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Expression during *Drosophila* Development of DER, a Gene Related to *erbB*-1 and *neu*: Correlations with Mutant Phenotypes

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We used *in situ* hybridization to study expression of the DER gene during *Drosophila* development. DER encodes a transmembrane cell-surface receptor with a cytoplasmic protein-tyrosine kinase domain, and resembles the vertebrate genes that encode the EGF receptor and the *neu* protein. We examined most stages of development in the *Drosophila* life cycle and found a substantial correlation between DER expression and the phenotypes associated with a variety of mutant alleles. Of particular note were high levels of expression in the primordia of the mouthparts, which are the embryonic tissues most sensitive to reductions in DER activity; discrete expression in a subset of neural cells essential for construction of the axonal scaffold, a structure that is deformed in mutant embryos; uneven expression in the eye disc, the development of which is abnormal in both mild hypomorphs and hypermorphs; and expression in the follicular epithelial cells of the ovary, which are responsible for producing the eggshell of developing oocytes and do so aberrantly in the mildest hypomorphs. However, DER transcripts were also detected in a subset of tissues that have not been reported to be abnormal in mutant organisms. Our findings agree with and extend recently reported results for the DER protein, indicating that DER is regulated primarily at the level of transcription, in contrast to previous suggestions. We conclude that the phenotypes displayed by recessive mutants can be attributed to loss of DER function in the affected tissues. © 1991 Academic Press, Inc.

INTRODUCTION

Multicellular organisms possess numerous protein-tyrosine kinases (PTKs) that apparently play a variety of roles in the determination of cellular phenotype through participation in signal transduction pathways (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. The physiological purposes of most PTKs are not well understood. One approach to this problem is to identify genes of *Drosophila melanogaster* that encode PTKs and seek mutations in them.

A number of genes encoding PTKs have been identified in the genome of *D. melanogaster*. One of these has been designated the *Drosophila* EGF receptor homolog or DER (Livneh *et al.*, 1985; Wadsworth *et al.*, 1985; A.L.K. and J.M.B. unpublished results) because its gene product shares sequence homology and general structural features with the EGF receptor of vertebrates, encoded by the gene *erb*B-1 (Ullrich *et al.*, 1984; Yarden and Ullrich, 1988). However, the functional relationship between the vertebrate and *Drosophila* genes is ambiguous since DER is equally related to another vertebrate gene, *neu* (Bargmann *et al.*, 1986). Moreover, the ligand (or ligands) for the *Drosophila* receptor has not been identified, and two of the known agonists for the EGF receptor, EGF and TGF-α, do not enhance the kinase activity of the DER protein (Hoffman, 1989). Finally, unlike *erb*B-1, DER transcripts are alternatively spliced to produce at least two messenger RNAs that could encode proteins differing at their amino termini (Schejter *et al.*, 1986; Schejter and Shilo, 1989).

Using diverse approaches, several different classes of DER mutants have been isolated and characterized. Schejter and Shilo (1989) screened mutagenized flies for recessive lethals that reside in the chromosomal vicinity of DER and identified a complementation group of embryonic lethals as DER mutants. Other alleles include *faint little ball* (*flb*), originally isolated as an embryonic lethal that displayed an abnormal pattern of larval cuticle (Nusslein-Volhard *et al.*, 1984; Price *et al.*, 1989); *torpedo* (*top*), generated in a screen for recessive female sterile mutations (Schupbach, 1987; Price *et al.*, 1989); and the first alleles isolated, *Ellipse* (*Elp*), dominant mutations affecting eye morphology (Lindsley and Grell, 1968; Baker and Rubin, 1989). Only a subset of tissues are defective in these mutants, raising the issue of whether this specificity is generated by the distribution of the unidentified ligand(s) or the receptor itself. In an effort to begin to address this question, we have examined the distribution of the DER message.

Two research groups have previously examined the RNA expression pattern of DER in a limited number of developmental stages (Schejter *et al.*, 1986; Kammer-

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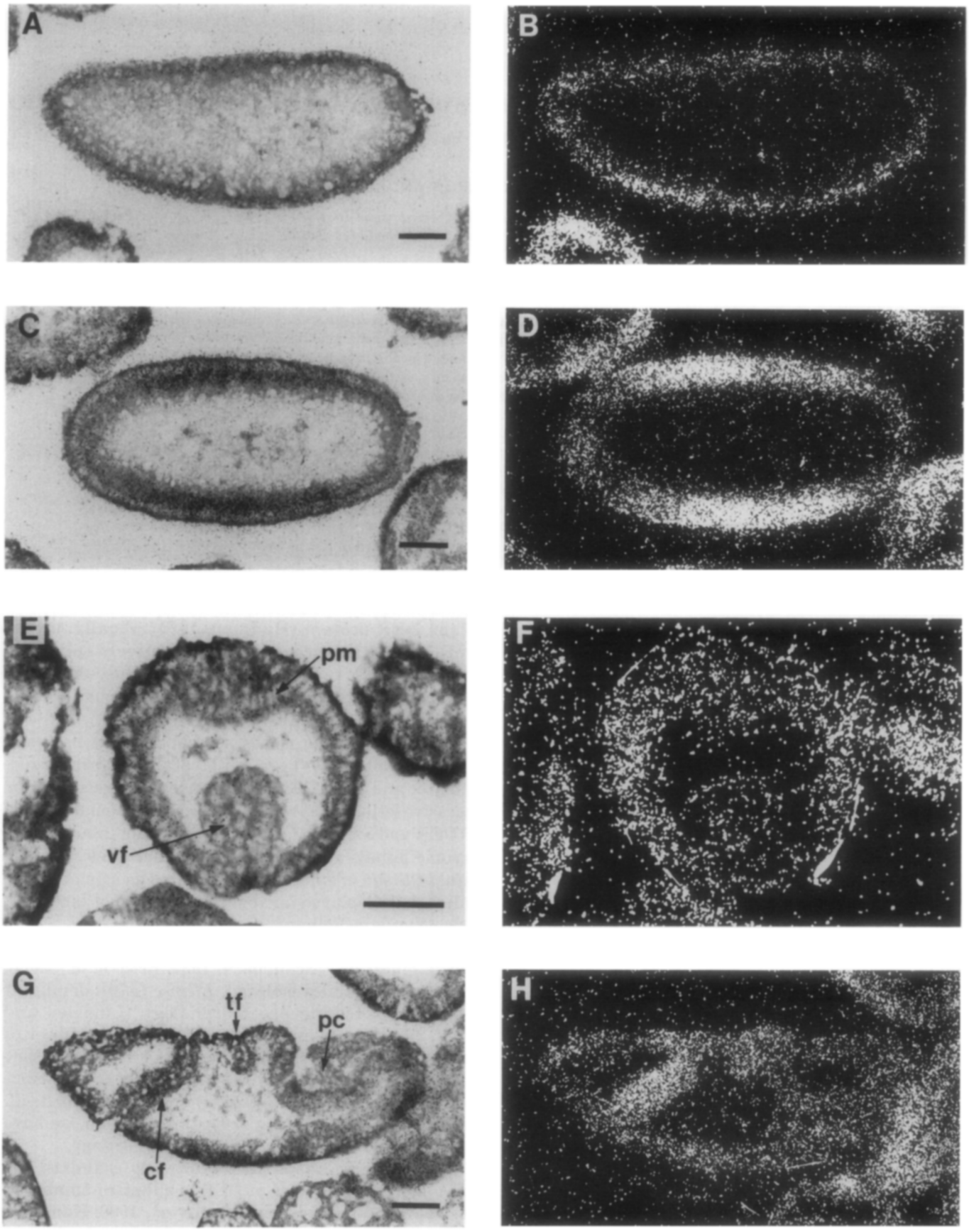
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meyer and Wadsworth, 1987). Their results revealed only limited correlation between the pattern of gene expression and the biological effects of genetic lesions. We now report a more extensive survey of the distribution of DER mRNA in embryos, third instar larvae, all stages of pupal development, and adults. Our results reveal a much closer relationship between expression patterns and mutant phenotypes than was previously thought to exist. The signaling pathway mediated by DER apparently serves diverse but specific purposes during the course of *Drosophila* development. This specificity is achieved, at least in part, by regulating the steady state levels of mRNA representing DER.

