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Diverse expression of *dsrc29A*, a gene related to *src*, during the life cycle of *Drosophila melanogaster*

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Summary

We used *in situ* hybridization to study the RNA expression of the *dsrc29A* gene during *Drosophila* development. This gene encodes two proteins differing at their amino termini. Both gene products contain a protein-tyrosine kinase domain and resemble the protein encoded by vertebrate *src*. We examined most stages of development in the *Drosophila* life cycle: embryos, third instar larvae, pupae and adults. Our results revealed that *dsrc29A* expression is specialized throughout development, being prominent at various times in neural tissue, phagocytic cells, dorsal vessel, ovaries, gut, developing salivary glands, imaginal discs and disc derivatives. These findings confirm and extend previous results for the distribution of *dsrc29A* protein, indicating that the regulation of this gene is primarily at the level of transcription. In some tissues expression is transient, whereas in others, it is continuous, and expression occurs in proliferative, differentiating and differentiated tissue. These patterns of expression demonstrate how a single protein-tyrosine kinase might play diverse roles at different times during development. Comparison of the expression of *dsrc29A* and other members of the protein-tyrosine kinase gene superfamily reveals that the genes are expressed in distinctive but sometimes overlapping patterns.

Key words: *dsrc29A*, protein-tyrosine kinase, proto-oncogene, oncogene, *Drosophila* development, gene expression.

Introduction

Multicellular organisms possess many protein-tyrosine kinases (PTKs) that serve a variety of functions in the determination of cellular phenotype (Hunter and Cooper, 1985). These enzymes fall into two broad classes, those that span the plasma membrane and serve as cell-surface receptors, and those that reside in the cytoplasm but are often associated with membranes.

Among the genes encoding cytoplasmic PTKs is a mini-family for which the proto-oncogene *src* serves as prototype. Two *Drosophila* genes related to *src* have been described (Simon *et al.* 1983, 1985; Hoffman *et al.* 1983; Wadsworth *et al.* 1985; Gregory *et al.* 1987). The more closely related of the two encodes a protein that possesses most of the signature sequences of its vertebrate counterpart (Hoffman *et al.* 1983; Simon *et al.* 1985). It has previously been called by various names, including *Drosophila* c-*src* and *Dsrc*. In order to avoid confusion, we will refer to this gene as *dsrc64B* to denote both its kinship to *src* and its chromosomal location (position 64B on the third chromosome). Expression of *dsrc64B* is prominent in developing retinal and other neural tissue and thus resembles that of vertebrate *src* (Cotton and Brugge, 1983; Levy *et al.* 1984; Simon *et al.* 1985).

The second *src*-related gene was the subject of the present study. It was originally identified in this laboratory and localized to position 29A on the second chromosome (Simon *et al.* 1983), but Gregory *et al.* (1987) reported that the gene was located at position 28C and termed the gene *Dsrc28C*. Subsequently, they have determined that the original localization at position 29A is correct (S. Wadsworth, personal communication). Therefore, we refer to this gene as *dsrc29A* to denote its kinship to *src* and its chromosomal location. While the gene product of *dsrc29A* shares many structural features with vertebrate *src* and is more related to members of the *src* gene family than to any other PTK genes, it also displays certain features that distinguish it from all other PTK genes described to date (Gregory *et al.* 1987).

Two protein products encoded by *dsrc29A*, p66 and p55 have been identified (Vincent *et al.* 1989). They differ at their amino termini and localize differently within the cell: p66 is associated with the plasma membrane, whereas p55 is uniformly distributed throughout the cytoplasm. Unlike all other known

members of the *src* family, neither protein contains a consensus signal sequence for myristylation at its amino terminus. Furthermore, neither has met and gly as its first two amino acids, a feature found in all other cytoplasmic PTKs characterized (Hunter, 1989; Cooper, 1990), and a sequence motif absolutely required for myristylation (Buss *et al.* 1986; Kaplan *et al.* 1988). A recent report has identified a mouse gene that may be the counterpart of *dsrc29A*, although further characterization will be required to confirm this relationship (Wilks *et al.* 1989).

Initial studies of *dsrc29A* RNA expression on a limited scale led to the conclusion that expression was general during embryonic and pupal development (Wadsworth *et al.* 1985; Gregory *et al.* 1987), findings that do not agree with results from more extensive protein analysis (Vincent *et al.* 1989; Wadsworth *et al.* 1990). We now report a more extensive survey of the pattern of *dsrc29A* mRNA distribution in embryos, larvae, pupae and adults. In contrast to previous reports, we find that *dsrc29A* is differentially expressed throughout most of development and that the pattern of RNA expression closely matches the distribution of gene products during embryogenesis. In addition, we identify several novel sites of expression in postembryonic development.

Materials and methods

Molecular cloning

Procedures and most of the reagents have been described previously (Katzen *et al.* 1985). The original lambda phage clones containing the *dsrc29A* gene were isolated previously using a *src* probe at relatively low stringency (Simon *et al.* 1983). Additional clones containing the gene were isolated using a *fps* probe prepared with a 1.3 kb *Pvu*II–*Sma*I fragment representing the bulk of v-*fps* (Hammond *et al.* 1985), also at low stringency (ALK and JMB, unpublished).

cDNA clones representing the *dsrc29A* transcript were isolated from libraries in lambda phage provided by L. Kauvar which had been prepared with polyadenylated RNA from pools of embryos (Poole *et al.* 1985). A 1.8 kb *Eco*RI–*Hinc*II fragment derived from one of these clones was inserted into pUC18. This pUC–*dsrc29A* construct was used to prepare the probe for *in situ* hybridization. The *Eco*RI site, which was generated in the process of constructing the cDNA library, is located approximately 220 nucleotides downstream of the initiation codon. The *Hinc*II site is located approximately 280 bases downstream of the termination codon (for a restriction map, refer to Gregory *et al.* 1987).

In situ *hybridization*

In situ hybridization to 8 μm sections of embryos, larvae, pupae and adults was performed by modification of the technique of Hafen *et al.* (1983) as described by Kornberg *et al.* (1985) with one addition. In order to reduce non-specific binding of probe to cuticle, especially in late pupae and adults, sections from postembryonic stages were acetylated immediately after they had been treated with pronase and fixed according to the method of Hayashi *et al.* (1978). The probes were prepared by nick translation of either pUC8 DNA or the pUC–*dsrc29A* construct described above (containing most of the coding domain of *dsrc29A*) in the presence of ^{35}S-labeled dATP and unlabeled dCTP, dGTP and TTP. Probe size was between 50 and 130 bases. Autoradiography was at 4°C for between 15 and 28 days. After developing the slides, sections were stained with Giemsa and mounted permanently with Permount (Fisher). The analysis employed serial sections of samples from various developmental stages. Photomicroscopy was performed with the Zeiss Axiophot. Selected sections were chosen to illustrate the principal conclusions.

Results

Tissue localization of dsrc29A *RNA*

We examined the spatial distribution of *dsrc29A* transcripts by *in situ* hybridization of RNA in frozen sections of developing embryos, larvae, pupae and adults. The probe was prepared from a plasmid containing a 1.8 kb fragment of a *dsrc29A* cDNA. This fragment contains most of the coding domain of the gene and recognizes transcripts encoding both identified protein products. To control for non-specific binding of the probe, parallel experiments were done in which sections were either pretreated with RNAse A or hybridized with a probe prepared with the pUC8 vector. In addition, the results were compared with those obtained with three other *Drosophila* proto-oncogene probes in parallel experiments (c-*myb*, DER, and *dps85D*, manuscripts submitted or in preparation). Each probe detected a different pattern of expression during *Drosophila* development. To ensure that the probe would not recognize messages from other PTK genes, a ^{32}P-labeled probe was prepared from the *dsrc29A* fragment and hybridized to cloned DNAs from four other PTK genes; no cross hybridization was detected (data not shown). Furthermore, under similar hybridization conditions, the *dsrc29A* probe did not recognize any other fragments in Southern blots prepared with *Drosophila* genomic DNA (data not shown). In the results and discussion sections, we will refer to varying levels of expression, but it should be understood that data obtained using the *in situ* technique reflect the levels of messenger RNA in a tissue at a given stage of development, but do not distinguish between differing levels of transcription and differential mRNA stability.

