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Temporal and spatial distribution of transcripts from the *Deformed* gene of *Drosophila*

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The *Deformed* gene of *Drosophila* is necessary for the proper development of epidermal pattern elements arising from the maxillary and mandibular segments of the head. We find one major transcript (2.8 kb) homologous to *Deformed* (*Dfd*) probes which is expressed continuously from 3 h of embryogenesis into adulthood. Localized transcript accumulation is first detected just prior to the formation of the cellular blastoderm in a single circumferential band at about 65–75% egg length. The zone of *Dfd* expression is approximately two segment primordia in width. At later stages of embryogenesis, *Dfd* transcripts accumulate in the posterior ectoderm of the mandibular segment, and in the ventro-lateral ectoderm of the maxillary segment. Transcripts are also detected in the mesoderm and neuromeres of the mandibular and maxillary segments. The distribution of *Dfd* transcripts supports the hypothesis that *Dfd* functions as a homeotic selector gene in the determination of posterior head segments.

Key words: homeotic genes/*Deformed*/expression

Introduction

Segmentation is one of the fundamental patterns in the organization of the insect body plan. Early in embryogenesis, 3 thoracic, 10 abdominal and approximately 6 head segments develop within the germ band of the fruit fly, *Drosophila melanogaster*. The selective determination of many, and perhaps all, of these metameric units is under the control of homeotic genes. These genes have been identified through mutant alleles that transform part or all of a segment into another. Two major clusters of homeotic genes exist on the *Drosophila* third chromosome. The Antennapedia complex (ANT-C) contains homeotic genes controlling head and thoracic region identity (Kaufman *et al.*, 1980; Kaufman, 1983; Wakimoto *et al.*, 1980) while the Bithorax complex (BX-C) controls posterior thoracic and abdominal pattern elements (Lewis, 1978; Karch *et al.*, 1985; Sanchez-Herrero *et al.*, 1985). Many of the protein-coding loci in these two complexes are evolutionarily related through the possession of homeo box sequences that have varying degrees of homology (McGinnis *et al.*, 1984a,b; Scott and Weiner, 1984; Regulski *et al.*, 1985).

The homeotic gene *Deformed* (*Dfd*) is a member of the ANT-C, and of the Antennapedia class of the homeo box gene family (Bridges and Morgan, 1923; Lewis *et al.*, 1980; Hazelrigg and Kaufman, 1983; Regulski *et al.*, 1985). Mutants with a homozygous loss of *Dfd* function exhibit homeotic transformation of larval atrium into pharynx (Regulski *et al.*, 1987). In addition, these mutants are missing most of the cuticular structures and sensory organs derived from the maxillary and mandibular segments of the embryonic head. In this paper we show that *Dfd* transcripts are expressed in those cells which give rise to the affected structures in mutant embryos, consistent with the proposed determinative function of *Dfd* in the development of specific metameric units of the *Drosophila* head.

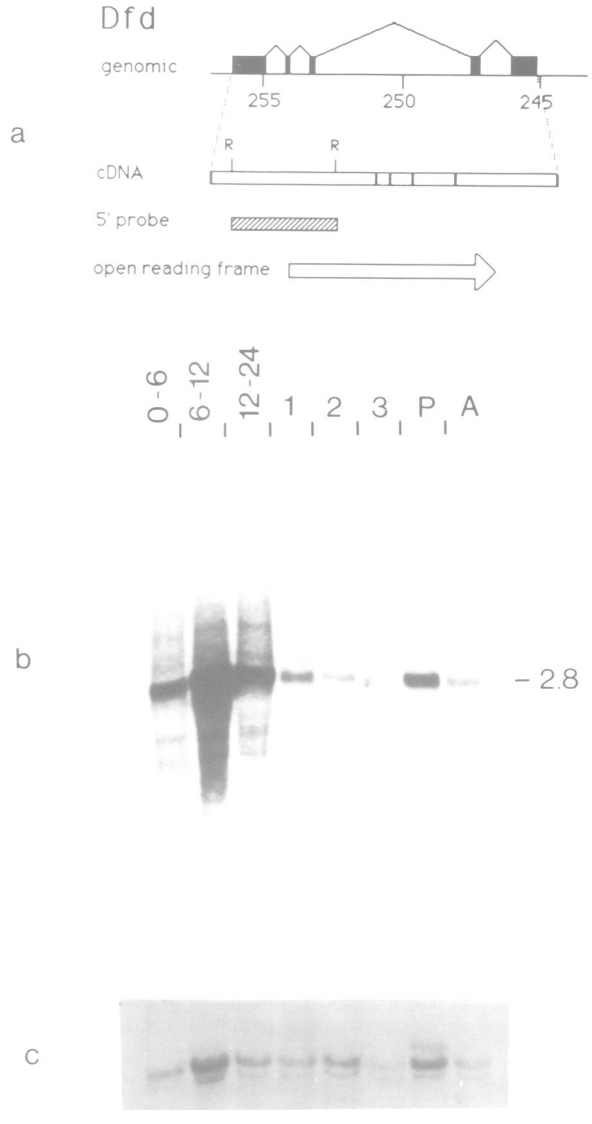

Fig. 1. Northern analysis of *Dfd* expression. **(A)** The *Dfd* gene has five exons (black boxes) which span 11 kb of genomic DNA and encode a 1758-bp open reading frame (Regulski *et al.*, 1987). The coordinates below the genomic map indicate the distance in kb from the *In(3R)Hu* chromosomal breakpoint (Garber *et al.*, 1983). **(B)** Poly(A)$^+$ RNA from 0–6 h, 6–12 h and 12–24 h embryos as well as first (1), second (2) and third (3) instar larvae, pupae (P) and adults (A), was electrophoretically resolved on a formaldehyde–agarose gel, blotted and hybridized with a ^{32}P-labeled probe from the 5′ region of the *Dfd* cDNA [indicated in **(A)** as the 5′ probe]. **(C)** The same Northern as shown in **(B)** was re-hybridized with an actin probe to give a rough measure of the amount of RNA in each lane (Fyrberg *et al.*, 1983). Gel scanning experiments show that while there is variable amounts of RNA from one lane to the next, *Dfd* is expressed at highest levels in 6–12 h embryos.

