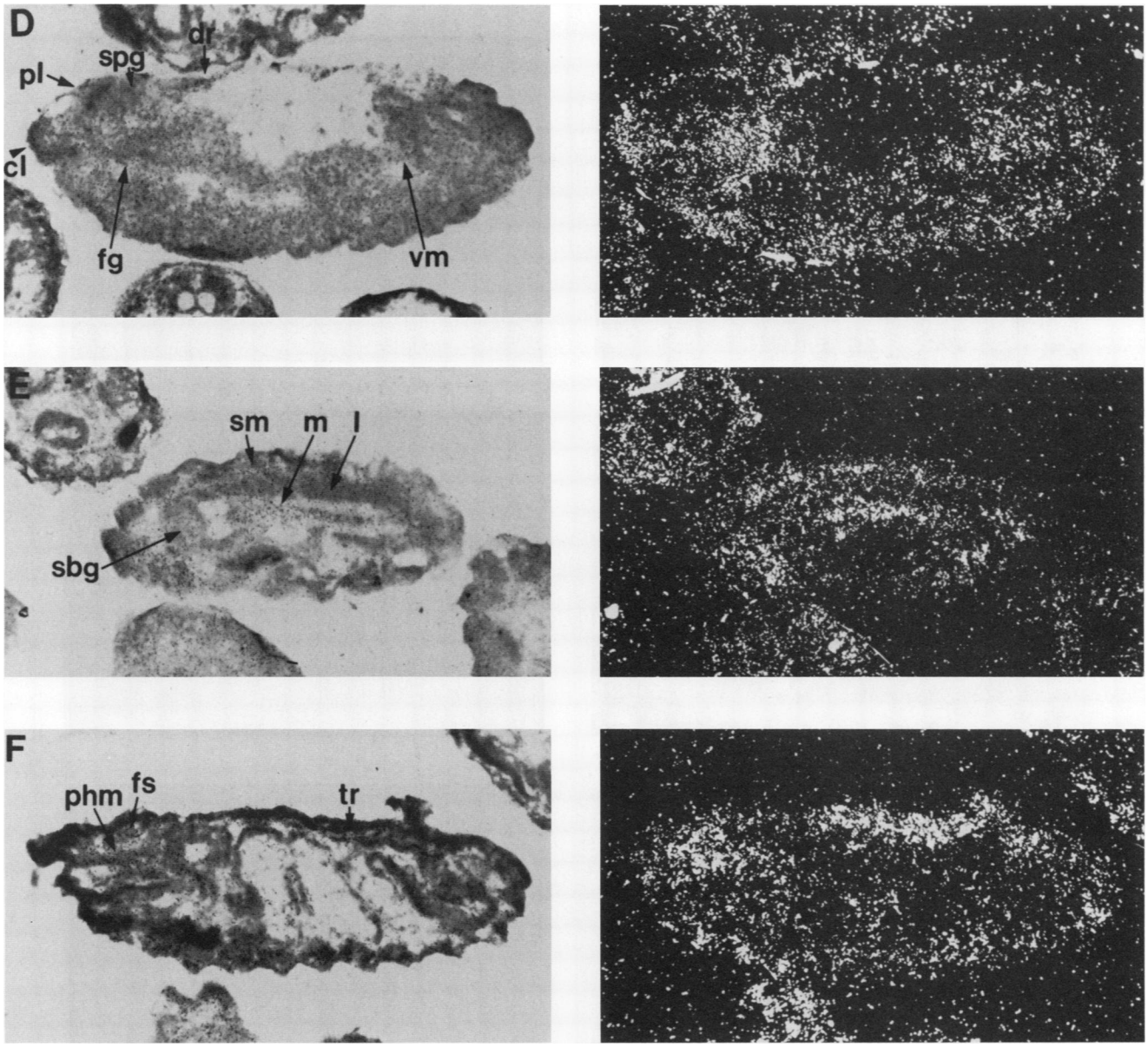
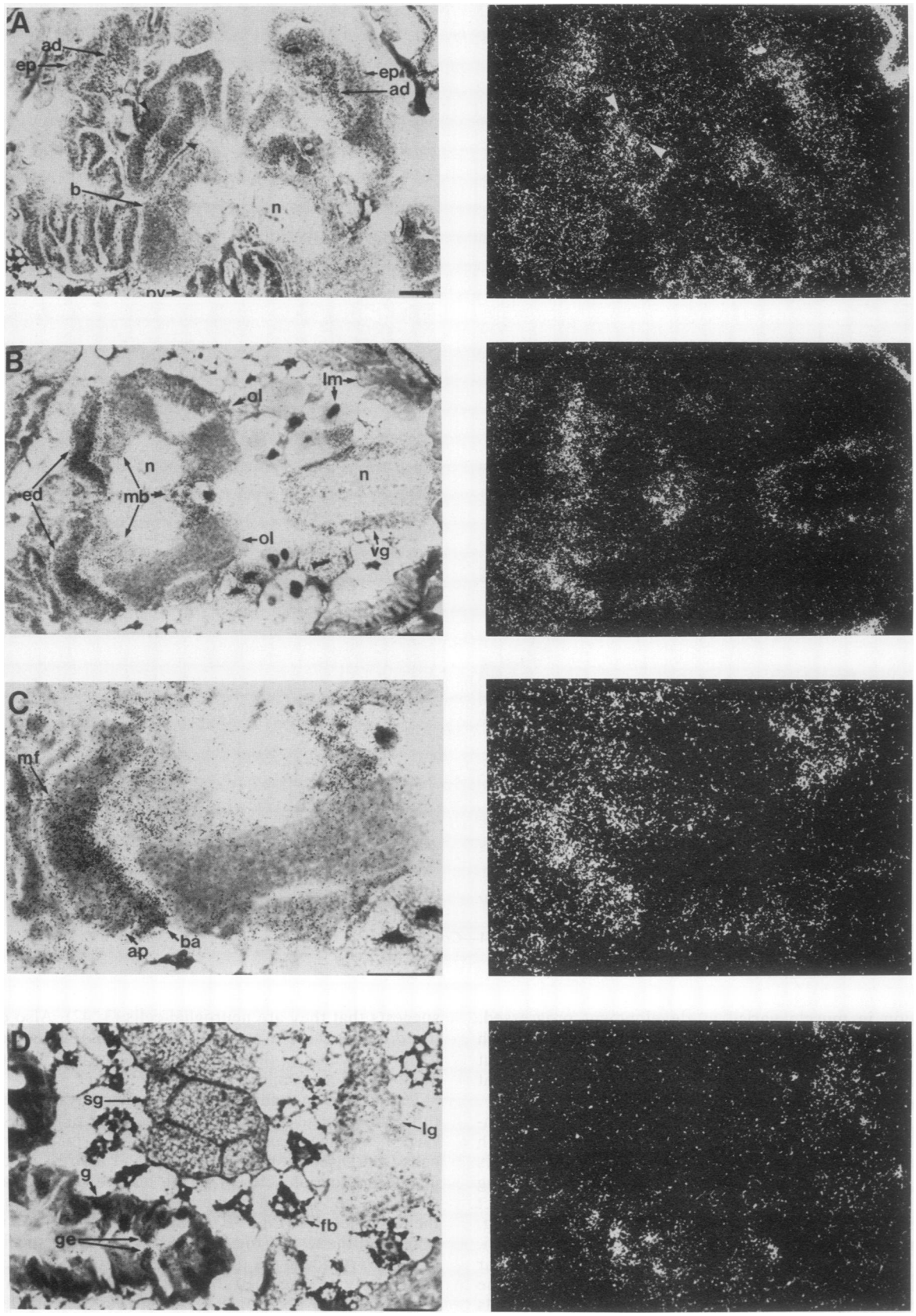