MATERIALS AND METHODS

Molecular Cloning

Procedures and sources of most of the reagents have been described previously (Katzen *et al.*, 1985). Lambda phage clones containing the DER gene were isolated from a recombinant DNA library of the *Drosophila* genome cloned into Charon 4 (Maniatis *et al.*, 1978), using a v-*fps* probe at low stringency. The probe was prepared from an isolated 1.3 kbp *Pvu*II/*Sma*I fragment representing the bulk of v-*fps* (Hammond *et al.*, 1985). Low stringency hybridizations were carried out as described elsewhere (Katzen *et al.*, 1991). We confirmed the identity of our clone by restriction mapping and nucleotide sequencing (Sanger *et al.*, 1977).

In Situ Hybridization

In situ hybridization to 8 μm sections of embryos, larvae, pupae, and adults was performed as described elsewhere (Katzen *et al.*, 1990, 1991). The probes were prepared by nick translation of either pUC8 DNA or a pUC construct containing the 3.5 kb *Hin*dIII/*Eco*RI fragment of the DER gene described in the text 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. The analysis employed serial sections of samples from various developmental stages. Photomicroscopy was performed with the Zeiss Axiophot.

RESULTS

Tissue Localization of DER RNA

We examined the spatial distribution of DER transcripts by *in situ* hybridization to RNA in frozen sections of developing embryos, larvae, pupae, and adults. The probe was prepared form a plasmid containing a 3.5 kb *Hin*dIII/*Eco*RI fragment of the DER locus derived from a genomic phage clone. This restriction fragment is identical or equivalent to those used in earlier studies (Schejter *et al.*, 1986; Kammermeyer and Wadsworth, 1987), contains most of the coding region of the gene, and recognizes all known DER transcripts. To control for any nonspecific binding of the probe, parallel experiments were done in which sections were either pretreated with RNAse A or hybridized with a probe prepared from pUC8. In addition, the results were compared with those obtained using three other *Drosophila* proto-oncogene probes in parallel experiments (c-*myb*, manuscript in preparation; *dsrc29A*, Katzen *et al.*, 1990; and *dfps85D*, Katzen *et al.*, 1991). Each probe detected a different pattern of expression during *Drosophila* development. In the Results and Discussion sections, we will treat the detection of RNA as a manifestation of gene expression, but we acknowledge that data obtained using hybridization *in situ* reflect only steady state levels of messenger RNA.

Embryos

The embryonic stages referred to are as described by Campos-Ortega and Hartenstein (1985). No DER message was detected in very early embryos (stage 1 to early stage 4), suggesting that there is no maternal contribution (data not shown). DER transcripts were first observed before cellularization in the syncytial blastoderm (late stage 4) and presumably represent the begin-

FIG. 1. Expression of DER in early embryos: fertilization to gastrulation. Bright-field (left) and corresponding dark-field (right) micrographs were taken after autoradiography. Horizontal bars correspond to 0.05 mm. (A and B) A lateral sagittal section (anterior left, dorsal up) of a syncytial blastoderm embryo (stage 4), showing the presence of DER transcripts prior to cellularization (no transcripts were detected in earlier embryos). (C and D) A slightly oblique horizontal section of a blastoderm embryo during cellularization (stage 5). Note that the levels of DER message were considerably lower at the anterior and posterior poles than in the midsection. (E and F) A cross section (dorsal up) of a gastrulating embryo through a region near the posterior end (ca 20% egg length) showing lower levels of expression in endoderm and mesoderm (represented by the invaginating primordia of the posterior midgut (pm) and the invaginated ventral furrow (vf), respectively) than in ectoderm. (G and H) A lateral sagittal section (anterior left, dorsal up) of a gastrulating embryo, showing especially strong expression in the cephalic furrow (cf), lower levels in the transversal furrows (tf) (which include the primordium of the amnioserosa), and no detectable expression in pole cells (pc).