Early embryos

The embryonic stages referred to in this section are as described by Campos-Ortega and Hartenstein (1985). *dsrc29A* transcripts were distributed uniformly in early embryos prior to the formation of the cellular blastoderm (stages 1 to 4, 0–2:10 h), suggesting that the message is of maternal origin (Fig. 1A and B). Once the cellular blastoderm formed (stage 5, 2:10–2:50 h), expression was no longer uniform (Fig. 1C, D, E and F). The *dsrc29A* message was distributed over all of the newly formed cells located at the periphery, but was especially abundant in the posterior half of the embryo in dorsomedial and dorsolateral positions, regions encompassing the anlage for the amnioserosa, the dorsal epidermis and the proctodeum. The yolk region

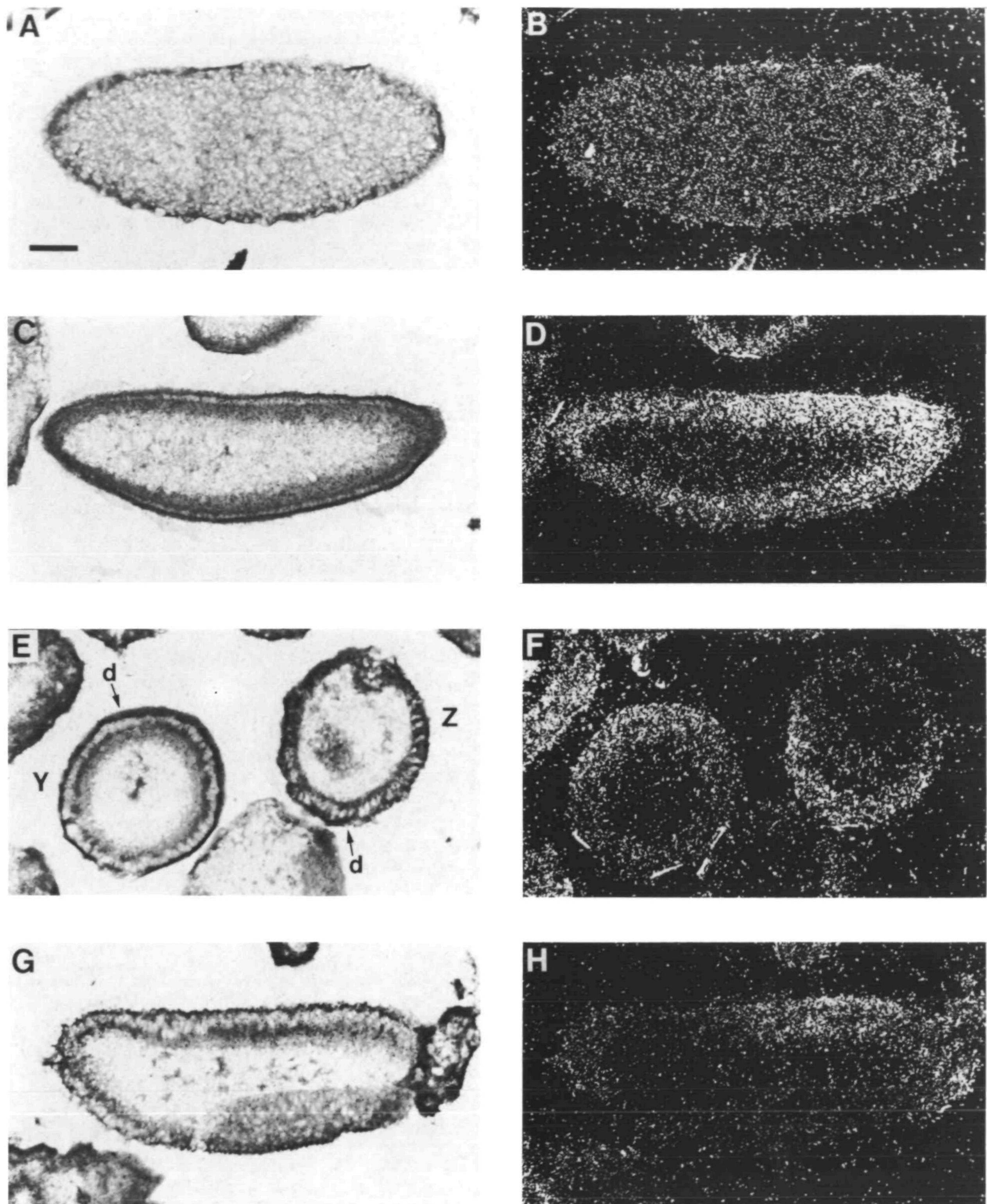


Fig. 1. Expression of *dsrc29A* in early embryos: fertilization to gastrulation. Bright-field (left) and corresponding dark-field (right) micrographs were taken after autoradiography. The horizontal bar in panel A corresponds to 0.05 mm. All embryos are shown at the same magnification. (A and B) A lateral parasagittal section (anterior left, dorsal up) of a preblastoderm embryo. (C and D) A lateral sagittal section (anterior left, dorsal up) of a cellular blastoderm embryo. (E and F) Cross sections of two embryos at different stages of development. Y, a cellular blastoderm embryo and Z, a gastrulating embryo. The dorsal surface (d) of each embryo is indicated. (G and H) A sagittal section (anterior left, dorsal up) of an embryo during early gastrulation (stage 6).

was virtually devoid of transcripts. During gastrulation (stages 6 and 7, 2:50–3:10 h), expression continued to be abundant in the posterior half of the embryo at the dorsal surface (Fig. 1E, F, G and H), but was greatly reduced elsewhere.

Germ band extension and shortening

During germ band elongation (stages 8 to 11, 3:10–5:20 h), novel expression in the ectoderm was observed in a segmentally reiterated pattern along the extent of the segmented germ band (Fig. 2A, B, C and D). While not especially strong, this expression was distinctive and reproducible. It was not observed along the entire extent of the germ band in most individual sections but, by examining many sections, we determined that each segment was represented by a 'stripe'. This expression pattern was observed throughout the period of germ band extension and continued to be observed in germ band shortened embryos, albeit only in quite lateral sections (Fig. 2E and F).

dsrc29A was also expressed in the procephalic lobe and amnioserosa during germ band extension (Fig. 2A and B). In the fully extended embryo and at the beginning of germ band shortening (stages 9 to early stage 12), the primordia of the anterior and posterior