FIG. 4—*Continued*

Expression in muscle varied as development progressed. For example, 48 h into the pupal stage, expression was high in the direct flight muscles and low in the indirect flight muscles. Later, expression in both types of muscle was at the same low level (data not shown).

dfps85D was expressed in the developing testes throughout pupal development (data not shown). Midway through the pupal period, expression declined to undetectable levels in the cortex of the midbrain (data not shown) but was strong in regions of the optic lobe. Subsequently, two distinct layers of the optic lamina expressed *dfps85D* strongly (Fig. 6D). The more distal hybridization (away from the brain, toward the eye) was located at the base of the lamina cellular cortex and is likely to represent expression from either the L4 or L5 cells (8). The more proximal expression was in cells located near the base of the lamina neuropile. Their location suggests that they are neuroglial cells (3, 42). Also expressing *dfps85D* were a small group of cells of undetermined identity, located at the junction of the medulla, lobula, and lobula plate neuropiles.

Expression in adult flies was especially strong in the retina, more so at the base than at the apex of the tissue (Fig. 7A), a layering that suggests expression in photoreceptor cells (see Discussion). The widespread expression detected in the thoracic muscles of pupae had now disappeared but was observed instead in the lateral tergosternal muscles of the abdomen (7) (Fig. 7B). Expression continued in some regions of the testes (Fig. 7C) and appeared for the first time in the ovary, localized to the follicular epithelium (Fig. 7D), particularly at stages 10 to 11 of oogenesis (24). As in the larval gut (see above), expression was detectable in the epithelium throughout the gut (Fig. 7D) and in specific



regions of the proventriculus (data not shown). Expression was not detected in any portion of the adult brain.

DISCUSSION

A *Drosophila* gene related to *fps/fes* and *fer*. We have used the retroviral oncogene v-*fps* as a probe to isolate several genes encoding PTKs from *Drosophila* DNA. Among these isolates was a previously unidentified gene that resides at chromosomal position 85D10-13 and that we have for convenience designated as *dfps85D*. We have isolated ca. 48 kbp of genomic DNA that may encompass the entirety of *dfps85D*, but we have yet to characterize this DNA in detail.