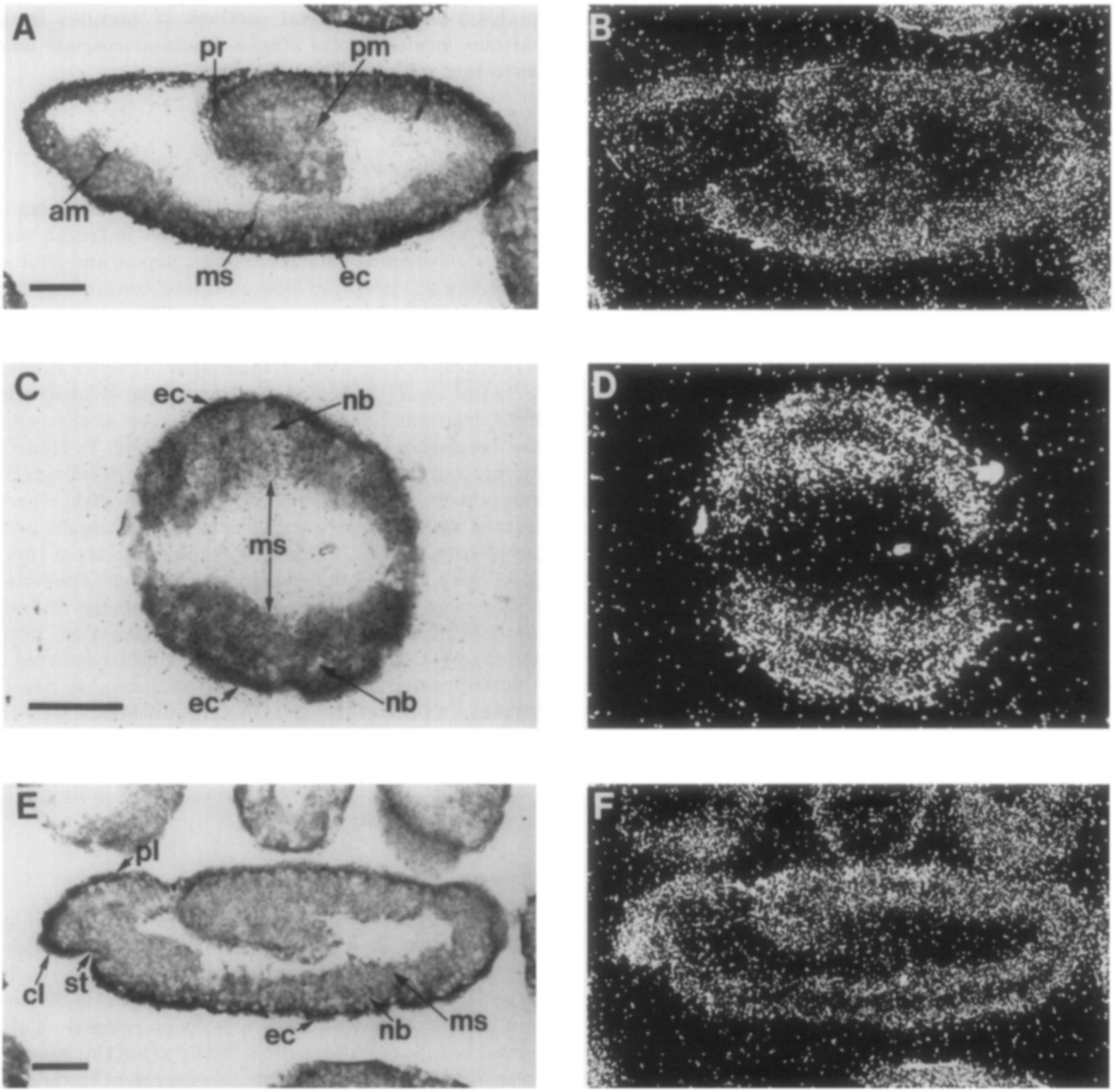


FIG. 2. Expression of DER in the extended germ band. Each pair of panels represents corresponding bright-field (left) and dark-field (right) micrographs. Horizontal bars correspond to 0.05 mm. (A and B) A sagittal section (anterior left, dorsal up) of an embryo during germ band elongation (late stage 8) showing higher levels of expression in ectodermal tissue (ec), including the primordium of the hindgut (or proctodeum, pr), than in mesoderm (ms) and endoderm. The latter comprises the anterior midgut (am) and posterior midgut (pm) primordia. (C and D) A cross section of a germ band extended embryo at a level of ca 35% egg length, showing especially low levels of DER message in segregating neuroblasts (nb). (E and F) A lateral sagittal section (anterior left, dorsal up) of an embryo at the end of the germ band extended stage (stage 11), showing roughly equivalent levels of DER expression in the ectoderm and mesoderm of the segmented germ band, the procephalic lobe (pl), and the stomadeum (st); relatively high levels in the clypeolabrum (cl); and especially low levels in segregating neuroblasts.

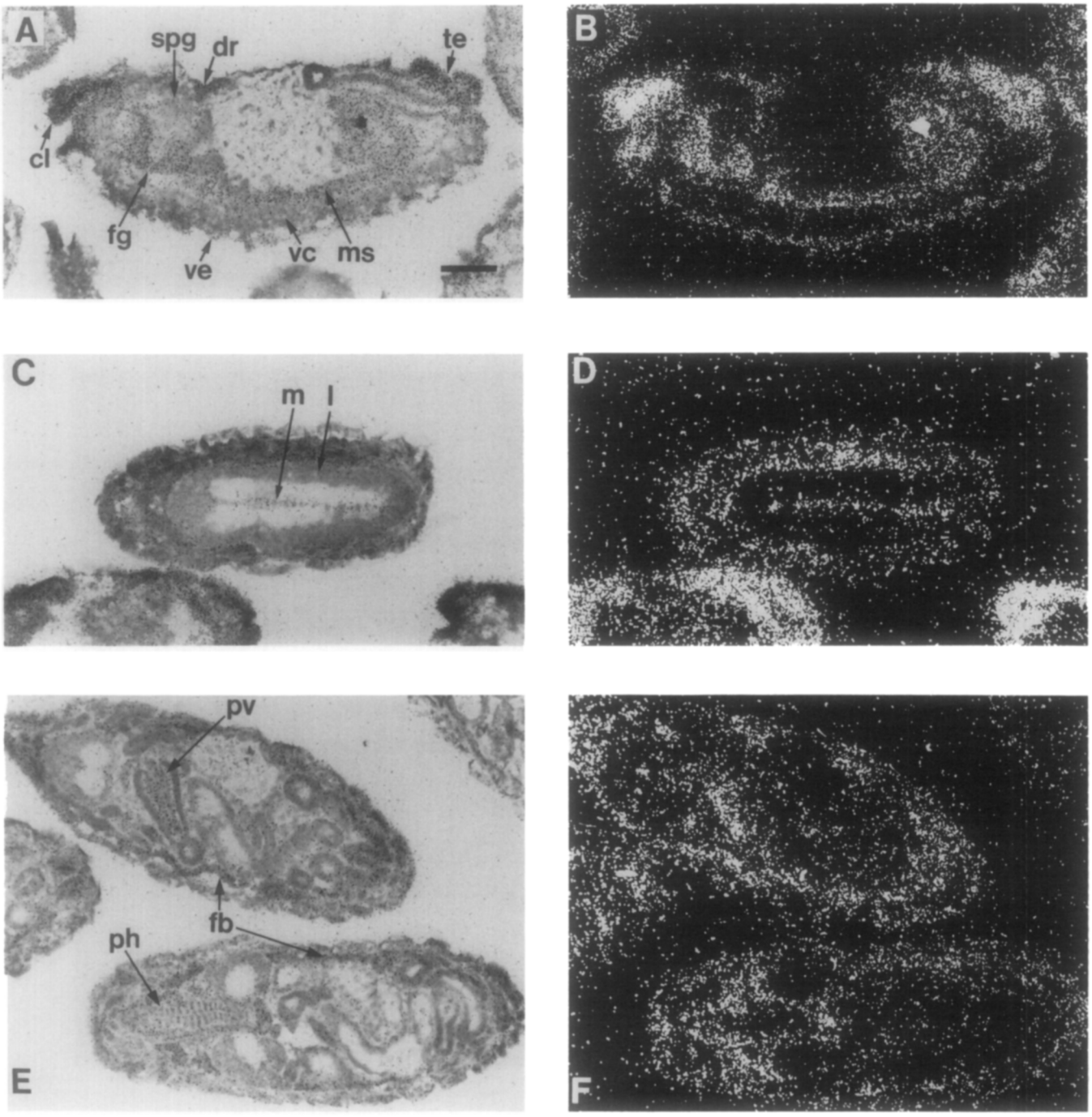
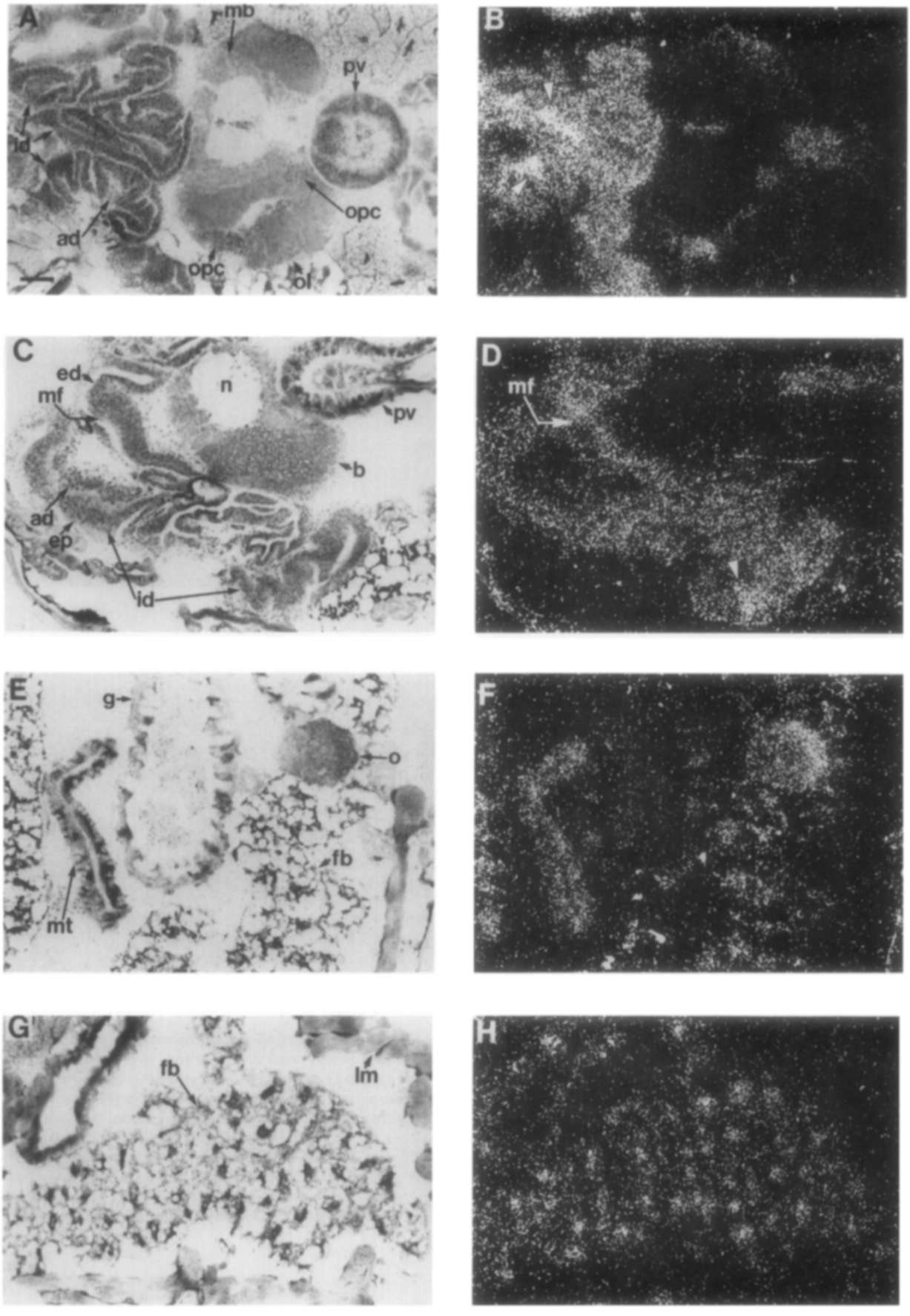