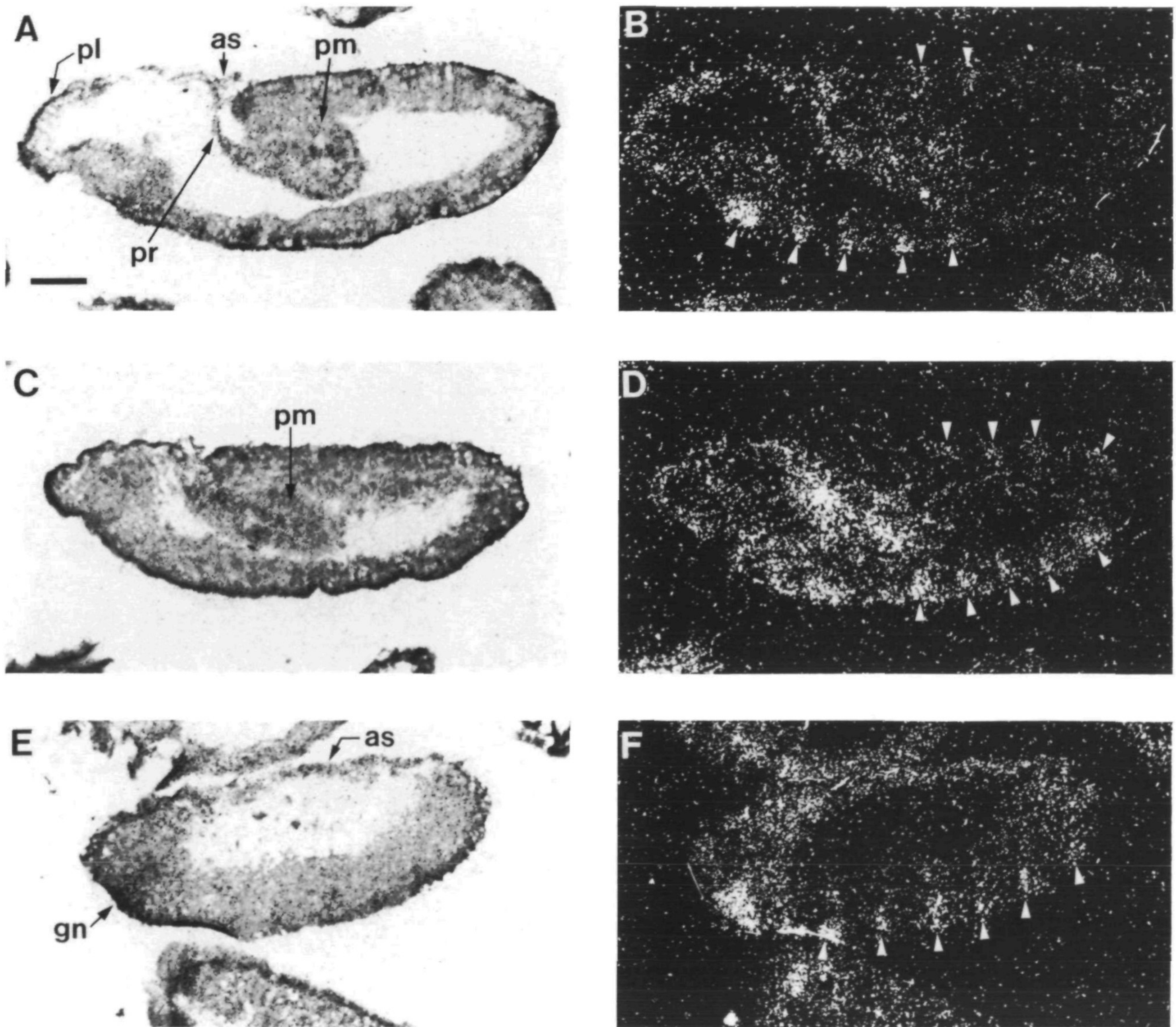


Fig. 2. Expression of *dsrc29A* in a segmentally reiterated pattern during germ band extension and shortening. Each pair of panels represents corresponding bright- (left) and dark-field (right) micrographs. Arrowheads in panels B, D, and F indicate regions of discrete expression representing the segmentally reiterated pattern. Orientation of all embryos is anterior to the left and dorsal side up. The horizontal bar in panel A corresponds to 0.05 mm. All embryos are shown at the same magnification. Abbreviations: as, amnioserosa; gn, gnathal segment; pl, procephalic lobe; pm, posterior midgut primordium; pr, proctodeal primordium. (A and B) A parasagittal section of an embryo undergoing germ band extension (early stage 8). (C and D) A sagittal section of a fully extended embryo (early stage 11). (E and F) A lateral sagittal section of a germ band shortened embryo (stage 13).

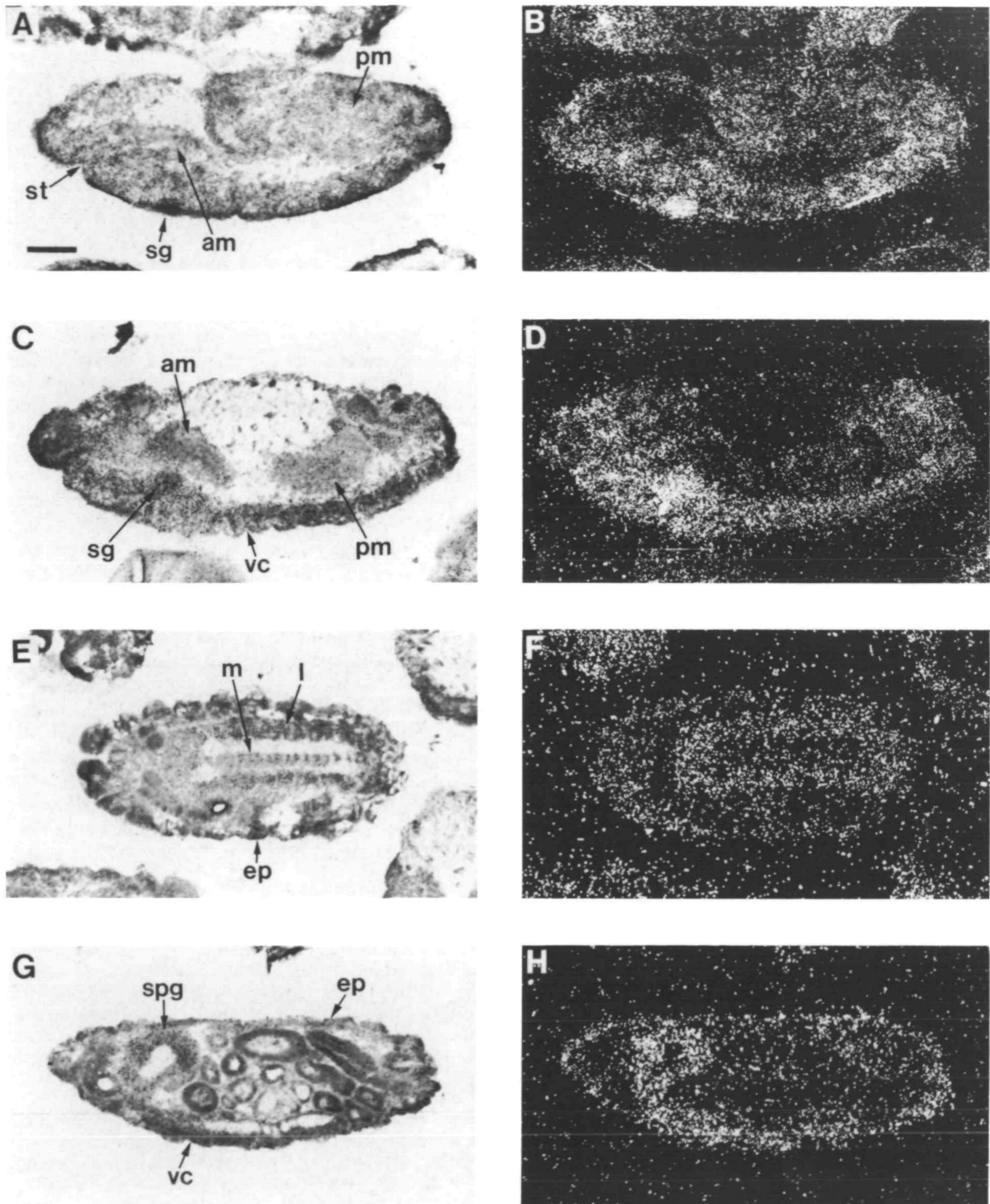


Fig. 3. Expression of *dsrc29A* in late embryos: germ band shortening to hatching. Each pair of panels represents corresponding bright- (left) and dark-field (right) micrographs. Orientation of all embryos is anterior to the left. The horizontal bar in panel A corresponds to 0.05 mm. All embryos are shown at the same magnification. Abbreviations include: am, anterior midgut; ep, epithelium; pm, posterior midgut; sg, salivary gland; st, stomadeum; spg, supraoesophageal ganglia; vc, ventral nerve cord. (A and B) A parasagittal section (dorsal up) of germ band shortening embryo (stage 12). (C and D) A parasagittal section (dorsal up) of germ band shortened embryo (stage 13).
(E and F) A horizontal section through the ventral nerve cord of a late embryo (ca. stage 16). Transcripts were detected over the lateral cell bodies (l), but not over cells residing at the midline (m) of the nerve cord. (G and H) A parasagittal section (dorsal up) of a nearly mature embryo (stage 17).

midguts and the proctodeum expressed the gene at especially high levels (Fig. 2C and D and data not shown).

During the process of germ band shortening (stage 12, 7:20–9:20 h), the segmentally reiterated 'stripes' became less distinctive in medial sections (Fig. 3A and B). Instead, more general expression was observed over developing tissues of ectodermal origin (including the ectoderm of the germ band, the developing nervous system, the anterior region of the invaginating stomadeum and the clypeolabrum), but the levels of expression were not entirely uniform. The developing salivary gland (a derivative of the labial segment) expressed *dsrc29A* at especially high levels during the shortening process and continued to do so upon its completion (Fig. 3A, B, C, and D). Over the same period, another region displaying especially high levels of expression was observed in lateral sections (Fig. 2E and F). This region corresponds to one of the gnathal segments, either the mandibular or maxillary bud. In contrast to increasing levels of *dsrc29A* RNA in most embryonic tissues during germ band shortening, the levels in the anterior and posterior midguts decreased (Fig. 3A, B, C, and D).

In germ band shortened embryos (stages 13 and 14, 9:20–11:20 h), the relative levels of *dsrc29A* expression in various tissues changed. For example, in stage 13 embryos, expression was stronger in developing salivary glands than in the adjacent nervous tissue (Fig. 3C and D), whereas in stage 14 embryos, the level of expression in the nervous system was considerably stronger than in the salivary glands (data not shown).