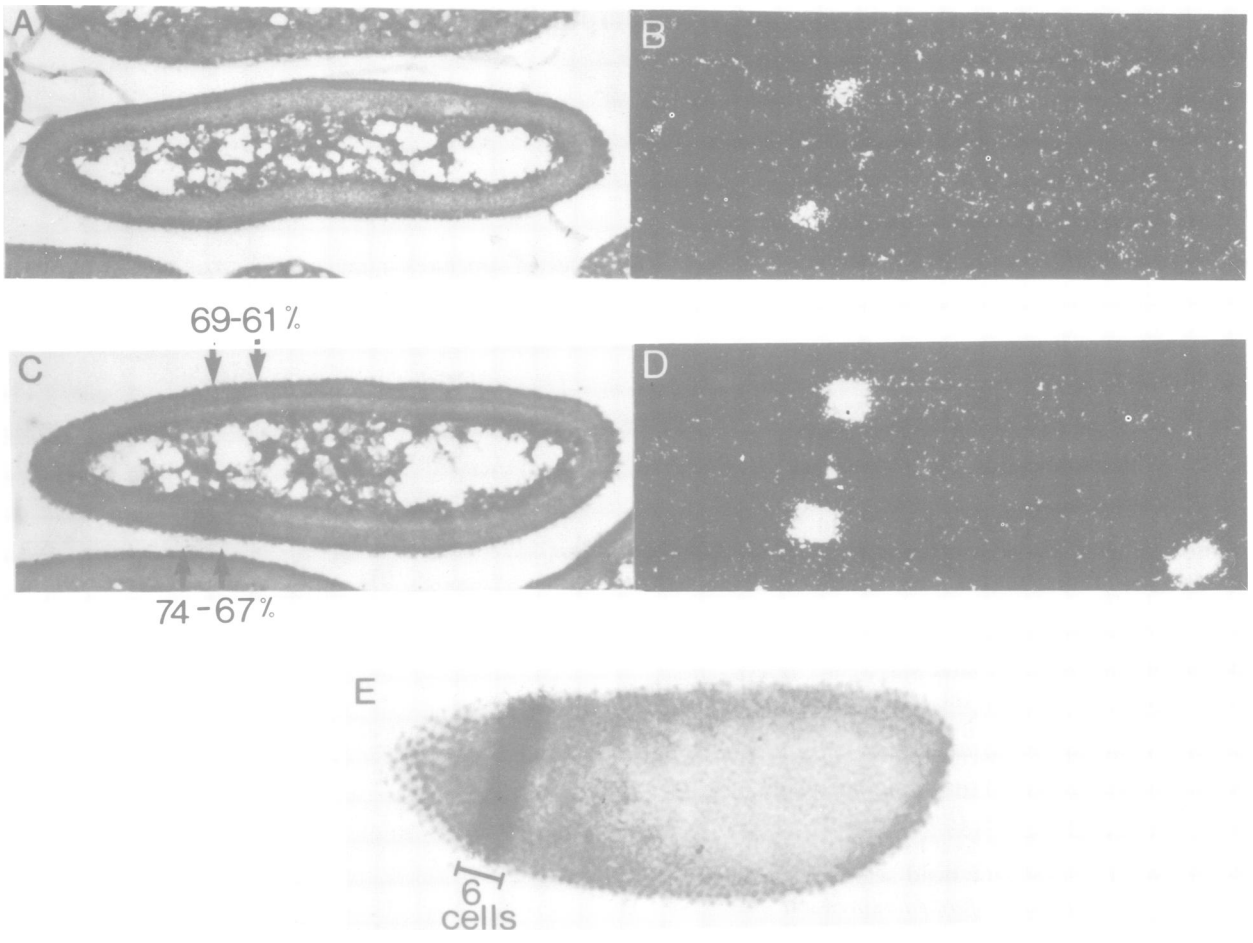

Fig. 2. *Dfd* transcript accumulation in blastoderm stage embryos. **(A)** Bright field photomicrograph of 2.75 h embryo that has just undergone the thirteenth nuclear division. **(B)** Dark field photomicrograph of the embryo shown in **(A)**. **(C)** Bright field photomicrograph of a 3 h embryo at the cellular blastoderm stage. The numbers indicate the region of *Dfd* expression on the dorsal (top) and ventral (bottom) sides of the embryo in per cent egg length as measured from the posterior pole. **(D)** Dark field photomicrograph of the section shown in **(C)**. **(E)** Bright field photomicrograph of a glancing section through a cellular blastoderm staged embryo. A band of six cells expresses *Dfd*.

Results

Transcripts from Dfd

To test for the size and temporal specificity of transcripts from *Dfd*, Northern blots of poly(A)$^+$ RNA from developmentally staged organisms were hybridized with *Dfd* genomic and cDNA fragments. When a probe from the 5′ region of the *Dfd* cDNA clone pcDfd41 (the 0.8-kb *Eco*RI fragment) is used, the developmental profile shown in Figure 1b is seen. A single major transcript of 2.8 kb is expressed throughout embryonic, larval and pupal stages of development and in adult flies. However, this RNA appears to be most abundant in the period from 6 to 12 h post-fertilization, a stage when the *Drosophila* germ band becomes segmented and retracts to the ventral side of the embryo. This is also the period during which several head segments fuse and involute through the stomodeum.