FIG. 3. Expression of DER in older embryos: germ band shortening to the end of embryogenesis. Corresponding bright-field (left) and dark-field (right) micrographs are oriented with anterior to the left. The horizontal bar in panel A corresponds to 0.05 mm. All embryos are shown at the same magnification. (A and B) A parasagittal section (dorsal up) of a stage 14 embryo, showing especially high levels of expression in the clypeolabrum (cl); slightly lower levels in the foregut (fg) and telson primordium (te); more moderate levels in the mesoderm (ms) and adjacent midgut lining, the dorsal ridge (dr) (derived from the labial gnathal segment and the progenitor of the dorsal fold), and the ventral epidermis (ve). DER transcripts were not detectable in the supraoesophageal ganglia (spg) or the ventral nerve cord (vc). (C and D) A horizontal section of a stage 14 or 15 embryo through 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). (E and F) Two horizontal sections of stage 17 embryos. The level of sectioning is slightly ventral in the upper embryo and more dorsal in the lower embryo. Prominent regions of expression included the internal part of the proventriculus (pv) (a part of the foregut), the epithelium of the pharynx (ph) (but not the pharangeal muscles), and differentiating fat body (fb).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ning of zygotic gene activity (Figs. 1A and 1B). We were unable to determine whether the DER gene is activated before or shortly after the last nuclear cleavage has taken place. During cellularization (stage 5), the level of expression increased substantially (Figs. 1C and 1D).

Examination of gastrulating embryos (stages 6 and 7) revealed unequal levels of DER expression. In comparison with ectodermal tissue, the signal was lower in both endoderm and mesoderm (Figs. 1E and 1F). DER expression was strongest in the cephalic furrow (an ectodermal tissue that roughly separates the head from the body and ultimately contributes to the mouthparts), lower in the transversal furrows, and undetectable in pole cells (Figs. 1G and 1H).

During germ band elongation (stages 8 and 9), DER transcripts continued to be detected in most ectodermal tissues and at lower levels in mesoderm, but not in the primordia of the anterior and posterior midguts (endodermal origin) (Figs. 2A and 2B). However, once neuroblasts began segregating from the ectodermal layer (late stage 9), some changes in the DER expression pattern occurred. The most striking feature was that DER transcripts were not detected in the new layer formed by the segregating neuroblasts (Figs. 2C–2F). This lack of expression in combination with increased expression in mesodermal tissue created the appearance of two stripes of expression along the full length of the germ band (Figs. 2E and 2F), a pattern that was retained through the beginning of germ band shortening.

During the latter part of germ band extension, the stomadeum (a structure that will form various parts of the foregut) begins to invaginate and the clypeolabrum becomes distinct. These tissues expressed DER at levels roughly equivalent to those found elsewhere in the embryo (Figs. 2E and 2F), but the levels increased during the next developmental stage and were very prominent in germ band-shortened embryos (stages 13 and 14) (Figs. 3A and 3B). Signal over the gnathal segments was also somewhat higher than elsewhere in the embryo (data not shown). Together with the high expression described earlier in the cephalic furrow, these results indicate that the DER gene is especially active in all primordial tissues of the mouthparts and foregut. Lower levels of expression were observed in ventral epidermis, mesoderm, in some dorsal tissues, and for the first time, in the lining of the midgut (Figs. 3A and 3B). In stage 14 embryos there was no longer detectable expression in the amnioserosa or most of the hindgut, but expression was observed at the base of the hindgut and dorsal to it, in a region where the posterior spiracles and the telson will form (Figs. 3A and 3B).

DER transcripts were not detected in the developing supra- and subesophogeal ganglia (tissue straddling the developing foregut) (Figs. 3A and 3B). The axonal scaffold of the ventral nervous system is established during this period (about 10:00–10:30 hr). Most of the cell bodies are located laterally to the neuropile; these did not express DER at detectable levels, (Figs. 3A and 3B). However, horizontal sections revealed hybridization to the midline of the developing ventral nervous system (Figs. 3C and 3D) where only a small population of cells reside, many of which play crucial roles in establishing the glial and axonal scaffolds (Jacobs and Goodman, 1989 a,b). Expression along the midline did not appear to be entirely contiguous, but the resolution was not refined enough to determine which subset of cells were actually expressing DER. The midline expression was observed throughout the remainder of embryogenesis (data not shown) and into later development (see below).

During the next periods of development, stages 15 and 16, the earlier expression pattern was generally maintained. However, novel DER expression was observed along the entire periphery of the midgut (data not shown). This transient activity was first observed at low

FIG. 4. Expression of DER in larvae. Each pair of panels represents corresponding bright-field (left) and dark-field (right) micrographs. All sections are from the climbing stage of third instar larvae and are shown at the same magnification. The horizontal bar in A corresponds to 0.05 mm. Abbreviations include: b, brain; lm, larval muscle; mb, midbrain; n, neuropile; ol, optic lobe. (A and B) A slightly oblique horizontal section (anterior left) showing several imaginal discs (id) and the developing brain. Expression was uneven in the disc epithelia (arrowheads in panel B indicate regions of especially high expression) and undetectable in the adepithelium (ad). In the brain, expression was limited to the outer proliferation center (opc) and the developing optic lamina (the latter, though not indicated explicitly, originates from the former). Expression was also observed in the valvular (internal) epithelium of the proventriculus (pv) and in imaginal cells of the foregut (located at the anterior end of the proventriculus). (C and D) A section (anterior left) showing expression in the epithelium (ep) of several imaginal discs (id), including the eye portion of the eye-antennal disc (ed). Above (anterior to) the morphogenetic furrow (mf) in the eye disc, transcripts were uniformly distributed throughout the disc; below the furrow, transcripts were mainly concentrated in the basal level. (This difference, though sometimes subtle, was consistently observed in many different eye discs examined.) The arrowhead in panel D indicates an abrupt change in the level of DER expression in the epithelium of another disc. (E and F) A horizontal section (anterior up) showing uneven expression in a developing ovary (o). Transcripts were more concentrated laterally than medially, an asymmetry that probably reflects the progressive differentiation of follicular epithelial cells to form egg tubes (Bodenstein, 1950). Expression was also observed in malphigian tubules (mt), but not in the larval gut (g). (G and H) A horizontal section (anterior left) showing expression in larval fat body (fb).