Late embryos

Over the next period of embryonic development (stage 15, 11:20–13:00 h), the level of *dsrc29A* transcripts decreased in most tissues but remained relatively high in the nervous system (data not shown). Transient expression was also observed in the tracheal epithelium and the posterior spiracle (data not shown). During the last two stages of embryogenesis (stages 16 and 17, 13:00 h–hatching), the most prominent region of expression was in the nervous system, but some expression was also observed in the epidermis (Fig. 3E, F, G, and H). In sagittal sections, *dsrc29A* expression appeared to be relatively uniform in the ventral nerve cord (Fig. 3G and H), but horizontal sections revealed a much lower level of expression in cell bodies residing at the midline than in those located laterally (Fig. 3E and F).

Larvae

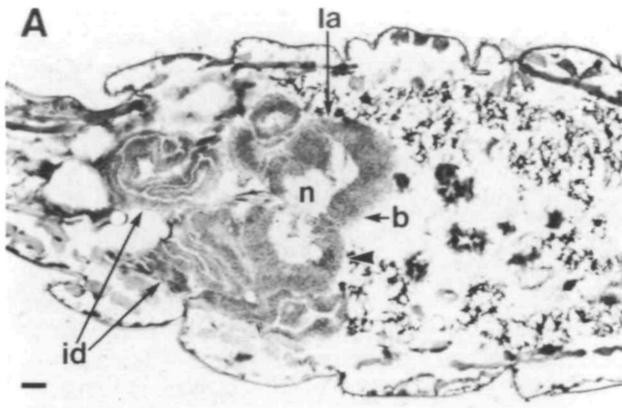
Three different types of tissue exist within *Drosophila* larvae (Bodenstein, 1950). Most of the cells necessary for proper larval function become polytene during larval development, do not divide after embryogenesis, and grow by cell enlargement. Conversely, most of the cells that will form the adult fly or imago are not required for larval development and function. These remain diploid and form either contiguous groups of cells termed imaginal discs or small pockets of cells. The former divide throughout larval development, whereas the latter, which include cells of the digestive system and the abdominal histoblasts, do not begin division until either late in third instar larvae or in the pre-pupal period. The final group of tissues are those that function both in the larva and with some modifications in the adult. This category includes neural tissue, both the brain and the ganglia.

When analyzed in the climbing stage of third instar larvae, *dsrc29A* was expressed in the tissues that will contribute to the adult organism (Fig. 4A–F). Strong expression was observed in the nervous system, but the levels were unevenly distributed (Fig. 4A and B). For example, a region of the midbrain expressed the gene at especially high levels, whereas the cells of the developing optic lamina expressed *dsrc29A* at very low levels. These differences in expression levels were further augmented in prepupal development (see below). Elsewhere in the brain (Fig. 4A and B) and in the ventral ganglia (including the midline) (data not shown), expression was more uniform.

The imaginal discs expressed *dsrc29A* at somewhat variable levels that were generally lower than those observed in the nervous system (Fig. 4A and B). The eye portion of the eye–antennal imaginal disc expressed especially low levels of the gene (data not shown, but see below). In the gonads, *dsrc29A* was expressed in the developing ovary (Fig. 4C and D), and at a low level at the apical pole of the developing testes (data not shown).

Low levels of *dsrc29A* expression were detected in cells (likely to be immature haemocytes) located within the lymph glands (Fig. 4E and F). The lymph glands are the presumptive blood-cell-forming organs of *Drosophila* larvae (Gateff, 1978; Rizki, 1978). In contrast to *dsrc29A* expression in most imaginal tissue, the majority of larval polytene tissues did not express the

Fig. 4. Expression of *dsrc29A* in larvae and prepupae. Each pair of panels represents corresponding bright- (left) and dark-field (right) micrographs. The horizontal bars correspond to 0.05 mm. Abbreviations: b, brain; ed, eye portion of the eye–antennal disc; id, imaginal discs; if, imaginal ring of foregut; la, optic lamina; lg, lymph gland; mb, midbrain; n, neuropile; ol, optic lobe; ov, ovary; pv, proventriculus; vg, ventral ganglia. (A and B) A slightly oblique horizontal section (anterior left) of a late third instar larva showing several imaginal discs and the developing brain. Corresponding arrowheads in panels A and B indicate a region of especially high expression in the midbrain. (C and D) A horizontal section (anterior up) of a late third instar larva showing the developing ovary. (E and F) A parasagittal section (anterior up, dorsal left) of a late third instar larva showing part of a lymph gland and the proventriculus. (G and H) A slightly oblique horizontal section (anterior up) of an early prepupa showing the developing brain and the eye portion of the eye–antennal disc. Arrowheads in panel G indicate regions of especially high expression in the midbrain. (I and J) A parasagittal section of prepupa (anterior left, dorsal up) showing the midbrain, ventral ganglia and some imaginal discs. Arrowhead in panel I indicates the region of especially high expression in the midbrain.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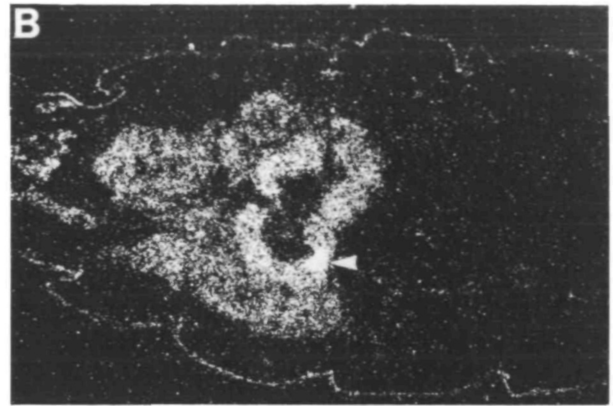
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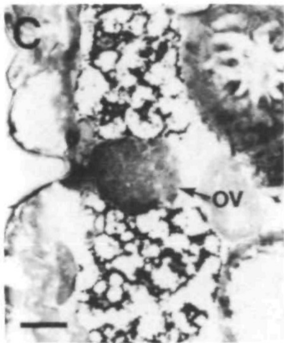
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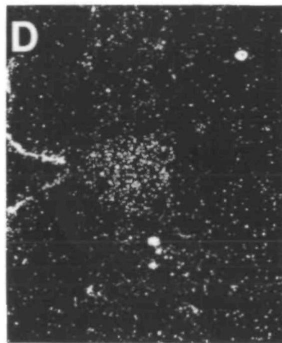
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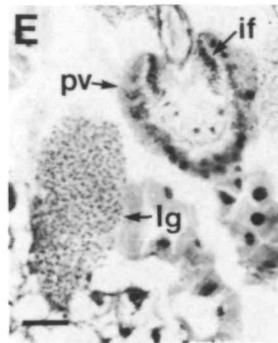
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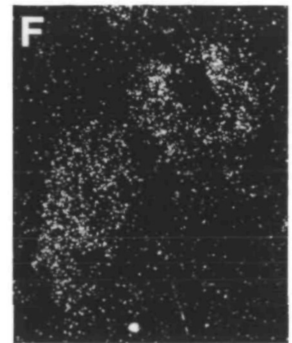
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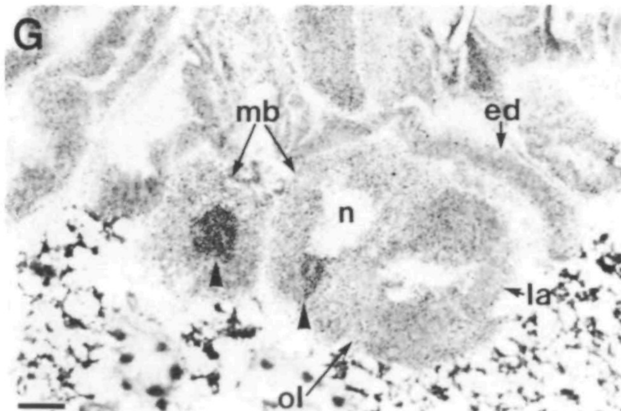
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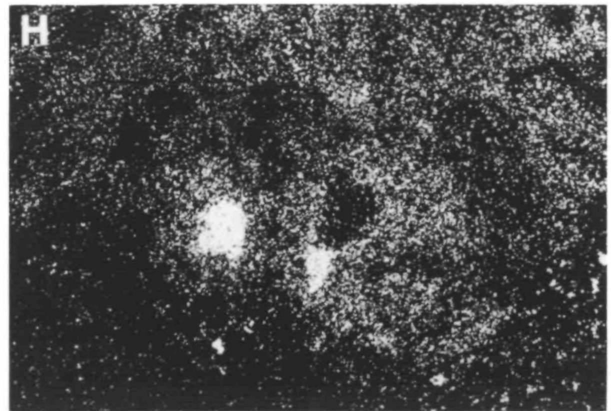
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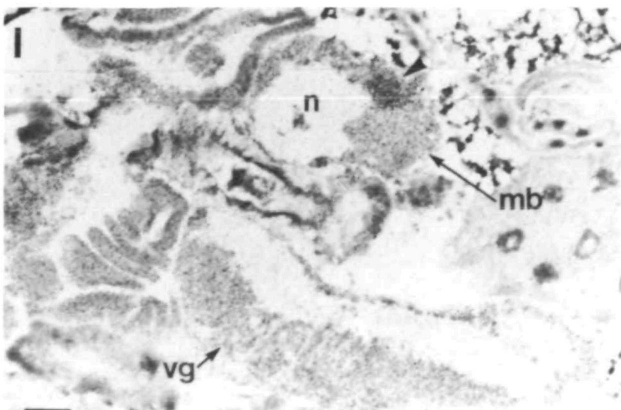
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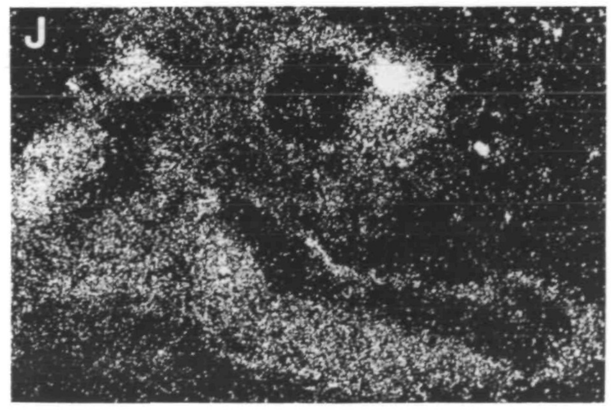
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gene at detectable levels (Fig. 4A–F). One exception to this was a subset of larval cells within the proventriculus that expressed *dsrc29A* at relatively low levels (Fig. 4E and F).