Faint signals appear in other molecular weight ranges, especially in the lanes with embryonic RNA. These are inconsistently detected, and presumably result from weak homology between the 5′ probe (which contains a region highly enriched in glycine codons, Regulski *et al.*, 1987) and a variety of different RNA species. Probes containing exons 4 and 5 yield patterns indistinguishable from that shown in Figure 1, except that a smear of hybridization also occurs in each lane, due to the presence of highly repeated transcribed sequences that fall within these exons (CAX or M or opa repeat and AAX repeat, McGinnis *et al.*, 1984a; Wharton *et al.*, 1985; Regulski *et al.*, 1986; Mlodzik and Gehring, personal communication). No transcripts were detected using intron probes (see Figure 1A and Regulski *et al.*, 1987). We conclude that *Dfd* consists of one major transcription unit encoding an RNA of ~2.8 kb.

Expression of Dfd in blastoderm stage embryos

After fertilization, seven to eight synchronous nuclear divisions occur before the nuclei migrate to the periphery of the egg. Near the beginning of this migration, 3−12 nuclei move to the posterior pole of the egg to form the presumptive germ cells, or pole cells (Sonnenblick, 1950; Foe and Alberts, 1983). Five more synchronous nuclear divisions occur before cell membranes simultaneously enclose the syncytial nuclei.

After the thirteenth nuclear division, but prior to cellularization of the syncytial blastoderm, localized *Dfd* transcripts are first detectable above background levels. Nuclei in the region of 65% egg length on the dorsal side and 70% egg length on the ventral side, as measured from the posterior pole of the embryo, are enriched in *Dfd* transcripts. Silver grains revealing these transcripts are found over both the nuclei and the surrounding cytoplasm; they are not concentrated in the subcortical cytoplasm as are *ftz* transcripts at this stage (Hafen *et al.*, 1984; Weir and Kornberg, 1985).