In the present report, we describe the topography and sequence of a cDNA clone representing *dfps85D*. The clone (obtained from a library representing embryonic RNA) appears to encompass virtually the entire length of the principal mRNA for *dfps85D*, because the cDNA has approximately the same length as the mRNA; the 5′ end of the cDNA falls within 100 nucleotides of the end of the mRNA when mapped by primer-initiated reverse transcription (20a); the cDNA appears to extend into the poly(A) at the 3′ end of the mRNA; and it encodes a protein that closely resembles previously described cytoplasmic PTKs.

Do vertebrates possess an authentic counterpart of *dfps85D*? The question cannot be answered decisively with the available data. Of the vertebrate PTK genes now known, none resembles *dfps85D* more closely than do *fps/fes* and *fer*. For example, the proteins encoded by *dfps85D* and vertebrate representatives of *fps/fes* and *fer* have similar sizes and share 57 to 60% identical amino acids, with the identities clustered in the kinase, SH2, and amino-terminal domains (Fig. 3 and Table 1). Moreover, *dfps85D* lacks the SH3 domain, a sequence of ca. 65 amino acids that adjoins the amino terminus of SH2 in a number of cytoplasmic PTKs but not in *fps/fes* or *fer* (29). The size of the encoded protein and the absence of SH3 place *dfps85D* outside the minifamily of PTK genes for which *src* serves as the prototype (20).

On the other hand, there are several reasons to question the identity of *dfps85D* with either *fps/fes* or *fer*. (i) Comparison of *fps/fes* alleles isolated from several vertebrate species has defined potential signatures of the gene (15, 46). These include exceptional conservation of the first 58 amino acids, a lysine-rich and highly hydrophilic region between residues 153 and 185, and conservation of the sequence between residues 273 and 312. *dfps85D* appears to possess only the first of these (Table 1); indeed, the similarities extend over the first 120 residues of the protein (Fig. 3 and Table 1).

(ii) The sequence between amino acids 325 and 427 in the *dfps85D* protein bears essentially no resemblance to the corresponding region in vertebrate *fps/fes* and the analogous region in *fer* (although this domain is also relatively diverged between avian and mammalian versions of *fps/fes*).

We conclude that the *Drosophila* gene we described here may not be the exact counterpart of either *fps/fes* or *fer*. But the three genes do appear to be the same genre, distinguished from other PTKs by the size of their gene products and clustered resemblances among their sequences. Nevertheless, our nomenclature for *dfps85D* is intended only as a convenience.

***dfps85D* encodes a PTK.** Although we have yet to identify the product of *dfps85D* in cells, there seems no reason to doubt that the gene encodes a PTK. The usual hallmarks of PTKs are present in the amino acid sequence of the gene product (see Fig. 3 and 4). In particular, the protein resembles cytoplasmic PTKs and thus may be associated with the plasma membrane and intracellular membranes. Amino acids 1 and 2 of the protein (Met and Gly) are characteristic of cytoplasmic PTKs that are myristylated, but the next five amino acids in the sequence are not (21). Work by others indicates that the protein product of vertebrate *fps/fes* is not tightly associated with the plasma membrane, suggesting that it is not myristylated (15).

Topography of *dfps85D*. Although we have isolated more than 48 kpb of DNA that may encompass the entirety of *dfps85D* (data not shown), we have yet to characterize the topography of the gene in detail. But analysis of heteroduplexes between cDNA and genomic DNA has revealed that the number, size, and arrangement of introns and exons are different from those of vertebrate *fps/fes* and related genes (unpublished data). Since substantial remodeling of gene structure occurred subsequent to the evolutionary radiation that gave rise to insects, the divergent topographies of *dfps85D* and *fps/fer* are of no assistance in evaluating the kinship of these genes. For example, most proto-oncogenes isolated to date from *D. melanogaster* do not have topographies resembling those of their vertebrate counterparts (for pertinent references, see references 19 and 35).

In work to be reported elsewhere, we have found evidence for a second form of mRNA representing *dfps85D* that arises by either alternative initiation of transcription or alternative splicing (28a). The protein encoded by this second mRNA would be an unusual version of PTKs, lacking regulatory domains normally found in the amino-terminal portions of typical cytoplasmic PTKs, and similar in this regard to the PTK encoded by the alternatively spliced form of mouse *fer* (9).

Expression and function of *dfps85D*. When tissues were analyzed en masse for RNA, expression of *dfps85D* was apparent from early in embryogenesis, became relatively abundant after 2 h, and then persisted at roughly the same level (with the possible exception of first- or second-instar larvae) through adulthood. But the higher resolution offered by hybridization in situ provided a more revealing picture.