Pupae

In prepupae, the expression patterns established in late third instar larvae were, for the most part, maintained. *dsrc29A* was expressed in most of the central nervous system, with especially strong expression in discrete regions of the midbrain. Specifically, each brain hemisphere contained one of these regions located in a medial (Fig. 4G and H) and dorsal (Fig. 4I and J) position. The optic lamina (and possibly part of the medulla) continued to express *dsrc29A* at very low levels as did the eye portion of the eye–antennal disc (Fig. 4G and H).

Novel *dsrc29A* expression was observed in a few tissues undergoing developmental changes during prepupal and early pupal development. The strongest expression during these periods was in cells we identified (by morphology and location) as phagocytic cells, termed lamellocytes. These terminally differentiated haemocytes derive from less mature forms immediately after puparium formation and play an important role in the histolysis of larval tissues (Crossley, 1978; Rizki, 1978). In prepupae, these cells were most frequently found in anterior regions of the organism (Fig. 5A and B), whereas shortly after cephalic eversion, they were generally observed in posterior regions (Fig. 5C and D), a temporal pattern that correlates with the systematic destruction of larval muscles (Bodenstein, 1950; Crossley, 1978). This is the only period of development in which there are large numbers of lamellocytes in the haemolymph, but blood cells expressing *dsrc29A* were occasionally observed in third instar larvae, later stages of pupal development and adults (data not shown).

Other cells that expressed *dsrc29A* during this period of development were the abdominal histoblasts (Fig. 5C and D), which undergo a period of rapid division immediately after puparium formation, and the epithelial cells of the developing imaginal gut, but not the myoblasts that will form the visceral mesoderm

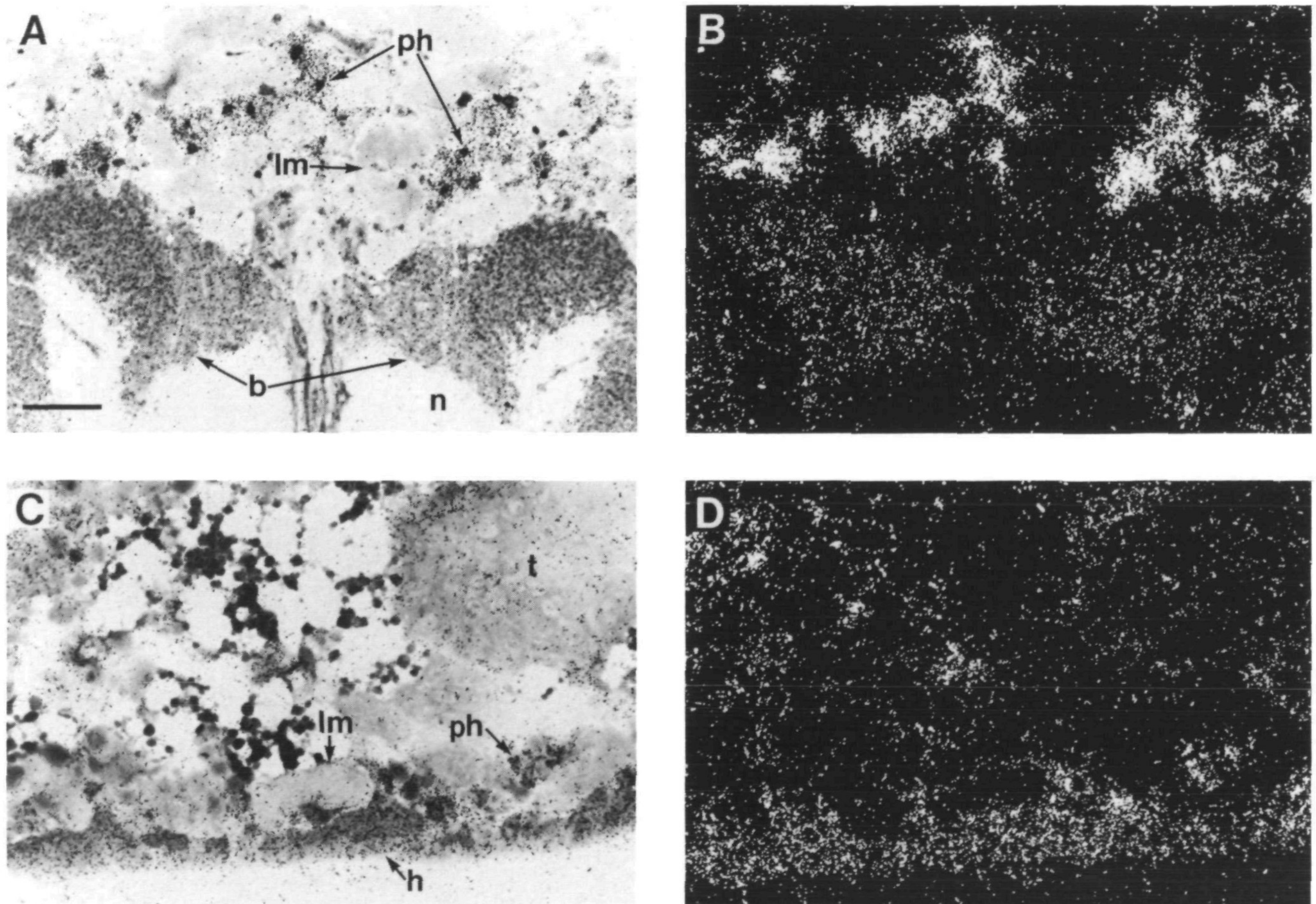

Fig. 5. Expression of *dsrc29A* at high levels in phagocytic cells. Each pair of panels represents corresponding bright- (left) and dark-field (right) micrographs. The horizontal bar in panel A corresponds to 0.05 mm. All sections are shown at the same magnification. Abbreviations: b, brain; h, abdominal histoblasts; lm, larval muscle; n, neuropile; ph, phagocytic cells; t, testes. (A and B) A horizontal section (anterior up) showing muscles undergoing histolysis in the anterior of a prepupa. (C and D) A section (anterior left) showing muscles undergoing histolysis in the abdomen of an early pupa. Also shown are abdominal histoblasts.

(data not shown). Various parts of the developing digestive system expressed *dsrc29A* during the remainder of pupal life (Fig. 6A, B, C and D).

After cephalic eversion, *dsrc29A* continued to be expressed at high levels in the nervous system, but the discrete regions of especially strong expression in the brain were no longer evident (Fig. 6A, B, C and D). Approximately two days after puparium formation the levels began to decline, but low levels of expression were still detectable in pharate adults (data not shown). Throughout pupal development, cells of the optic lamina continued to express the gene at lower levels than elsewhere in the nervous system (Fig. 6A and B and data not shown).