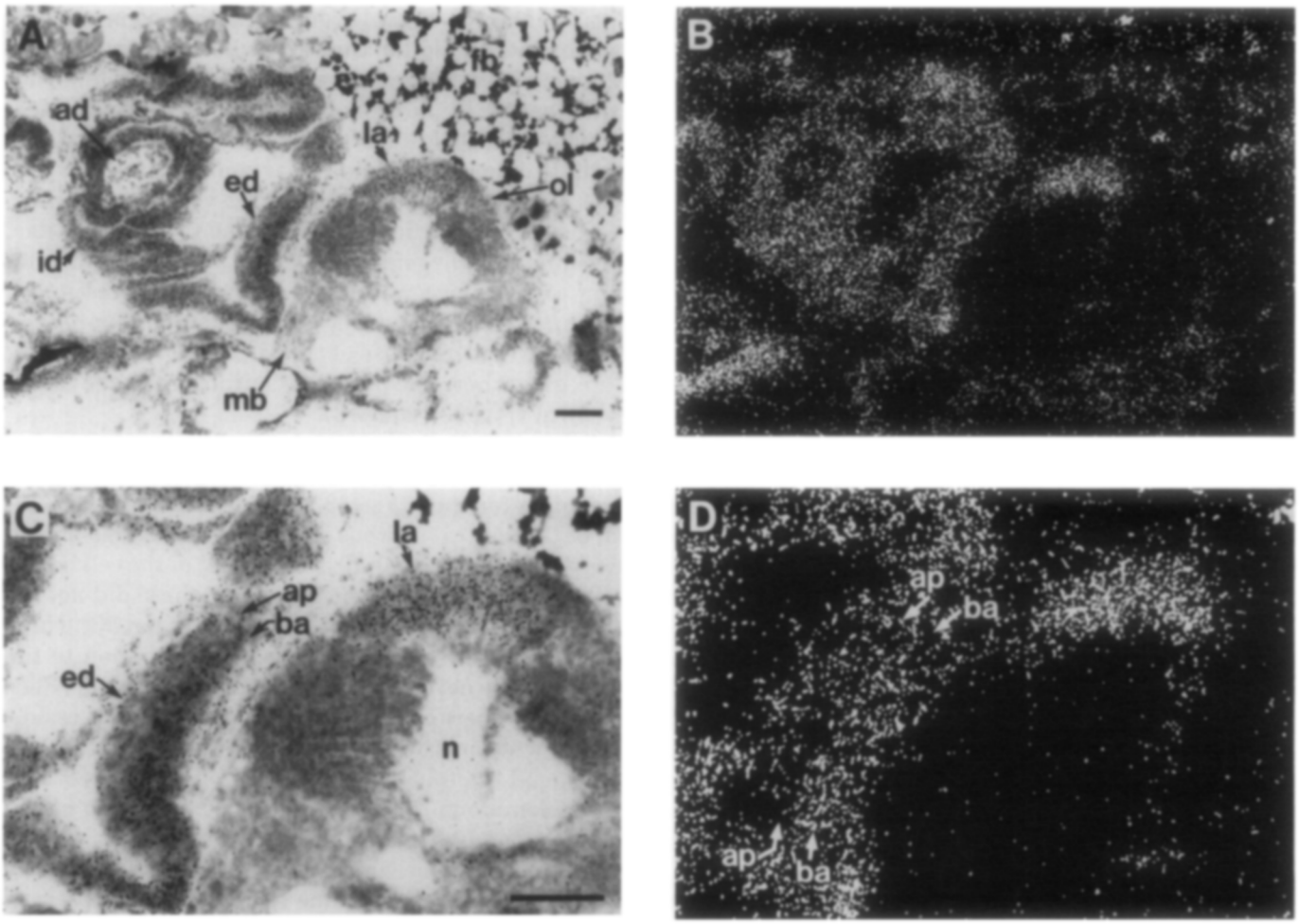


FIG. 5. Expression of DER in prepupae. Corresponding bright-field (left) and dark-field (right) micrographs are oriented with anterior to the left. Horizontal bars correspond to 0.05 mm. (A and B) A horizontal section (anterior left) of a prepupa showing expression in the optic lamina (la), but not in the rest of the developing optic lobe (ol) or the midbrain (mb). Expression was also observed in the eye disc (ed) and other imaginal discs (id), but not in the adepithelium (ad). Lower levels of expression were detected in the larval/pupal fat body (fb). (C and D) A higher magnification view of the eye disc and optic lobe (including the lamina) shown in A and B. The morphogenetic furrow has completely traversed the eye disc. DER transcripts were more concentrated in the basal surface (ba) than the apical surface (ap) of the eye disc. This pattern is somewhat obscured in D by the presence of transcripts in a thin layer of tissue immediately beneath the eye disc and is therefore more readily observed in C, where the grains can be visualized directly. (n, neuropile).

levels in the stage 14 embryo (Figs. 3A and 3B) and was no longer evident in mature embryos (Figs. 3E and 3F). In the final stage of embryogenesis (stage 17), the most prominent regions of expression included the internal part of the proventriculus (a part of the foregut), the epithelium of the pharynx (but not the pharangeal muscles), and differentiating fat body (Figs. 3E and 3F).

Larvae

In the climbing stage of third instar larvae, the DER gene was expressed in all of the imaginal discs (tissues of ectodermal origin), but the signal was not evenly distributed among the discs or even within a single disc (Figs. 4A–4D). Examination of the eye portion of the eye-antennal disc revealed that anterior to the morphogenetic furrow, the message was abundant and evenly distributed, but posterior to the furrow, the signal was localized over the basal level of the disc (Figs. 4C and 4D). Only very low levels of expression were detected over the apical level, where the developing photoreceptor cells are located. In other imaginal discs, DER transcripts appeared to be restricted to epithelial as opposed to the adepithelial tissue (Figs. 4A–4D). The latter contributes to imaginal muscle tissue (Poodry, 1980). DER message was also detected in imaginal nest cells of the

digestive system (Figs. 4A and 4B). DER was expressed, albeit unevenly, in developing ovaries (Figs. 4E and 4F); whereas expression was undetectable in developing testes, even in long exposures (data not shown). The lack of testicular expression remained constant throughout pupal development and into adulthood.

DER transcripts were detected in restricted regions of the central nervous system. We observed very specific hybridization to a subset of cells in the cellular cortex of the developing optic lobe (Figs. 5A and 5B). Kammermeyer and Wadsworth (1987) previously identified such regions and proposed that they correspond to the outer and inner proliferation centers (White and Kankel, 1978; Kankel *et al.*, 1980). Our results agreed, but also revealed expression in cells of the developing optic lamina, which originates from the outer proliferation center. This identification is supported by continued DER expression in the lamina of prepupae when the structure is more readily identified (see below). No signal was detected over the rest of the brain (Figs. 4A–4D) or over most of the cellular cortex of the ventral ganglia (except the midline) (data not shown, but see below). These data contradict the results of Schejter *et al.* (1986), who reported low level expression over the entire nervous system in third instar larvae and in adults.