RNA representing *dfps85D* was undetectable in sections of embryos until the late cellular blastoderm. It therefore appears that expression of *dfps85D* is largely if not entirely zygotic in origin. The most notable feature of expression was the diversity of developmental periods, tissues, and cells in which it occurred. Nevertheless, expression was especially

FIG. 5. Expression of *dfps85D* in larvae. Each panel includes corresponding bright-field (left) and dark-field (right) photomicrographs. All sections are from climbing stage third-instar larvae. The horizontal bars correspond to 0.05 mm. Abbreviations: b, brain; ed, eye portion of the eye-antennal imaginal disk; fb, fat body; g, larval gut; ge, epithelial cells of the gut; lg, lymph glands; lm, larval muscle; mb, cellular cortices of the midbrain; ol, optic lobe; n, neuropile; pv, proventriculus; sg, salivary gland; vg, ventral ganglia. (A) Parasagittal section (anterior up) showing several imaginal disks. In the indicated disks, much higher concentrations of grains were observed over the adepithelial tissue (ad) than over the epithelial tissue (ep). Arrowheads indicate abrupt changes in the levels of *dfps85D* expression in the epithelium of a single disk. (B) Cross-section (dorsal left) through the brain and eye disks. (C) Higher magnification of one of the eye disks and part of the brain shown in panel B. In the eye disk, anterior is above the morphogenetic furrow (mf) and posterior is below; apical (ap) and basal (ba) surfaces are indicated. (D) Oblique section (anterior up) showing part of the larval gut, lymph gland, and salivary gland.