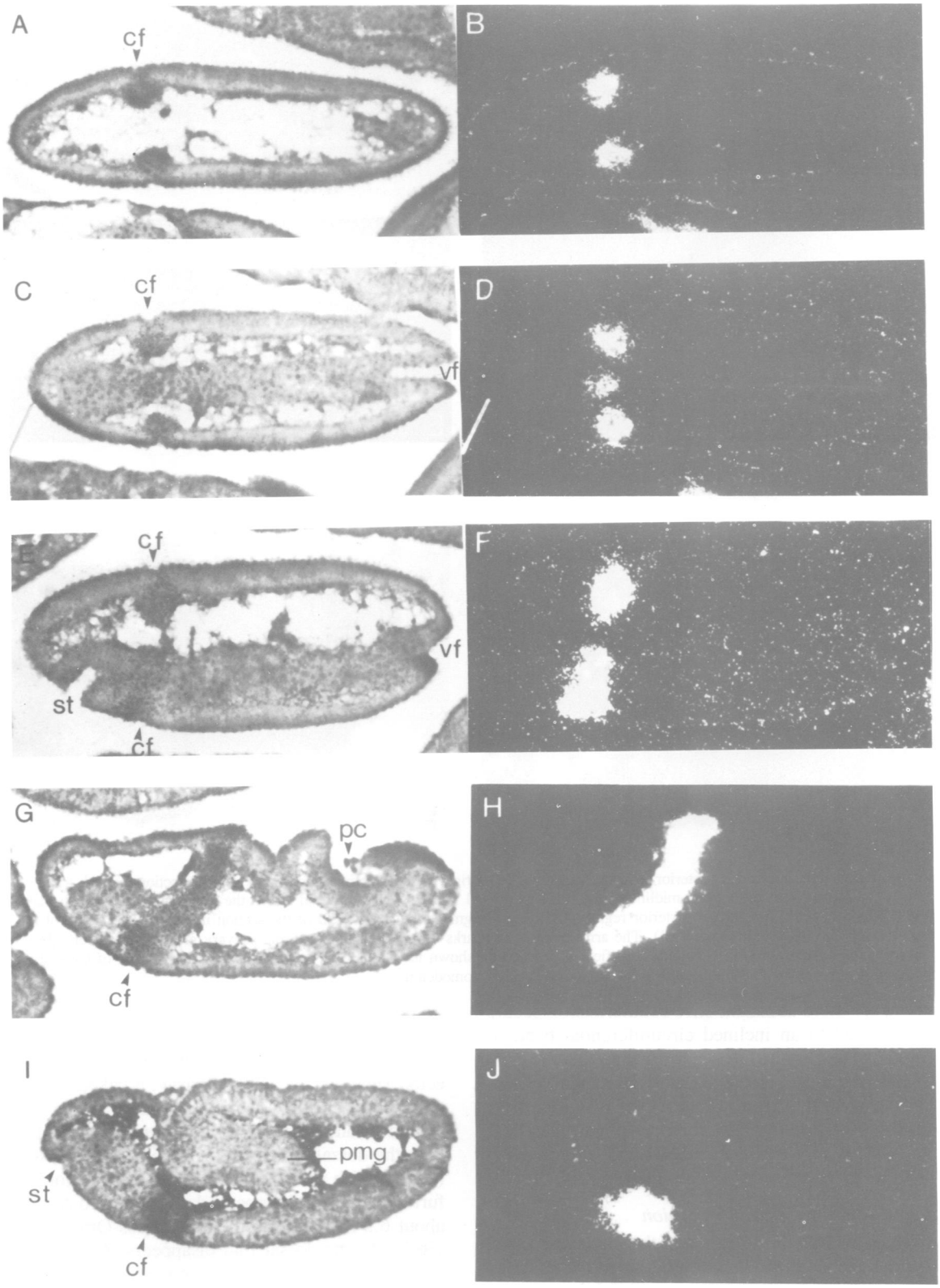

Fig. 3. *Dfd* transcript accumulation in gastrulation and germ band extension staged embryos. **(A)** Bright field photomicrograph of a horizontal medial section through a 3 h embryo at the beginning of gastrulation. **(B)** Dark field photomicrograph of the section shown in **(A)**. **(C)** Bright field photomicrograph of a more ventral section through the same embryo as that shown in **(A)** and **(B)**. **(D)** Dark field of the same section shown in **(C)**. **(E)** Bright field photomicrograph of a sagittal section through an embryo of about the same developmental stage as those shown in **A–D**. The germ band has not begun to elongate. **(F)** Dark field of the same section shown in **(E)**. **(G)** Bright field photomicrograph of a parasagittal section through an embryo as the germ band begins to elongate (about 3.5 h post-fertilization). **(H)** Dark field of the section shown in **(G)**. **(I)** Bright field photomicrograph of a parasagittal section through an embryo where the germ band is full extended (about 4 h post-fertilization). **(J)** Dark field of the same section shown in **(I)**. Abbreviations: cf = cephalic furrow, pc = pole cells, pmg = posterior midgut primordia, st = stomodeum, vf = ventral furrow.

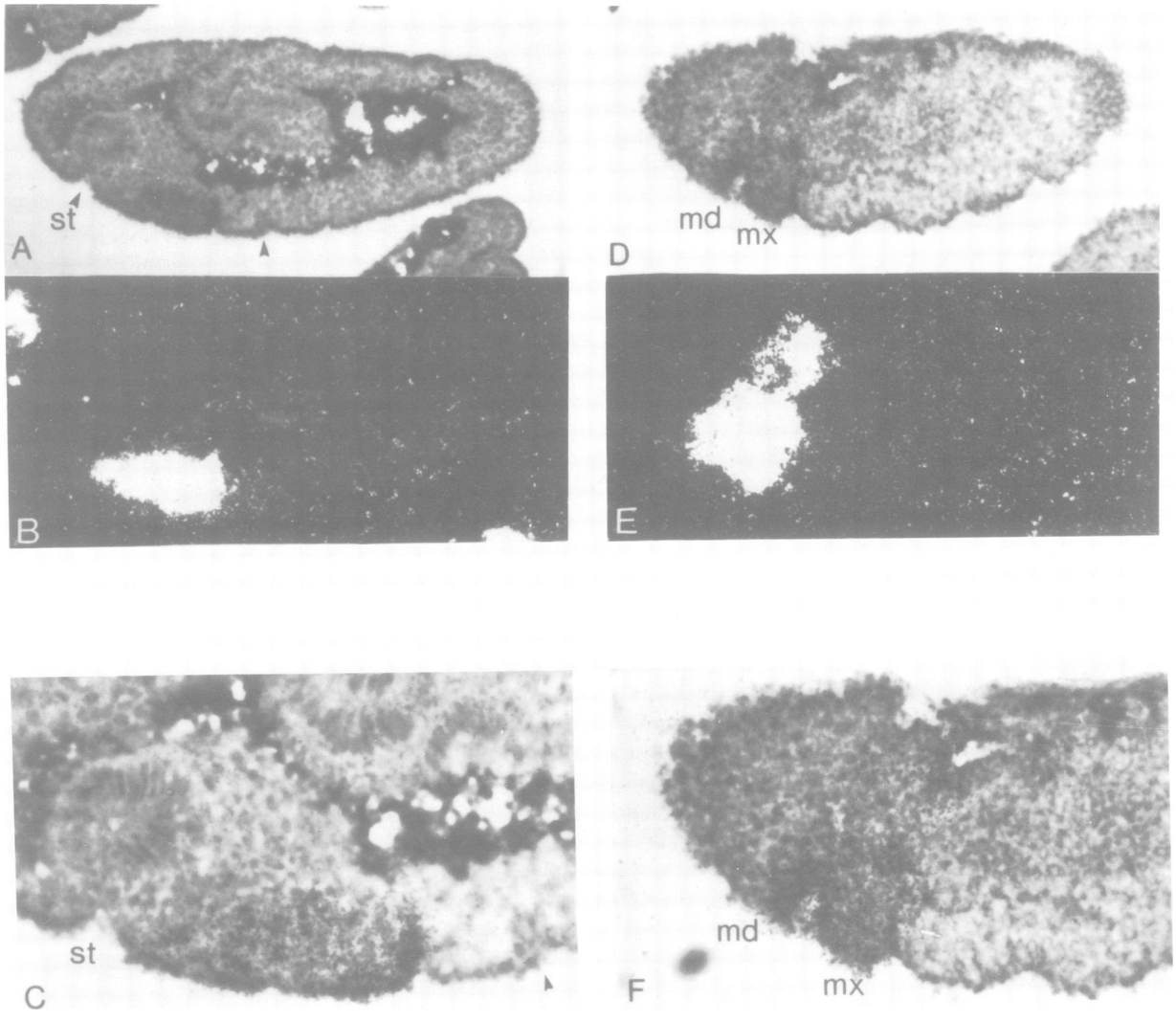

Fig. 4. *Dfd* is expressed in three lobes just posterior to the stomodeum. **(A)** Bright field photomicrograph of a sagittal section through a 6–7 h embryo where segmental grooves have formed near the ventral midline of the primordial head region. The arrowhead marks the position of cells which may form the salivary gland as it begins to invaginate in the posterior region of the labial segment. **(B)** Dark field of the section in **(A)**. **(C)** Close-up of the region expressing *Dfd* in the embryo shown on **(A)** and **(B)**. The arrowhead again marks the position of a possible salivary gland invagination. **(D)** More lateral section of the same embryo shown in **(A)**–**(C)**. **(E)** Dark field of the section shown in **(D)**. **(F)** Close-up of the anterior portion of the embryo shown in **(D)** and **(E)**. Abbreviations: md = mandibular lobe, mx = maxillary lobe, st = stomodeum.