Contrary to previous reports (Schejter *et al.*, 1986; Kammermeyer and Wadsworth, 1987), we found that DER expression was not limited to imaginal cells of the larva. Substantial levels of expression were observed in a subset of polytene larval tissues including the valvular epithelium of the proventriculus (Figs. 4A–4D) and larval fat body (or adipose tissue) (Figs. 4G and 4H). Expression in both of these tissues was observed earlier in development (late embryos) and was maintained in adults (see below). Relatively low levels of expression were also observed in the salivary glands (data not shown) and a subset of cells in the malphigian tubules (Figs. 4E and 4F), but no expression was detected in larval muscle cells (Figs. 4G and 4H), most of the gut (Figs. 4E and 4F), or the lymph glands (data not shown).

Pupae

In prepupae, the expression patterns established in late third instar larvae were, for the most part, maintained: in imaginal discs, DER expression continued to be observed in the epithelium and undetectable in the adepithelium (Figs. 5A and 5B); in the eye disc, transcripts were more concentrated basally than apically (Figs. 5A–5D); and in the central nervous system, expression was still observed in the developing optic lamina (Figs. 5A–5D) and the midline of the ventral ganglia (data not shown). Expression also continued in larval/pupal fat body (Figs. 5A and 5B) and was activated in the developing midgut where imaginal replacement epithelial cells proliferate (see below).

In early pupae, the disc epithelia continued to express DER at approximately the same levels displayed in larvae and prepupae (Figs. 6A and 6B), though the tissues were no longer mitotic (Postlethwait, 1978; Poodry, 1980). Expression was particularly prominent over the epithelia that form the border between the head and thorax; whereas it was especially low in the everted eye disc (Figs. 6A and 6B). The levels of expression in disc epithelia began to decline midway through pupal development (ca 48 hr after puparium formation) and were no longer detectable after another 24 hr. DER transcripts were not detected in developing muscle tissue, which is derived from the adepithelium (data not shown).

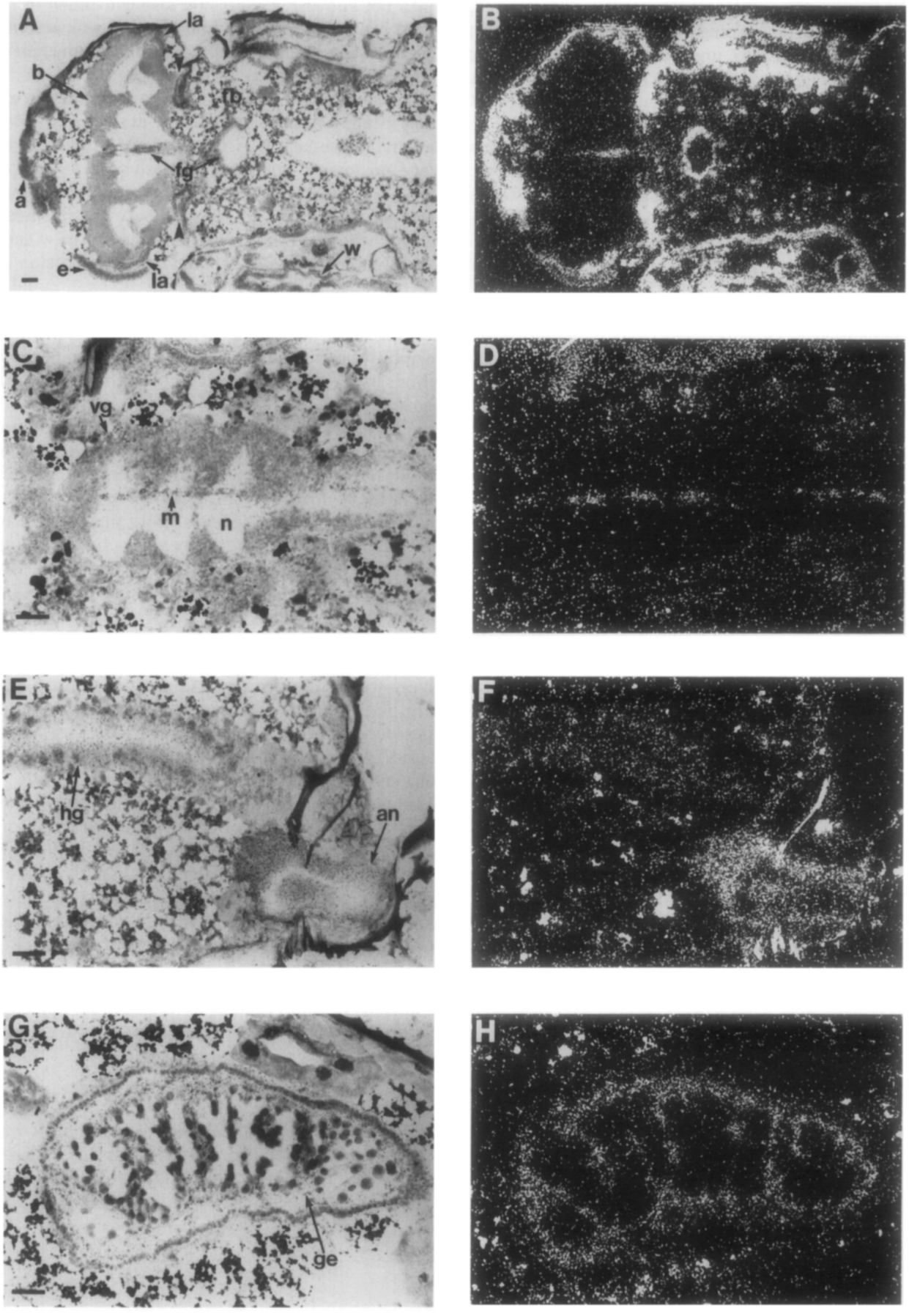
DER expression continued in the optic lamina as it moved out toward the eye (Figs. 6A and 6B), but was no longer detectable when the lamina reached its final position (data not shown). Expression was maintained in cells residing at the midline of the ganglia (Figs. 6C and 6D) during the time that they were observed (approximately the first 2 days of pupal development). No DER transcripts were detected in any neural tissue in later pupae or in adults.

Expression of DER was observed around each ovarian egg chamber in early pupae, but the signal was relatively weak and remained so until the pupae matured into pharate adults (data not shown). Developing tissues derived from the genital discs of both sexes produced DER transcripts during the first 2 days of pupal development (Figs. 6E and 6F).

In very early pupae, epithelial cells of the developing midgut expressed DER, but the myoblasts that form the visceral muscles did not (Figs. 6G and 6H). Epithelial expression in the midgut declined rapidly, but expression was observed in regions of the fore- and hindguts later in the pupal period. During early pupal development, expression continued in larval fat body (Figs. 6A and 6B), but then declined, and was undetectable in pharate adults (data not shown). However, DER was expressed in small regions of imaginal fat body discernible in pharate adults.

Adults

Expression of the DER gene in adult flies was primarily restricted to three types of tissues: imaginal fat body (Figs. 7A, 7B, 7E, and 7F), valvular (internal) epithelium of the proventriculus (Figs. 7C and 7D), and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follicular epithelium of the ovary (Figs. 7E and 7F). DER transcripts detected in the terminally differentiated fat body and valvular epithelium were not due to residual message from pupal development, since they were found in mature adults (3-5 days after eclosion) as well as in newly emerged flies.

Unlike the other two adult tissues that expressed DER, follicular epithelial cells continually undergo division, differentiation, and cell death. In agreement with the previous study reported by Kammermeyer and Wadsworth (1987), we found that DER was expressed in these cells from early in oogenesis through at least stage 10B (Figs. 7E and 7F), a period during which they divide, become polyploid, migrate over the oocyte, and secrete the vitelline membrane (King, 1970; Mahowald and Kambysellis, 1980).