Immediately after cephalic eversion, most of the everted imaginal discs continued to express *dsrc29A* (Fig. 6A and B), but shortly thereafter, expression became more discrete within each disc (Fig. 6C and D). Midway through pupal development, relatively strong expression was observed in specific regions of the developing wing, leg and thoracic epidermis (Fig. 6E and F). Afterwards, *dsrc29A* expression began to decline and was undetectable in these disc derivatives after the third day of pupal development. Thoracic muscle tissue, which is derived from the adepithelium of imaginal discs, also expressed low levels of *dsrc29A* in early pupae (Fig. 6C and D), but expression was no longer detectable after two days of pupal development (data not shown).

Low levels of *dsrc29A* expression were observed in the ovaries during most of pupal development (Fig. 6G and H), with the levels increasing in pharate adults (data not shown). Portions of genital discs of both genders expressed *dsrc29A* throughout most of the pupal period (Fig. 6G and H and data not shown). When the developing disc derivatives attached to the gonads (ca. 30 h after puparium formation), strong expression was observed transiently at the periphery of both the ovary (Fig. 6G and H) and the testes (data not shown). Otherwise, the testes did not express *dsrc29A*.

Adults

dsrc29A was expressed in a variety of adult tissues. Expression was not due to residual mRNA from pupal development, since it was observed in mature (3–5 days after eclosion) as well as newly emerged adults. The highest concentration of transcripts was present in the ovaries, specifically in nurse cells and developing oocytes (Fig. 7A and B), indicating that the message present in preblastoderm embryos was maternally derived. Low, but detectable levels of expression continued in the adult nervous system, where it was somewhat higher in the midbrain than in the optic lobes (Fig. 7C and D), and in the abdominal ganglia than in the thoracic ganglia (Fig. 8A and B).

Two specialized epithelial cell types expressed *dsrc29A*. These were the tall epithelial cells of the proventriculus (Fig. 7E and F) and the epithelium of the ejaculatory bulb, a derivative of the genital disc (Fig. 7G and H). Both of these tissues appeared to express the gene at higher levels in the adult than in late pupae (data not shown).

dsrc29A was also expressed in the dorsal vessel, both at the junction of the heart and the aorta (Fig. 8A and B) and along the extent of the heart (Fig. 8C and D). The specific cells expressing the gene were not identified, but we suspect that they are the pericardial cells. One other cell type present in the adult abdomen expressed *dsrc29A* at relatively high levels (Fig. 8E and F). These cells were observed in very few sections, indicating that there are a small number of them in the organism. Although their appearance is very distinctive, we were unable to identify them.

Discussion

Summary of dsrc29A *expression during development*

We examined the pattern of *dsrc29A* expression during most periods of *Drosophila* development, using a probe that recognizes transcripts encoding both of the identified gene products. The only period in which transcripts are uniformly distributed is in the early embryo, when the message is of maternal origin. Throughout the rest of development, *dsrc29A* is

Fig. 6. Expression of *dsrc29A* in pupae. Each pair of panels represents corresponding bright- (left) and dark-field (right) micrographs. The horizontal bars correspond to 0.05 mm. Abbreviations of developing tissues: an, anus; b, brain; g, gut; gd, genital disc derivative; im, imaginal muscle; l, leg; la, optic lamina; n, neuropile; ov, ovary; pv, proventriculus; th, thoracic epithelium; vg, ventral ganglia; w, wing. (A and B) A horizontal section (anterior left) showing the developing head and thorax of a young pupa. Arrowheads in panel A indicate everted imaginal discs. (C and D) A parasagittal section (anterior up, dorsal right) showing the developing thorax of a slightly older pupa than shown in panels A and B. (E and F) A horizontal section (anterior left) of a pupa midway through development (ca. 2 day after puparium formation) showing a developing wing and leg. Arrowhead in panel E indicates a region of especially high expression in the thoracic epithelium. (G and H) An oblique section (anterior left) of a young pupa (ca. 30 h after puparium formation) showing the developing ovary and genital disc derivatives, including the anus.

Fig. 7. Expression of *dsrc29A* in adults. Each pair of panels represents corresponding bright- (left) and dark-field (right) micrographs. The horizontal bar in panel A corresponds to 0.05 mm. All sections are shown at the same magnification. Note that the apparent signal over some cuticular structures in dark-field micrographs is due to light refraction caused by the cuticle itself. Abbreviations: e, eye; eb, ejaculatory bulb; mb, midbrain; n, neuropile; nc, nurse cells; o, oocytes; ol, optic lobe; pv, proventriculus; t, testes; te, tall epithelial cells of the proventriculus. (A and B) A horizontal section (anterior left) showing a mature ovary. (C and D) A horizontal section (anterior up) showing an adult head. (E and F) A horizontal section (anterior left) showing an adult thorax, including the proventriculus. (G and H) A section (anterior left) showing the abdomen of an adult male, including part of the testes and the ejaculatory bulb.