Dfd transcripts are more abundant once cellularization occurs when they are limited to an inclined circumferential band of approximately six cells (Figure 2E). Expressing cells are positioned at 61–69% egg length on the dorsal surface and 67–74% egg length on the ventral surface. This region of the cellular blastoderm contains the primordia for the ectodermal, mesodermal and neural tissues of the posterior head segments (Underwood *et al.*, 1980; Hartenstein *et al.*, 1985; Jürgens *et al.*, 1986).

Gastrulation and early germ band extension

Gastrulation begins with the invagination of the mesodermal and endodermal primordia along the ventral midline of the embryo, creating the ventral furrow. A simultaneous infolding of cells at about 67% egg length forms the cephalic furrow (Underwood *et al.*, 1980). At the earliest gastrula stage, *Dfd* transcripts appear in the cells that first invaginate to form the cephalic furrow. These cells are rapidly pulled into the cleft of the furrow so that *Dfd* expression is detected deep within the cephalic furrow (Figure 3). In addition to expression throughout the cephalic furrow, *Dfd* transcripts appear in the ventral aspect of the embryo in the primordial mesodermal cells which arise within the ventral furrow (Figure 3C–F).

The mesodermal primordium, and the associated overlying ectoderm along the ventral furrow, is called the germ band (Sonnenblick, 1950). As the cells within the germ band divide they extend around the posterior of the embryo, pushing the pole cells onto the dorsal surface. When the germ band is fully extended (at about 4 h post-fertilization), the dorsal fold of the cephalic furrow begins to disappear; the ventral fold remains visible until about 6.5 h of development (Campos-Ortega and Hartenstein, 1985). As the dorsal fold disappears, *Dfd* transcripts also disappear from the extreme dorso-medial cells of the embryo. On the ventral side of the embryo *Dfd* transcripts are found in cells that flank the cephalic furrow. In medial sections approximately one cell anterior and seven cells posterior to the vanishing cephalic furrow express *Dfd* transcripts. These cells form the primordia for the hypopharyngeal (also known as the intercalary or premandibular), mandibular and maxillary segments (Turner and Mahowald, 1977; Underwood *et al.*, 1980; Technau and Campos-Ortega, 1985).