In contrast to Schejter *et al.* (1986), we were unable to detect DER transcripts in any region of the brain (Figs. 7A and 7B) or thoracic ganglia (data not shown). Our only explanation for this discrepancy is that adipose tissue lies very close to both the brain and the thoracic ganglia, and this tissue expressed DER in abundance (see above).

DISCUSSION

Correlation of the DER Expression Pattern with Mutant Phenotypes

Previous studies of the distribution of DER transcripts focused on a limited number of developmental stages and concluded that once zygotic transcription is initiated, DER expression is general throughout embryogenesis (Schejter *et al.*, 1986; Kammermeyer and Wadsworth, 1987). These results indicated that the expression pattern provides no basis for understanding the mutant phenotypes which affect various tissues quite differently. These results have been rationalized by a variety of explanations such as translational control, protein stability, and differential tissue sensitivity. Our results show that these rationalizations are not necessary, that the expression of DER is differentially regulated in a manner that can explain the susceptibility of certain tissues to the effects of mutant alleles. During preparation of this manuscript, Zak *et al.* (1990) reported that the DER protein is not uniformly distributed among tissues, at least in embryos (the only stage of development examined for protein). The close correlation between the distribution of DER mRNA and protein indicates that at least during embryogenesis, expression of the gene is regulated primarily at the level of transcription (or mRNA stability). The same may be true in later stages of development, although data on protein localization in these stages are not yet available.

Embryonic Derivatives of the Ectoderm (Nonneural)

Phenotypes associated with recessive embryonic lethal mutations in DER (DER/*flb/top* mutants) have been studied in detail (Clifford and Schupbach, 1989; Price *et al.*, 1989; and Schejter and Shilo, 1989). Embryos homozygous for severe alleles appear normal through germ band extension. Abnormalities become evident at the onset of stomadeal invagination and worsen through the remainder of embryogenesis. Defects are limited to a subset of tissues of ectodermal origin and are due, at least in part, to cell death, indicating that DER function is necessary for the maintenance of these tissues.

During early embryogenesis DER is not expressed in endodermal tissues, a finding that correlates with the observation that even severe alleles exhibit no endodermal defects (Price *et al.*, 1989). In contrast, DER is expressed at varying levels in much of the ectoderm and its derivatives, and the levels show a direct correlation with the severity of defects in mutant embryos. For example, the most sensitive tissues, deformed even in weak alleles, are those that form the head regions, especially the mouthparts (Price *et al.*, 1989). Correspondingly, DER is expressed at very high levels in the cephalic furrow as it forms during early gastrulation, in the gnathal segments, and in the clypeolabrum. All of these regions contribute to formation of the mouthparts and make some contribution to the foregut. DER is expressed at somewhat lower levels in the ectoderm of the

FIG. 6. Expression of DER in pupae. Corresponding bright-field (left) and dark-field (right) micrographs are oriented with anterior to the left. Horizontal bars correspond to 0.05 mm. (A and B) A horizontal section showing the developing head and thorax of a young pupa. DER expression in the epithelia of developing disc derivatives is shown (a, antenna; e, eye; and w, wing). Arrowheads in (A) indicate the epithelium that forms the border between the head and thorax, a region where expression was especially prominent. DER transcripts were also detected in the optic lamina (la), but not elsewhere in the brain (b); in the foregut (fg); and in fat body (fb). (C and D) A horizontal section showing the ventral ganglia (vg) in the thorax of a young pupa. Cells residing at the midline (m) of the ganglia continued to express DER. (n, neuropile). (E and F) A parasagittal section through the posterior of a young pupa showing expression in the developing anus (an) but not in the hindgut (hg), both of which are derivatives of the genital disc. (G and H) An oblique section of a very early pupa showing expression in the developing imaginal gut epithelium (ge).

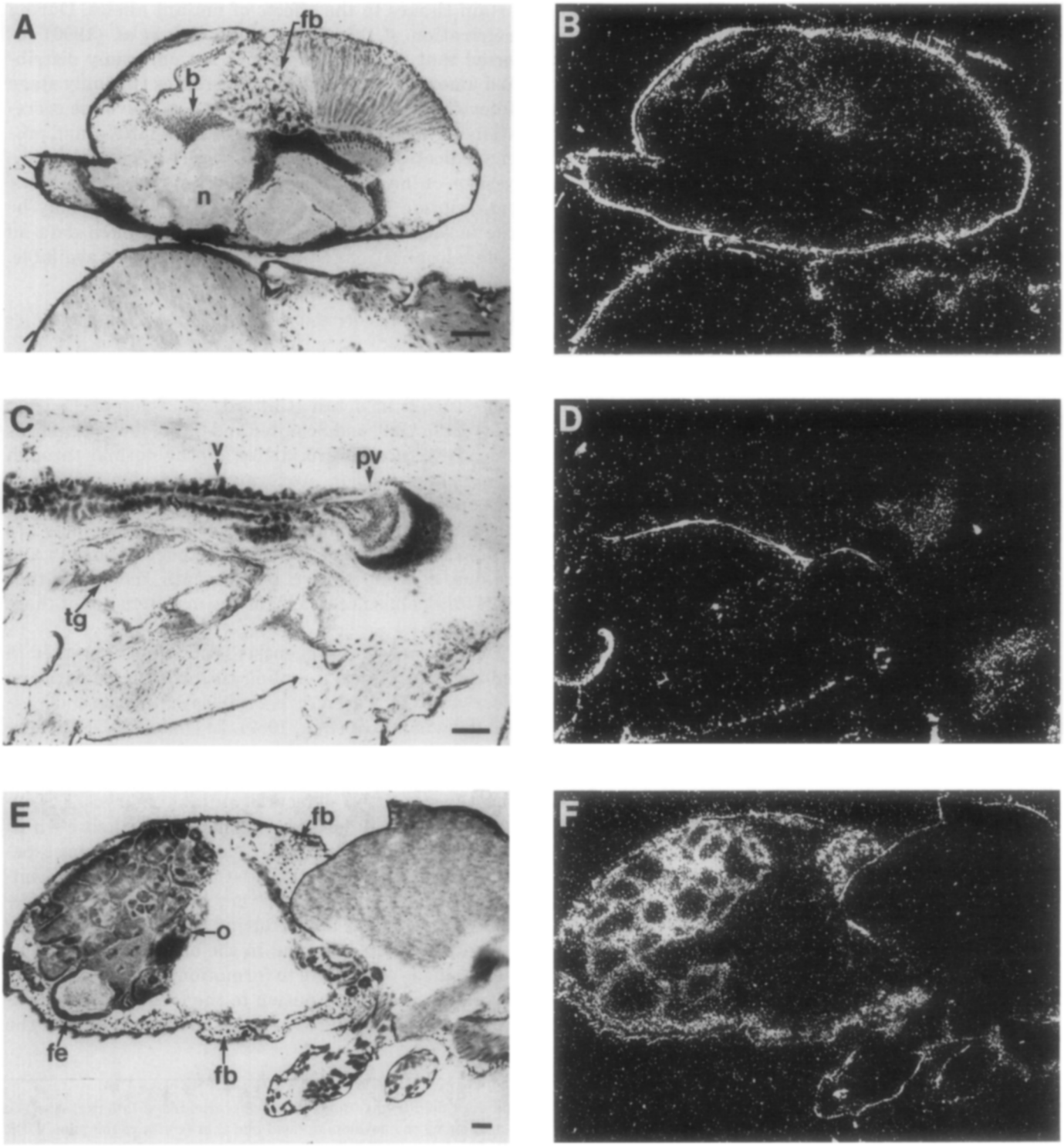

FIG. 7. Expression of DER in adults. Each pair of panels represents corresponding bright-field (left) and dark-field (right) micrographs. Horizontal bars correspond to 0.05 mm. Abbreviations include: b, brain; n, neuropile; tg, thoracic ganglia; v, ventriculus. (A and B) A parasagittal section (anterior up, dorsal left) through the head of a mature adult, showing expression in fat body (fb). (C and D) A parasagittal section (anterior right, dorsal up) through the thorax of a mature adult, showing expression in the valvular epithelium of the proventriculus (pv). (E and F) A parasagittal section (anterior up) through the thorax and abdomen of a mature female adult, showing expression in the follicular epithelium (fe) of the ovary (o) and in fat body (fb).

segmented germ band, the primordia of the amnioserosa, and the primordia of the telson, all of which are affected in the more severe mutant alleles (Price *et al.*, 1989; Schejter and Shilo, 1989).