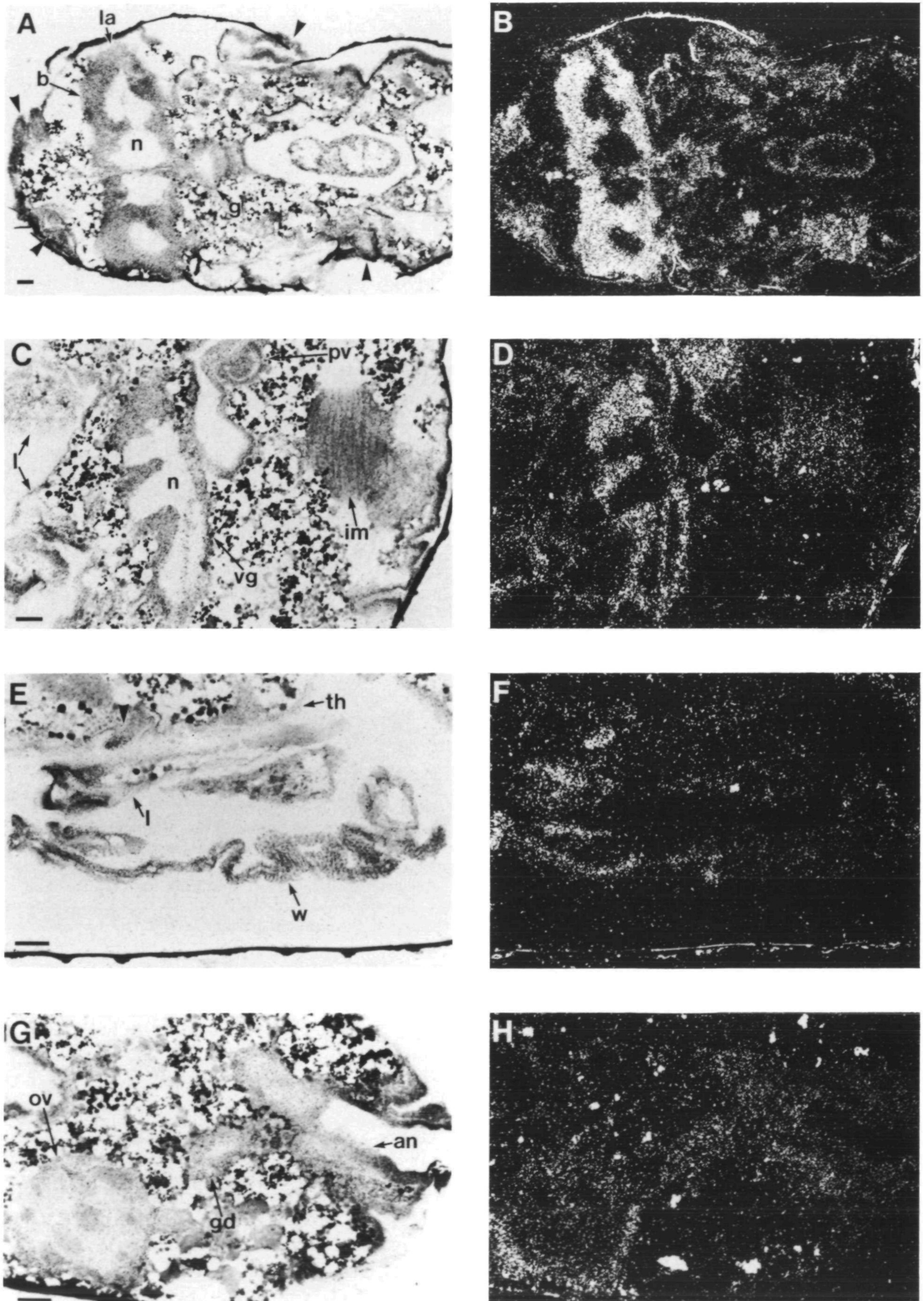

Fig. 6. For legend see p. 1177

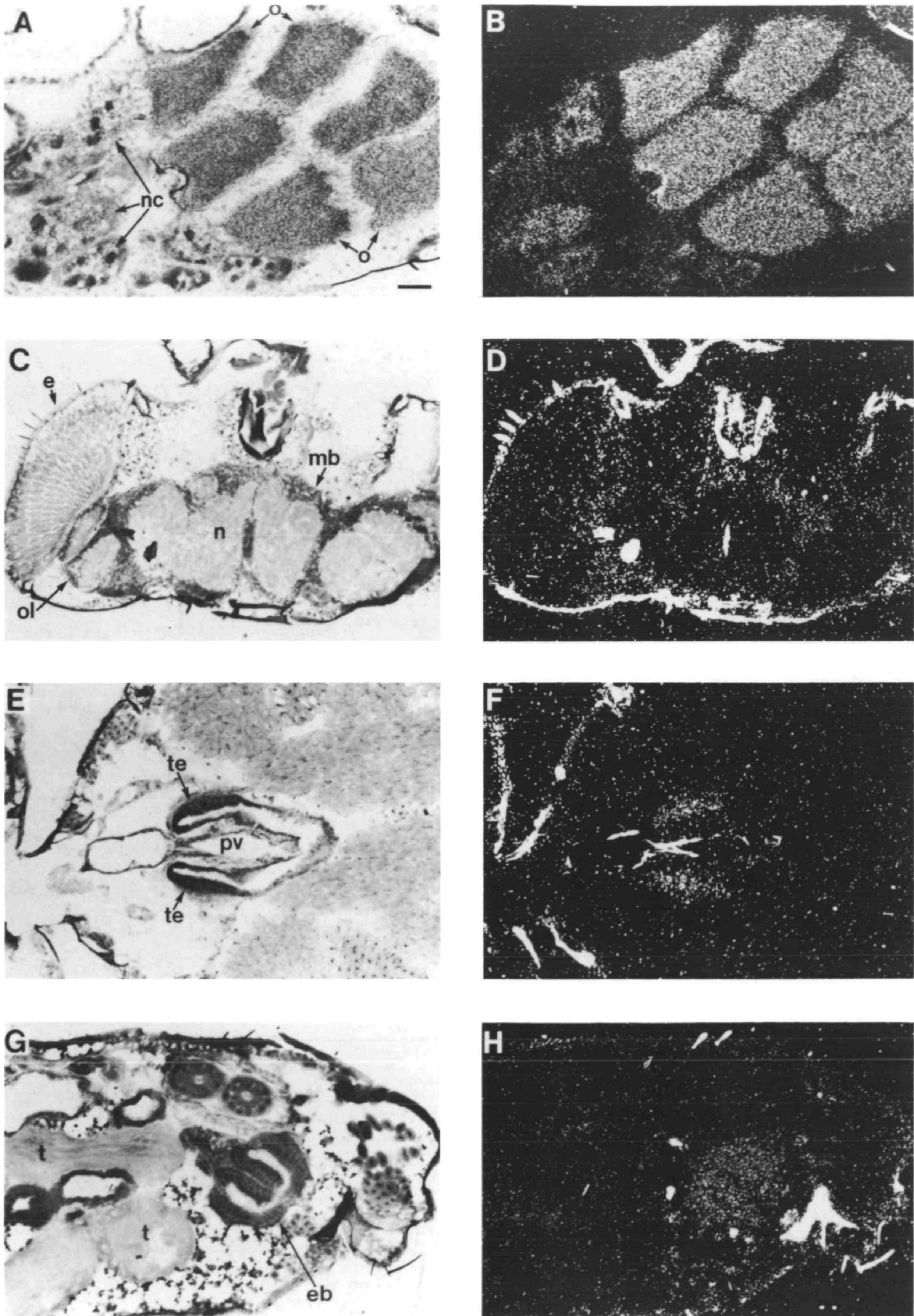

Fig. 7. For legend see p. 1177

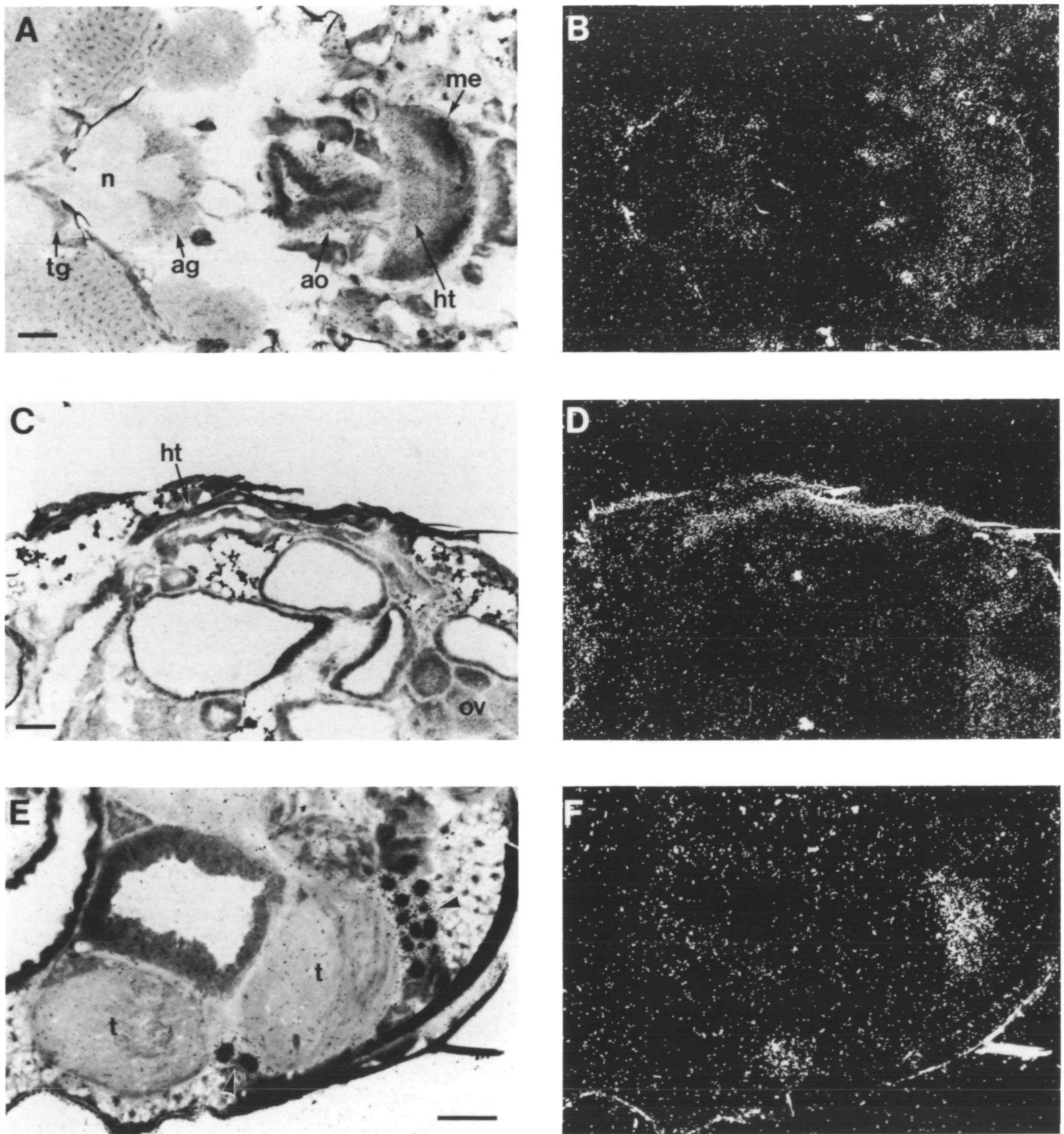

Fig. 8. Expression of *dsrc29A* in adults. Each pair of panels represents corresponding bright- (left) and dark-field (right) micrographs. The horizontal bars correspond to 0.05 mm. Abbreviations: ag, abdominal ganglia; ao, aorta (thoracic portion of dorsal vessel); ht, heart (abdominal portion of dorsal vessel); me, mesophragma; n, neuropile; ov, ovary; t, testes; tg, thoracic ganglia. (A and B) A horizontal section (anterior left) showing the junction of the heart and aorta of the dorsal vessel. Also shown is a portion of the thoracic and abdominal ganglia. (C and D) A parasagittal section (anterior left, dorsal up) showing the dorsal half of an adult abdomen, including part of the heart. (E and F) A parasagittal section (anterior left, dorsal up) showing *dsrc29A* expression in unidentified cells, indicated by arrowheads, located in the abdomen of an adult male.

preferentially expressed in a variety of proliferative and differentiated tissues. Expression is transient in some tissues, including the developing salivary gland, gut epithelium, tracheal epithelium, imaginal discs (and imaginal nests) and muscle tissue. Especially provocative is the segmentally reiterated pattern observed in

the ectoderm (and possibly mesoderm) of the germ band extended embryo. In other tissues, expression is more sustained, being as strong (or stronger) when the tissues reach maturity as it is during their development. These tissues include terminally differentiated haemocytes (phagocytes or lamellocytes), the dorsal vessel (specifically the heart region), tall epithelial cells of the proventriculus, epithelium of the ejaculatory bulb, and nurse cells and oocytes in the ovary. *dsrc29A* is abundantly expressed in the nervous system throughout most of development and continues to be expressed at low levels in the adult.