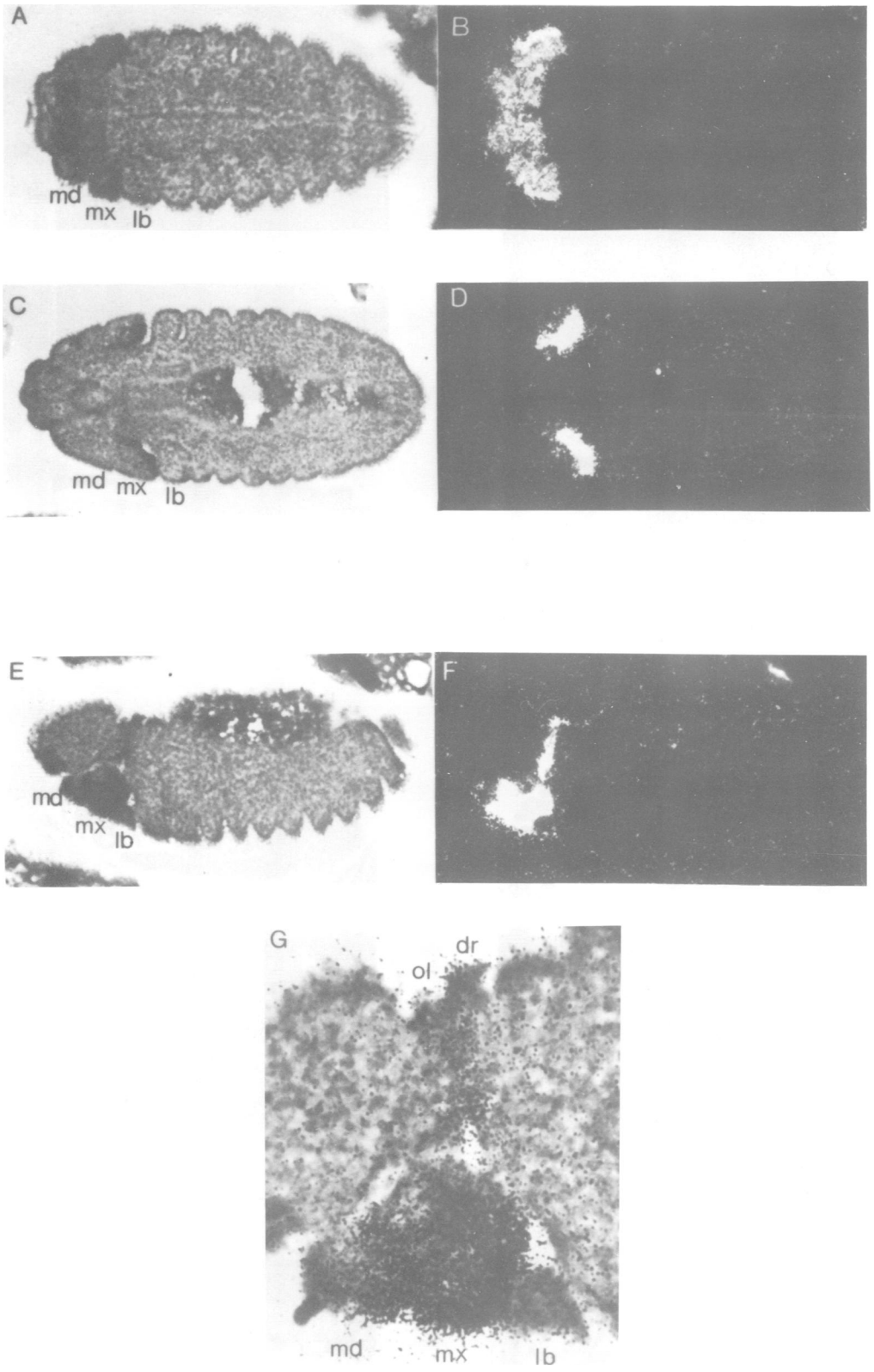

Fig. 5. *Dfd* expression during germ band retraction. **(A)** Ventral horizontal section of 8.5−9.5 h embryo which is undergoing germ band retraction. **(B)** Dark field of the section shown in **(A)**. **(C)** Dorso-medial section from the same embryo in **(A)** and **(B)**. **(D)** Dark field of the section shown in **(C)**. **(E)** Very lateral parasagittal section of an embryo at ~9.5 h post-fertilization where the germ band is almost fully retracted. **(F)** Dark field of the section shown in **(E)**. **(G)** Close-up of the gnathal region of the section shown in **(E)**. Abbreviations: dr = dorsal ridge, lb = labial lobe, md = mandibular lobe, mx = maxillary lobe, ol = optic lobe.