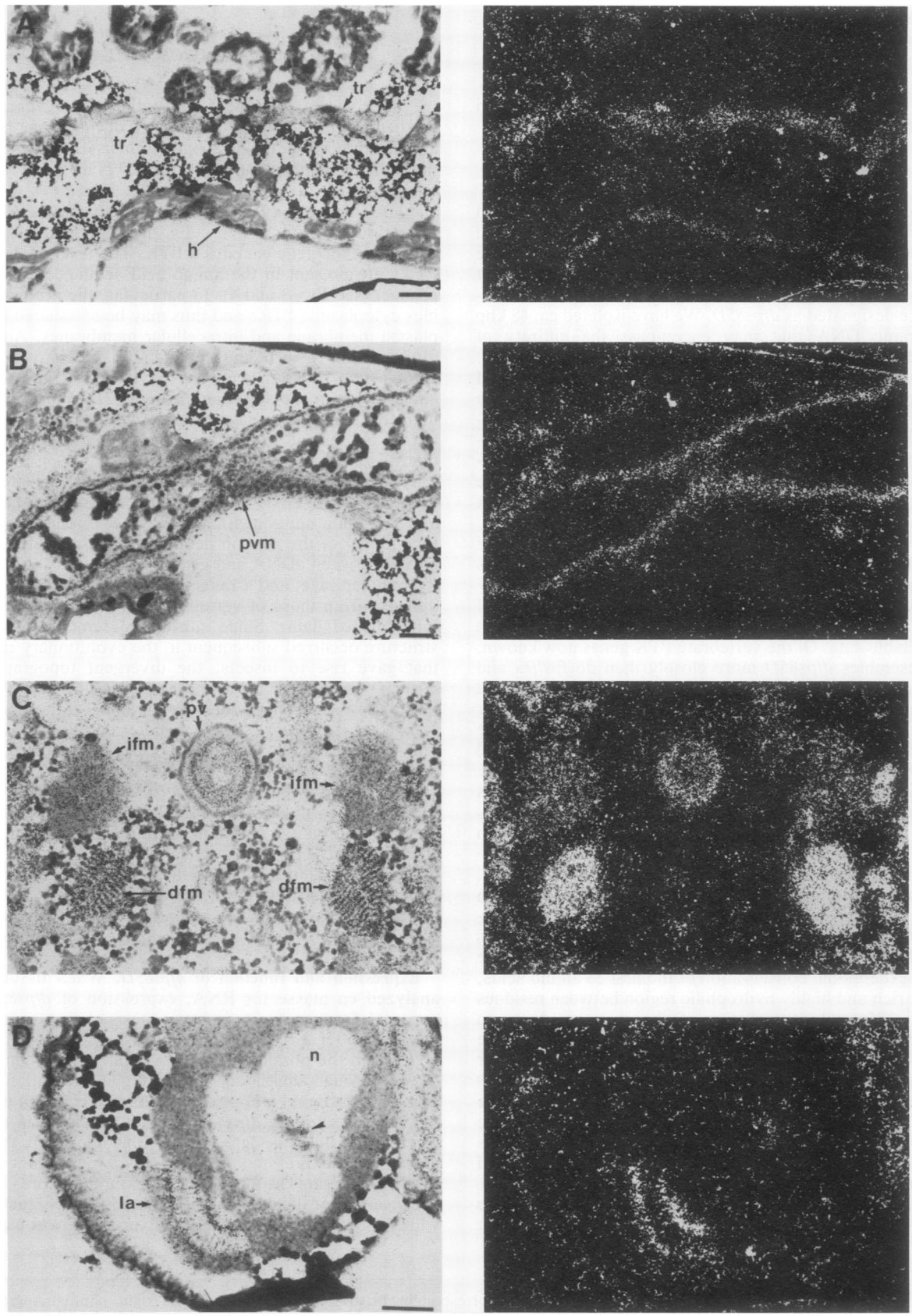


FIG. 6. Expression of *dfps85D* in pupae. Each panel includes corresponding bright-field (left) and dark-field (right) photomicrographs. The horizontal bars correspond to 0.05 mm. Abbreviations of developing imaginal tissues and organs: dfm, direct flight muscles; h, abdominal histoblasts; ifm, indirect flight muscles; la, optic lamina; n, neuropile; pv, proventriculus; pvm, precursors of visceral muscle; tr, trachea. (A) Horizontal section of a prepupa (anterior left) showing the most posterior region of the thorax and the anterior region of the abdomen (including developing trachea). (B) Parasagittal section of a very early pupa (anterior left) showing the developing imaginal gut. (C) Horizontal section (anterior up) showing developing muscles in the thorax midway through pupal development (ca. 48 h after puparium formation). (D) Horizontal section (anterior left) showing the developing optic lobe approximately 60 h after puparium formation. The arrowhead indicates signal over a small group of cells located at the junction of the medulla, lobula, and lobula plate neuropiles.