Embryonic Nervous System

The nervous system provides the most intriguing correlation between expression pattern and mutant phenotype. In wild type embryos, the ventral nerve cord is composed of an axonal scaffold, consisting of axon bundles arranged as two bilaterally symmetrical longitudinal connectives that are joined in each segment by an anterior and posterior commissure (Thomas *et al.*, 1984). In DER mutant embryos, the axonal scaffold is disorganized, often displaying fusion of longitudinal tracks and horizontal commissures, a phenotype that suggests a defect in the midline of the nervous system (Schejter and Shilo, 1989).

Our results show that while DER is not expressed in cell bodies located laterally to the neuropile, it is expressed in some of the cells residing at the midline. These include the very cells essential to the establishment of the axonal scaffold (Jacobs and Goodman, 1989a,b): midline precursors and their descendents, median neuroblasts, corner cells, and RP neurons. Also residing at the midline are glial cells that participate in the formation of the glial scaffold, a structure established prior to and apparently necessary for formation (and possibly maintenance) of the axonal scaffold. Our observations suggest that loss of DER function in one or more of these cell types results in disruption of this complex process. The recent report by Zak *et al.* (1990) localized the DER protein to the midline of embryos as well and indicated that it was probably restricted to the glial cells. Expression in these cells suggests that DER may be under the transcriptional regulation of *single-minded*, a gene required for expression of several other genes known to be important for development of the midline (Nambu *et al.*, 1990).

Imaginal Discs

To begin assessing the role of the DER gene product in postembryonic stages of development, Clifford and Schupbach (1989) studied several weaker DER/*top* mutant alleles that survive embryogenesis. Some imaginal discs dissected from mature third instar larvae (including the eye portion of the eye-antennal imaginal disc, the wing disc, and the haltere disc) are severely reduced in size; whereas others (including the antennal portion of the eye-antennal disc and all three pairs of leg discs) appear to be morphologically normal. However, the latter apparently suffer from more subtle patterning defects, since their derivatives display abnormalities in viable adults homozygous for even weaker mutant alleles.

Earlier reports (Schejter *et al.*, 1986; Kammermeyer and Wadsworth, 1987), indicated that all imaginal discs express DER evenly. In contrast, we found that although all discs express DER, the levels of expression vary within and among them. Both mutant phenotypes and expression patterns suggest that DER plays a role in the development and patterning of most imaginal discs. Defects in apparently asymptomatic discs might become evident if mosaic analysis were used to study the more severe embryonic lethal alleles in larvae. Baker and Rubin (1989) used this approach to study the eye disc and showed that lack of DER activity had a much more detrimental effect than moderate reductions or increases. Alternatively, since most epithelial tissues express DER well into pupal development, it may be that for some tissues the crucial periods of DER activity occur later than third instar larvae. A combination of further phenotypic analysis along with detailed studies of uneven expression patterns within specific imaginal discs should help provide insights into the molecular basis of mutant phenotypes.

The Developing Compound Eye

DER is not expressed at unusually high levels in the eye disc, but abnormalities are observed in the adult eye of both relatively weak hypomorphs (Clifford and Schupbach, 1989) and hypermorphs (Baker and Rubin, 1989). The latter, represented by the *Ellipse* (*Elp*) alleles, give rise to defective eyes with a decreased number of ommatidia and an increased number of pigment cells and mechanosensory bristles. The ommatidia contain the appropriate number and arrangement of cells, indicating that once photoreceptor determination has been initiated, ommatidial development progresses normally.

Ommatidial determination is initiated at the position of the morphogenetic furrow (Tomlinson and Ready, 1987). Differentiating photoreceptor and cone cells rise to the apical surface while undifferentiated cells (which will eventually become pigment and bristle forming cells) remain in the basal level. DER transcripts are uniformly distributed in the undifferentiated cells of the eye disc anterior to the morphogenetic furrow and are restricted to the basal level posterior to it. Considered in conjunction with the mutant phenotype observed in hypermorphic *Elp* alleles, these results suggest that DER plays a role in keeping these cells in an

undifferentiated state, and that reducing the levels of DER mRNA is an important step in initiating ommatidial determination.

The Follicular Epithelium of the Ovaries

DER expression in the follicular epithelium of the ovary has been reported previously (Kammermeyer and Wadsworth, 1987) and its implications with respect to the female sterility of some *torpedo* alleles have been discussed (Price *et al.*, 1989). Briefly, the top^1 (and top^{CJ}) mutation's ventralizing effect on eggshell patterning and resulting embryos has been shown to be a maternal effect due to reduction of wild type DER activity in the follicular epithelium (Schupbach, 1987; Price *et al.*, 1989). The ventralizing phenotype apparently results from shifts in follicle cell fate rather than cell death, suggesting a different function for DER in these cells than in embryonic tissues. In agreement with Kammermeyer and Wadsworth (1987), we found that DER is abundantly expressed in the follicular epithelium but not at detectable levels in germ line cells, confirming the correlation between the wild type expression pattern and the affected tissue in mutants. In addition, we found that DER is expressed in the developing ovary from at least third instar larvae onward, suggesting that the receptor may also play a role in ovarian development.

Expression without Mutant Phenotype

DER is expressed in a variety of tissues that display no obvious abnormalities in any of the mutants, including larval and imaginal fat body, epithelial cells of the developing gut, valvular epithelium of the proventriculus, developing optic lamina, and cells residing at the midline of the ventral ganglia (in postembryonic development). It is possible these tissues have not yet been examined in enough detail, or that in hypomorphic alleles that survive to the pertinent stages of development, sufficient DER activity is retained. Alternatively, these tissues may not require the DER gene product for proper function and/or maintenance. Detailed mosaic analysis using amorphic alleles (or generation and analysis of a *ts* allele) will be required to distinguish between the last two possibilities.

Conclusion

Our results demonstrate that the spatial and temporal distribution of DER transcripts is more specific than was previously thought, and reveal a substantial correlation with mutant phenotypes. This work also demonstrates that in some settings, at least, the DER gene is regulated at the level of transcription (or mRNA stability): in embryos, there is strong correlation between the distribution of DER mRNA (present results) and protein (Zak *et al.*, 1990). Since tissues deformed by recessive mutant alleles express DER in the wild type organism, defects can be directly attributed to loss of DER function in those tissues.

The presence of DER transcripts in postmitotic undifferentiated and differentiated cells reinforces the evidence from mutant phenotypes that the role of this gene is not limited to mitotic events. The diverse patterns of DER expression and the pleiotropic effects caused by lesions in the gene suggest that it may participate in multiple pathways throughout *Drosophila* development. Further molecular and genetic investigations, along with identification of the receptor's ligand(s), should help uncover these pathways and elucidate DER's function during development. Although these results with DER cannot be directly extrapolated to the gene's vertebrate counterparts (*erb*B-1 and *neu* encoding the EGF receptor and *neu* protein), they suggest the possibility that the vertebrate gene products may participate in multiple developmental processes which can only be revealed by analysis in the organism as a whole.

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