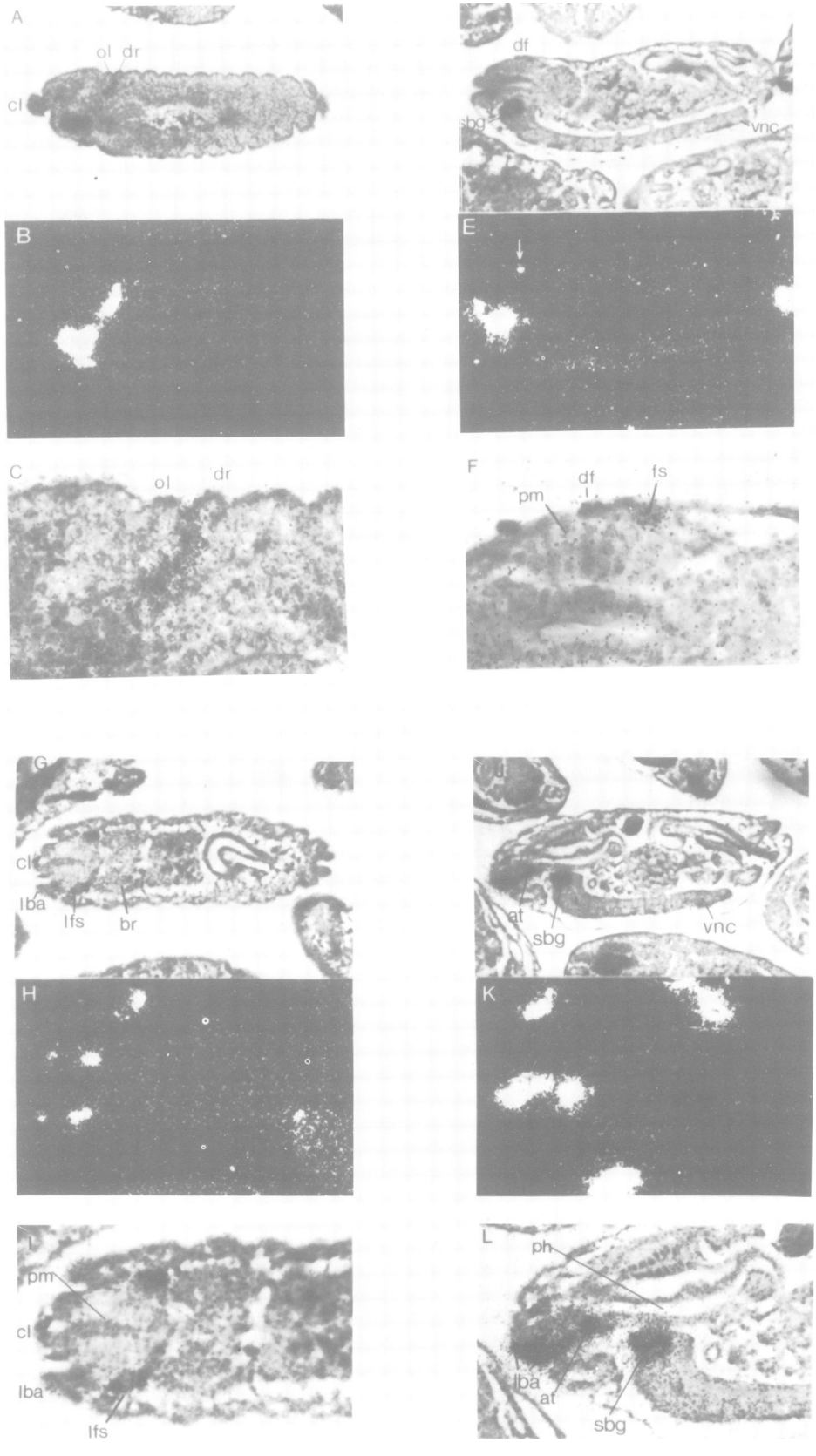

Fig. 6. *Dfd* expression late in embryogenesis. **(A)** Parasagittal section through an embryo where the germ band is fully retracted (about 10 h post-fertilization). **(B)** Dark field of the same section shown in **(A)**. **(C)** Close-up of the dorsal aspect of the section shown in **(A)**. **(D)** Parasagittal section of a 13 h embryo. **(E)** Dark field of the section shown in **(D)**. **(F)** Close-up showing *Dfd* expression in the dorsal fold. **(G)** Horizontal section through the dorsal region of a 15 h embryo. **(H)** Dark field of the section shown in **(G)**. **(I)** Close-up of the anterior region of the embryo shown in **(G)** and **(H)**. **(J)** Parasagittal section through a 16–20 h embryo. **(K)** Dark field of the section shown in **(J)**. **(L)** Close-up of the anterior region of the section shown in **(J)**. Abbreviations: a = atrium, br = brain, cl = clypeolabrum, df = dorsal fold, dr = dorsal ridge, lba = lateral border atrium, lfs = lateral frontal sac, ol = optic lobe, ph = pharynx, pm = pharyngeal muscles, sbg = subesophageal ganglion, vnc = ventral nerve cord.

Germ band extension

The first signs of segmentation occur shortly after the germ band has completely elongated. By 5.5−6 h, superficial grooves have become obvious along the ventral midline of the embryo. A sagittal section through a 6−7 h embryo indicates that *Dfd* transcripts are detected in the ventral regions of the three lobes that form just posterior to the lower lip of the stomodeum (Figure 4A−C). These correspond to the primordia for the hypopharyngeal, mandibular and maxillary segments (Turner and Mahowald, 1977; DiNardo *et al.*, 1985; Jürgens *et al.*, 1986). Hypopharyngeal expression is transient, and is not detected at later stages. A parasagittal section from the same embryo, which does not include the mesodermal cells found along the ventral midline, indicates that in dorso-lateral regions most *Dfd* transcripts are restricted to the ectoderm of the mandibular and maxillary lobes (Figure 4D−F).

Martinez-Arias and Lawrence (1985) and Akam and Martinez-Arias (1985) propose that the embryonic lobes formed during germ band extension are actually parasegments, not segments, and that the two metameric units lie out-of-phase with each other by the width of approximately a half segment. Segment boundaries form later in development at the positions of the tracheal pits. Although the expression of *engrailed* (*en*) strongly supports this view in the thoracic and abdominal metameres, the head appears to be differently organized. Each gnathal lobe acquires a characteristic shape by 6−7 h of development. At this time *en* protein is localized only in the posterior region of each gnathal lobe (DiNardo *et al.*, 1985). Since *en* has been shown to be necessary for the formation of posterior compartments of all segments (Lawrence and Morata, 1976; Kornberg, 1981; Eberlin and Russell, 1983), the gnathal lobes correspond to segments at this stage. Therefore, at the extended germ band stage, the posterior boundary of *Dfd* transcript accumulation lies at, or very near, the posterior boundary of the maxillary segment.

Germ band retraction

The germ band remains extended until about 7.5−8 h post-fertilization. During the next hour of development the germ band retracts around the posterior pole of the embryo until all segmental primordia are positioned along the ventral surface (Campos-Ortega and Hartenstein, 1985; Ede and Counce, 1956). Horizontal sections from an embryo that has undergone germ band retraction indicate that the majority of transcripts from *Dfd* have become restricted ventrally to the mesoderm and ectoderm of the maxillary segment and the posterior region of the mandibular segment (Figure 5A and B). Note that the internal labeling, representing *Dfd* transcripts in the presumptive subesophageal ganglion and mesoderm, is offset anteriorly by approximately half a segment. This might represent a parasegmental frame of expression for *Dfd* in the neural and mesodermal elements of the posterior head. A more dorsal section from the same embryo shows that *Dfd* expression becomes further localized in the dorso-medial region of the embryo to the posterior region of the maxillary segment (Figure 5C and D). *Dfd* transcripts, at this time, are not found in the anterior-lateral regions of the maxillary lobe. However, *Dfd* transcripts appear in a stripe of cells between two newly-formed lobes: the optic lobe and the dorsal ridge (Figure 5E−G). Figure 5G is a close-up which summarizes epidermal *Dfd* expression during germ band retraction: *Dfd* transcripts appear in the posterior region of the mandibular lobe, throughout the maxillary lobe except in cells of the antero-lateral region, and in cells lying between the optic lobe and dorsal ridge.

Late stage embryos

By approximately 9 h post-fertilization, the germ band has fully retracted. In an embryo of this stage *Dfd* is strongly expressed in the primordia for the subesophageal ganglia, the ventral ectoderm of the maxillary segment and in the cells that lie between the optic lobe and the dorsal ridge (Figure 6A−C). Soon after the optic lobes are formed, which occurs at about 9 h of development, they invaginate and eventually merge with the ventrocaudal region of the brain. The dorsal fold, which forms from a fusion of the two dorsal ridge lobes, slides over the procephalon and clypeolabrum enclosing them in a pocket called the frontal sac. Figure 6D−F shows that a small cluster of cells within the frontal sac express *Dfd* in a 13 h embryo. These cells are presumably derived from the cells expressing *Dfd* at earlier stages in the region of the dorsal ridge. A horizontal section through an older embryo demonstrates that *Dfd* is expressed bilaterally deep within the frontal sac. This is the region from which the eye-antennal imaginal discs arise (Figure 6G−I) (Chen, 1929; Enzmann and Haskins, 1938; Poulson, 1950; Turner and Mahowald, 1979).