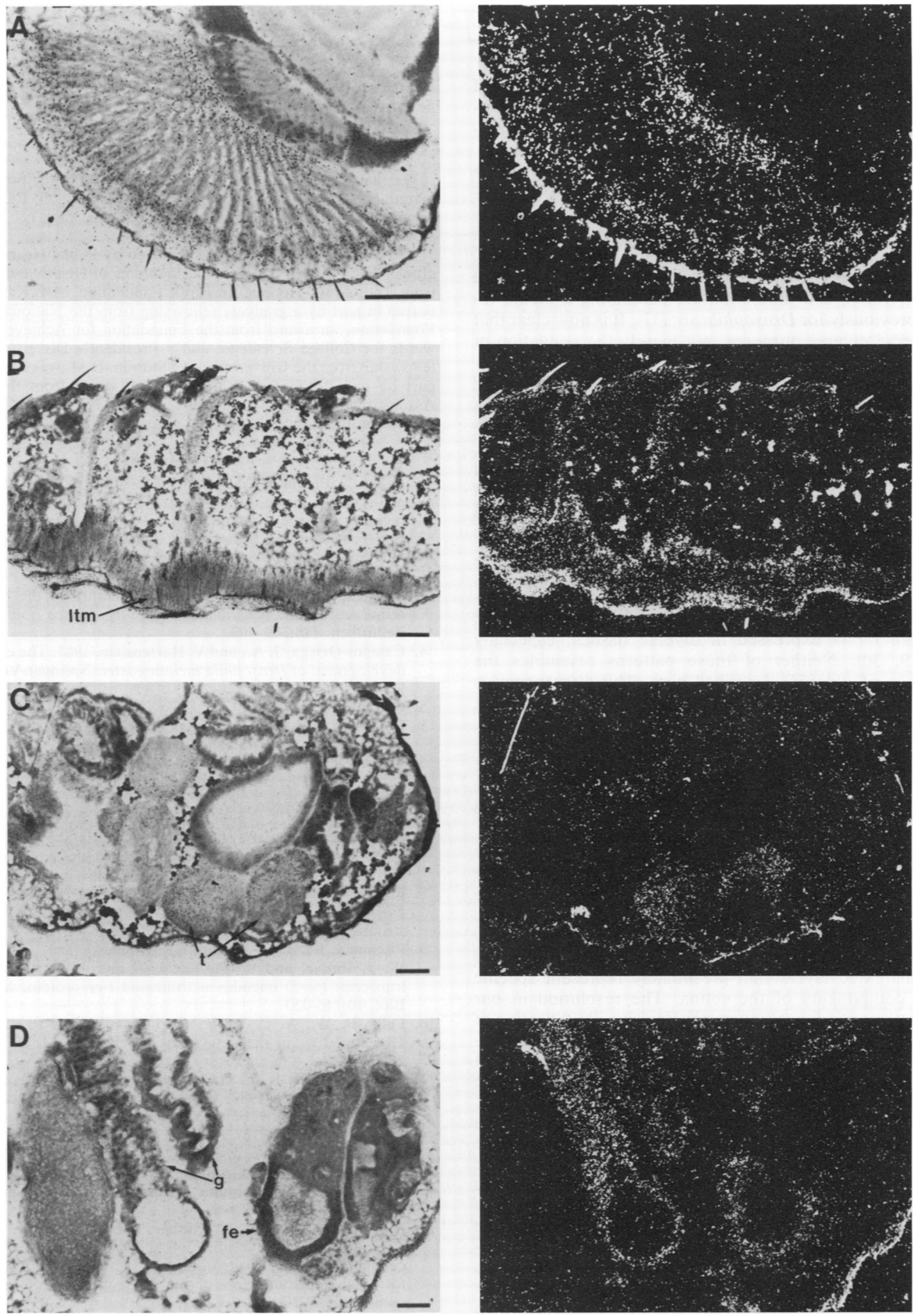


FIG. 7. Expression of *dfps85D* in adult flies. Each panel includes corresponding bright-field (left) and dark-field (right) photomicrographs. The horizontal bars correspond to 0.05 mm. Abbreviations: fe, follicular epithelium of the ovary; g, adult gut; ltm, lateral tergosternal muscles; t, testes. (A) Compound eye of a mature (3- to 5-day-old) adult fly (anterior left). (B) Lateral sagittal section (anterior left, dorsal up) through the abdomen of a young (<1-day-old) adult, including the lateral tergosternal muscles. (C) Abdomen of a mature adult male (anterior left), including portions of the testes. (D) Abdomen of a mature adult female (anterior up), showing part of the gut and developing oocytes.

prominent in muscular and retinal tissue. The expression of *dfps85D* is exceptionally diverse and dynamic when compared with vertebrate *fps* and the other PTKs of *D. melanogaster* studied to date.

The patterns of expression took several forms. In some tissues, (central nervous system, tracheal epithelium, skeletal muscles, and follicular epithelium of the ovary), expression was transient. By contrast, in tissues such as the retina and the epithelium of the gut, expression was continuous. Many of the tissues in which expression occurred were nonproliferative and differentiated, continuing a theme reported previously for *Drosophila src* (37). It is now clear that PTKs serve diverse purposes, not merely the regulation of cellular proliferation.

The evolving patterns of *dfps85D* expression dramatize how a single PTK can serve diverse but specific purposes during development, with the specificity of expression shifting from one embryological lineage and tissue to another, and with the same enzyme serving in both mitotic and terminally differentiated cells. The sites and periods of expression for *dfps85D* are more diverse than those found for two other *Drosophila* genes that encode cytoplasmic PTKs, *src* (37) and the related gene *Dsrc28C* (11, 21a, 43, 44).

Vertebrate *fps/fer* is expressed principally in hematopoietic cells of the granulocyte and macrophage lineages (15); vertebrate *fer* is expressed in diverse tissues, especially testes (9, 30). Neither of these patterns resembles the expression of *dfps85D* described here. The discrepancy is provocative because the expression of *src* (another gene that encodes a cytoplasmic PTK) shares major features in *D. melanogaster* and vertebrates (6, 10, 37, 38). The contrast offers yet another reason to doubt that *dfps85D* is the exact counterpart of either *fps/fes* or *fer*, although it remains possible that all three genes serve the same physiological purpose in whatever context they are expressed.

The transient and specific expression of *dfps85D* in the eye disk, the optic lamina, and elsewhere in the optic lobe suggests that the gene plays a role in development of the visual system, in the brain as well as in the developing retina. The pattern of expression in the adult retina is especially provocative. RNA transcribed from the gene occurs in two well-demarcated layers that presumably represent specific cellular components of the retina. The resolution in our analysis was not adequate to permit decisive identification of those components, but we suggest that they are photoreceptor cells—R8 at the base of the retina, and one or more of R1 to R7 at the apex (41). Immunocytochemistry could provide a test of this suggestion.

Previous work has implicated three other PTKs in the development of the *Drosophila* retina, one encoded by the counterpart of the vertebrate proto-oncogene *src* (37), the second by sevenless (33), and the third by *DER* (1). The product of *Drosophila src* is found in photoreceptor cells of the developing retina, where it is first expressed at the time of neurite extension and persists in the fiber tracts connecting the retina to the brain rather than in the cell bodies (35a). Despite this detailed description, no hint of function for *src* in retinal cells has emerged. The product of sevenless is a transmembrane receptor whose function is required for the differentiation of the R7 photoreceptor cell (2, 36). Dominant variants of *DER* cause specific defects in retinal development (1). From the data presented here, it appears likely that *dfps85D* also plays an important role in the development and function of the *Drosophila* retina. We hope to learn the nature of that role and other functions of *dfps85D* from genetic analyses now in progress.