Correlation of dsrc29A *expression with previous studies of RNA and protein expression*

Previous studies of *dsrc29A* RNA expression during development included Northern and *in situ* analysis. Northern analysis showed that the levels of *dsrc29A* message per microgram of total RNA are highest during embryogenesis and in the adult ovary (Wadsworth *et al.* 1985; Gregory *et al.* 1987). *In situ* analysis showed that the transcripts in the ovary are located in nurse cells and oocytes, and represent the synthesis of maternal message (Gregory *et al.* 1987). Our results are in agreement with these. Though no data were shown, Gregory *et al.* (1987) also stated that *in situ* analysis of RNA in embryos and 24 h pupae revealed no preferential expression. More recent analysis revealed that the *dsrc29A* protein is not uniformly distributed throughout the tissues during embryogenesis (Vincent *et al.* 1989; Wadsworth *et al.* 1990). Several possible explanations for the discrepencies between the RNA and protein data were offered, including selective translation or selective turnover of the protein.

In contrast to the previous reports, we found that *dsrc29A* RNA is expressed selectively during embryogenesis and that the expression pattern correlates well with the reported distribution of the protein (Vincent *et al.* 1989; Wadsworth *et al.* 1990). These results indicate that the *dsrc29A* gene is regulated primarily at the level of transcription (or possibly RNA stability). Since regulation is likely to be transcriptional, the generation of a segmentally reiterated pattern during germ band extension suggests that, at this stage, *dsrc29A* expression may be (at least partially) under control of one of the segmentation genes (Ingham, 1988).

An interesting exception to the correspondence between the message and the protein occurs in embryos prior to the formation of the syncytial blastoderm. During this period, there is an abundance of maternally derived transcript, but no protein is detectable (Wadsworth *et al.* 1990), indicating that the message is not translated at this early stage of development. A few other apparent discrepancies between the distribution of mRNA and protein exist. For example, Wadsworth *et al.* (1990) reported that among cells of the cellular blastoderm, the distribution of protein is relatively uniform, whereas we found that the message is distributed unevenly (see Fig. 1, panels C–F). Since, during subsequent stages of development, the protein pattern agrees with that of the message and neither is distributed uniformly, we suspect that this transient difference reflects a lag in changes of the protein pattern with respect to the mRNA pattern (possibly due to longer half-life of the protein). Our observation of an especially high concentration of *dsrc29A* transcripts in one of the gnathal segments during germ band shortening (Fig. 2E and F) was not reported for the protein. This may represent a real distinction or slight differences in the developmental stages of the animals examined. Finally, Wadsworth *et al.* (1990) detected *dsrc29A* protein in the peripheral nervous system, but we have not reported the same finding for the message. This is almost certainly due to the technical difficulty of detecting and identifying mRNA in cell bodies of peripheral neurons, using the method of *in situ* hybridization to sectioned material. Although *dsrc29A* expression is at its highest overall levels during embryogenesis and in the adult ovary (Wadsworth *et al.* 1985; Gregory *et al.* 1987), our findings (of specific expression throughout development and in the adult) dispute the conclusion of Gregory *et al.* (1987) that the gene's function is largely embryonic.

Comparison of dsrc29A *expression with that of other genes encoding PTKs*

The expression pattern of *dsrc29A* is both similar to and different from the expression of *dsrc64B*, another gene closely related to *src* (Simon *et al.* 1985). Similarities include maternally inherited message and preferential expression in both embryonic and postembryonic neural tissue. Both genes are also expressed at especially high levels in discrete regions of the larval and prepupal midbrain. The locations of these regions are similar and may be overlapping, but a direct experimental comparison will be required to determine this. However, there are also several distinctions between the expression patterns of the two genes. One of these is that *dsrc64B* is preferentially expressed in differentiating photoreceptor cells of the eye portion of the eye–antennal disc, and only at low levels elsewhere in this and other imaginal discs. In contrast, *dsrc29A* is expressed at very low levels in the eye disc and at higher levels in all other discs. Since *dsrc64B* expression was not studied at stages later than early pupae, no comparison can be made for late expression between the two genes.

The expression of several other PTK genes has been characterized to varying extents (Schejter *et al.* 1986; Banerjee *et al.* 1987; Hafen *et al.* 1987; Kammermeyer and Wadsworth, 1987; Garofalo and Rosen, 1988; Sprenger *et al.* 1989; Elkins *et al.* 1990; Katzen *et al.* unpublished data). Most of these, like the two *src*-related genes, are preferentially expressed in the nervous system during development, but each exhibits its own particular pattern. Examples include the following. (i) While *dsrc29A* is expressed at higher levels in the lateral cell bodies of the embryonic ventral nerve cord than at the midline, two other PTK genes – one encoding a cytoplasmic protein denoted *dfps85D* and the other encoding a cell surface receptor denoted DER – are preferentially expressed at the midline, but

not laterally (Katzen *et al.* unpublished data). (ii) During larval and prepupal development, *dsrc29A* is not expressed (or is expressed only at very low levels) in the optic lamina, but is expressed throughout the rest of the central nervous system. In contrast, DER is only expressed in the optic lamina (Kammermeyer and Wadsworth, 1987; Katzen *et al.* unpublished data).

The especially low expression of *dsrc29A* in the developing eye disc (noted above) appears to be an unusual feature for a PTK gene. In addition to *dsrc64B*, at least three other PTK genes are expressed at high levels in the eye disc before or after the morphogenetic furrow. These are *dfsp85D*, DER and another cell surface receptor, *sevenless* (Simon *et al.* 1985; Banerjee *et al.* 1987; Hafen *et al.* 1987; Katzen *et al.* unpublished data). Additionally, genetic analysis of the *sevenless* and DER loci has shown that both genes serve a function in retinal development (Tomlinson and Ready, 1986; Baker and Rubin, 1989; Rubin, 1989). Two features of *dsrc29A* gene expression appear to be unique: expression in terminally differentiated haemocytes or phagocytes and in cells of the dorsal vessel. No other PTK gene has been reported to be expressed in these cell types.

We studied the expression of *dsrc29A*, *dfps85D* and DER in parallel and discovered another intriguing example of the diversity and complexity of PTK gene expression. All three genes are expressed in the adult proventriculus, but each in a different and non-overlapping subset of cells: *dsrc29A* in the tall epithelial cells, *dfs85D* in cells at the junction of the fore and midgut derivatives, and DER in the internal valvular epithelium. It is not obvious why these genes display such precise patterns of expression in the proventriculus, an organ that has the dual function of acting as a valve to prevent regurgitation of food and of producing the peritrophic membrane (Miller, 1950).

The work discussed here illustrates some of the complexities involved in regulating PTK genes. Though many of these genes are active in the same types of tissues, each appears to be under a separate set of controls. Some of the PTK gene expression patterns are partially or completely overlapping, whereas others appear to be mutually exclusive. The relative expression patterns among the PTK genes is reminiscent of the relationships between expression of early pattern formation genes (Ingham, 1988). These observations suggest the possibility that certain developmental pathways may require the combinatorial activities of two or more PTK genes.

Conclusion

Since *dsrc29A* is specifically expressed in adults as well as during development, it is likely that the gene provides a function to the organism throughout its life cycle. As has been observed for several other PTK genes, expression occurs in dividing, differentiating and terminally differentiated tissues, adding to the mounting evidence that the function of these genes is not limited to regulation of cell proliferation. The changing patterns of *dsrc29A* expression illustrate how a single PTK might serve diverse yet specific purposes during development, functioning in both mitotic and differentiated cells.

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