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REFERENCES

1. **Baker, N. E., and G. M. Rubin.** 1989. Effect on eye development of dominant mutations in *Drosophila* homologue of the EGF receptor. Nature (London) **340**:150–153.
2. **Basler, K., and E. Hafen.** 1988. Control of photoreceptor cell fate by the *sevenless* protein requires a functional tyrosine kinase domain. Cell **54**:299–311.
3. **Boschek, C. B.** 1971. On the fine structure of the peripheral retina and lamina ganglionaris of the fly, *Musca domestica*. Z. Zellforsch. **118**:369–409.
4. **Campos-Ortega, J. A., and V. Hartenstein.** 1985. The embryonic development of *Drosophila melanogaster*. Springer-Verlag KG, Berlin.
5. **Cavener, D. R.** 1987. Comparison of the consensus sequence flanking translational start sites in *Drosophila* and vertebrates. Nucleic Acids Res. **15**:1353–1361.
6. **Cotton, P. C., and J. S. Brugge.** 1983. Neural tissues express high levels of the cellular *src* gene product pp60$^{c\text{-}src}$. Mol. Cell. Biol. **3**:1157–1162.
7. **Crossley, C.** 1978. The morphology and development of the *Drosophila* muscular system, p. 499–560. *In* M. Ashburner and T. R. F. Wright (ed.), The genetics and biology of *Drosophila*, vol. 2b. Academic Press, Inc., New York.
8. **Fischbach, K.-F., and A. P. M. Dittrich.** 1989. The optic lobes of *Drosophila melanogaster*. I. A Golgi analysis of wild-type structure. Cell Tissue Res. **258**:441–475.
9. **Fischman, K., J. C. Edman, G. M. Shackleford, J. A. Turner, W. J. Rutter, and U. Nir.** 1990. A murine *fer* testis-specific transcript (*ferT*) encodes a truncated Fer protein. Mol. Cell. Biol. **10**:146–153.
10. **Fults, D. W., A. C. Towle, J. M. Lauder, and P. F. Mannes.** 1985. pp60$^{c\text{-}src}$ in the developing cerebellum. Mol. Cell. Biol. **5**:27–32.
11. **Gregory, R. J., K. L. Kammermeyer, W. S. Vincent III, and S. C. Wadsworth.** 1987. Primary sequence and developmental expression of a novel *Drosophila melanogaster src* gene. Mol. Cell. Biol. **7**:2119–2127.
12. **Hafen, E., K. Basler, J.-E. Edstroem, and G. M. Rubin.** 1987. *Sevenless*, a cell-specific homeotic gene of *Drosophila*, encodes a putative transmembrane receptor with a tyrosine kinase domain. Science **236**:55–63.
13. **Hafen, E., M. Levine, R. Garber, and W. Gehring.** 1983. An improved *in situ* hybridization method for the detection of cellular RNAs in *Drosophila* tissue sections and its application for localizing transcripts of the homeotic *Antennapedia* gene complex. EMBO J. **2**:617–623.
14. **Hammond, C. I., P. K. Vogt, and J. M. Bishop.** 1985. Molecular cloning of the PRCII sarcoma viral genome and the chicken proto-oncogene c-*fps*. Virology **143**:300–308.
15. **Hanafusa, H.** 1988. The *fps/fes* oncogene, p. 39–51. *In* E. P. Reddy, A. M. Skalka, and T. Curran (ed.), The oncogene handbook. Elsevier Science Publishers B.V. (Biomedical Division), Amsterdam.

16. **Hao, Q.-L., N. Heisterkamp, and J. Groffen.** 1989. Isolation and sequence analysis of a novel human tyrosine kinase gene. Mol. Cell. Biol. **9**:1587–1593.
17. **Hayashi, S., I. C. Gillam, A. D. Delaney, and G. M. Tener.** 1978. Acetylation of chromosome squashes of *Drosophila melanogaster* decreases the background in autoradiographs from hybridization with [^{125}I]-labeled RNA. J. Histochem. Cytochem. **26**: 677–679.
18. **Henkemeyer, M. J., R. L. Bennett, F. B. Gertler, and F. M. Hoffmann.** 1988. DNA sequence, structure and tyrosine kinase activity of the *Drosophila melanogaster* Abelson proto-oncogene homolog. Mol. Cell. Biol. **8**:843–853.
19. **Hoffmann, F. M.** 1989. Roles of *Drosophila* proto-oncogene and growth factor homologs during development of the fly. Curr. Top. Microbiol. Immunol. **147**:1–29.
20. **Hunter, T., and J. A. Cooper.** 1985. Protein-tyrosine kinases. Annu. Rev. Biochem. **54**:897–931.
20a. **Jackson, J.** Unpublished data.
20b. **Jackson, J., and R. F. Paulson.** Unpublished data.
21. **Kaplan, J. M., G. Mardon, J. M. Bishop, and H. E. Varmus.** 1988. The first seven amino acids encoded by the v-*src* oncogene act as a myristylation signal: lysine-7 is a critical determinant. Mol. Cell. Biol. **8**:2435–2441.
21a. **Katzen, A. L., T. Kornberg, and J. M. Bishop.** 1990. Diverse expression of *dsrc29A*, a gene related to *src*, during the life cycle of *Drosophila melanogaster*. Development, in press.
22. **Katzen, A. L., T. B. Kornberg, and J. M. Bishop.** 1985. Isolation of the proto-oncogene c-*myb* from *Drosophila melanogaster*. Cell **41**:449–456.
23. **Kemphues, J. J., E. C. Raff, and T. C. Kaufman.** 1983. Genetic analysis of B2t, the structural gene for a testis specific β-tubulin subunit of *Drosophila melanogaster*. Genetics **105**:345–356.
24. **King, R. C.** 1970. Ovarian development in *Drosophila melanogaster*. Academic Press, Inc., New York.
25. **Kornberg, T., I. Siden, P. O'Farrell, and M. Simon.** 1985. The engrailed locus of *Drosophila*: *in situ* localization of transcripts reveals compartment-specific expression. Cell **40**:45–53.
26. **Livneh, E., L. Glazer, D. Segal, J. Schlessinger, and B.-Z. Shilo.** 1985. The *Drosophila* EGF receptor gene homolog: conservation of both hormone binding and kinase domains. Cell **40**:599–607.
27. **McLachlan, A. D.** 1971. Tests for comparing related amino-acid sequences. Cytochrome *c* and cytochrome c_{551}. J. Mol. Biol. **61**:409–424.
28. **Meckling-Hansen, K., R. Nelson, P. Branton, and T. Pawson.** 1987. Enzymatic activation of Fujinami sarcoma virus *gag-fps* transforming proteins by autophosphorylation at tyrosine. EMBO J. **6**:659–667.
28a. **Paulson, R. F.** Unpublished data.
29. **Pawson, T.** 1988. Non-catalytic domains of cytoplasmic protein-tyrosine kinases: regulatory elements in signal transduction. Oncogene **3**:491–495.
30. **Pawson, T., K. Letwin, T. Lee, Q.-L. Hao, N. Heisterkamp, and J. Groffen.** 1989. The *fer* gene is evolutionarily conserved and encodes a widely expressed member of the *fps/fes* protein-tyrosine kinase family. Mol. Cell. Biol. **9**:5722–5725.
31. **Petruzzelli, L., R. Herrera, R. Arenas-Garcia, R. Fernandez, M. J. Birnbaum, and O. M. Rosen.** 1986. Isolation of a *Drosophila* genomic sequence homologous to the kinase domain of the human insulin receptor and detection of the phosphorylated *Drosophila* receptor with an anti-peptide antibody. Proc. Natl. Acad. Sci. USA **83**:4710–4715.
32. **Poole, S. J., L. M. Kauvar, B. Drees, and T. Kornberg.** 1985. The engrailed locus of *Drosophila*: structural analysis of an embryonic transcript. Cell **40**:37–43.
33. **Rubin, G. M.** 1989. Development of the Drosophila retina: inductive events studied at single cell resolution. Cell **57**:519–520.
34. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
35. **Shilo, B.-Z.** 1987. Proto-oncogenes in *Drosophila melanogaster*. Trends Genet. **3**:69–73.
35a. **Simon, M. A., and J. M. Bishop.** Unpublished data.
36. **Simon, M. A., D. D. L. Bowtell, and G. M. Rubin.** 1989. Structure and activity of the *sevenless* protein—a protein tyrosine kinase receptor required for photoreceptor development in *Drosophila*. Proc. Natl. Acad. Sci. USA **86**:8333–8337.
37. **Simon, M. A., B. Drees, T. Kornberg, and J. M. Bishop.** 1985. The nucleotide sequence and the tissue-specific expression of *Drosophila* c-*src*. Cell **42**:831–840.
38. **Sorge, L. K., B. T. Levy, and P. F. Maness.** 1984. pp60$^{c\text{-}src}$ is developmentally regulated in the neutral retina. Cell **36**:249–257.
39. **Sorsa, V.** 1988. Chromosome maps of *Drosophila*, vol. II. CRC Press, Inc., Boca Raton, Fla.
40. **Sprenger, F., L. M. Stevens, and C. Nusslein-Volhard.** 1989. The *Drosophila* gene *torso* encodes a putative receptor tyrosine kinase. Nature (London) **338**:478–483.
41. **Tomlinson, A., and D. F. Ready.** 1987. Cell fate in the *Drosophila* ommatidium. Dev. Biol. **123**:264–275.
42. **Trujillo-Cenoz, O.** 1965. Some aspects of the structural organization of the intermediate retina of dipterans. J. Ultrastruct. Res. **13**:1–33.
43. **Vincent, W. S., R. J. Gregory, and S. C. Wadsworth.** 1989. Embryonic expression of a *Drosophila src* gene: alternate forms of the protein are expressed in segmental stripes and in the nervous system. Genes Dev. **3**:334–347.
44. **Wadsworth, S. C., F. A. Muckenthaler, and W. S. Vincent III.** 1990. Differential expression of alternate forms of a *Drosophila src* protein during embryonic and larval tissue differentiation. Dev. Biol. **138**:296–312.
45. **Weinmaster, G., M. J. Zoller, M. Smith, E. Hinze, and T. Pawson.** 1984. Mutagenesis of Fujinami sarcoma virus: evidence that tyrosine phosphorylation of P130$^{gag\text{-}fps}$ modulates its biological activity. Cell **37**:559–568.
46. **Wilks, A. F., and R. R. Kurban.** 1988. Isolation and structural analysis of murine c-*fes* cDNA clones. Oncogene **3**:289–294.
47. **Zuker, C. S., A. F. Cowman, and G. M. Rubin.** 1985. Isolation and structure of a rhodopsin gene from *D. melanogaster*. Cell **40**:851–858.