Head involution begins as the germ band begins to retract. During the process of head involution the gnathal segments migrate anteriorly to form the borders of the atrial opening (larval mouth) and parts of the atrium itself (Turner and Mahowald, 1979; Campos-Ortega and Hartenstein, 1985; Jürgens *et al.*, 1986). The first lobe to move into the stomodeal opening is the hypopharyngeal lobe which migrates into the oral cavity to form the floor of the pharynx. Meanwhile the labial lobes have fused along the ventral midline, creating a single, common salivary duct. The salivary duct then follows the hypopharyngeal lobe into the atrium, moving into the opening until it reaches its ultimate location at the border between the atrium and the pharynx. The remaining labial cells form the ventral border of the atrium.

The maxillary and mandibular lobes fuse during germ band retraction, and the dorsal regions of both lobes also merge with the procephalon at about this time. Later, the fused maxillary-mandibular segments are displaced along the sides of the embryo so that some cells of mandibular, and possibly maxillary, origin move into the oral opening to create the lateral wall of the atrium (Turner and Mahowald, 1979; Campos-Ortega and Hartenstein, 1985). Several external head structures derive from the maxillary segment including the mouth hooks, cirri and most of the papillae of the maxillary sense organ. The mandibular segment appears to give rise to at least one of these papillae but most other structures of mandibular origin lie within the oral cavity (Jürgens *et al.*, 1986). Figure 6I−J shows *Dfd* transcripts in the subesophageal ganglion as well as in cells of the lateral walls and mouth of the atrium.

Dfd expression in eye-antennal imaginal discs

Imaginal discs each consist of an infolding of a single layer of larval epidermal cells which remain contiguous with the epidermis through a thin stalk (Russell, 1982). Most discs are clustered near the brain of the developing larvae. Cells fated to become discs can first be identified late in embryogenesis. In the larval instar stages, larval tissues grow by polytenization, while imaginal discs remain as pockets of dividing diploid cells, each disc taking on a characteristic size and shape. During pupariation, most of the larval tissues are replaced by imaginal disc cells. Fate maps have been constructed indicating which cells within the imaginal disc give rise to specific adult structures (Ouweneel, 1970; Sprey and Oldenhave, 1974; Bryant, 1978; Ransom, 1982). The eye-antennal imaginal disc gives rise to most of the adult head capsule

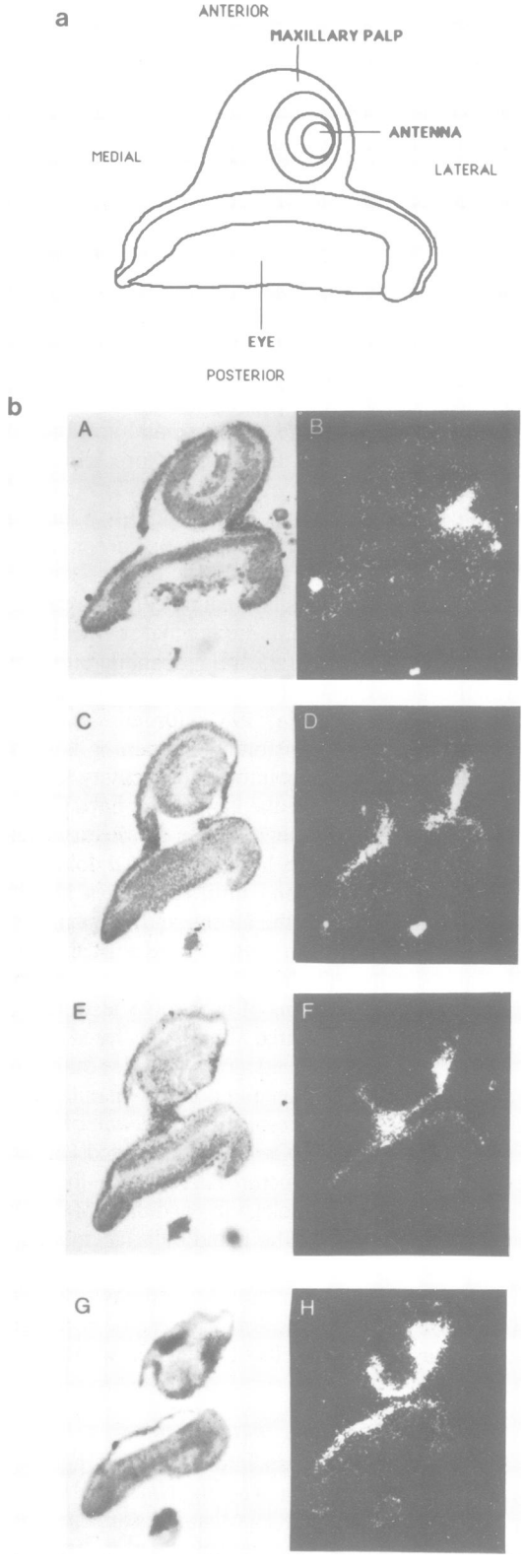

Fig. 7. *Dfd* expression in the eye-antennal disc. **(a)** A schematic drawing of an eye-antennal (head) disc which has the same orientation as the sections shown in **(b)**. A rudimentary fate map of some of the adult structures which arise form specific regions of the disc is also shown. **(b)** *Dfd* transcripts accumulation in head discs. Sections are from the same eye-antennal disc and are shown in sequential order moving from the face of the disc towards the peripodal membrane. Therefore A (and B) includes more cells of the disc proper while G (and H) includes cells that lie mostly in the peripodal membrane. Each section is shown on the left in bright field with its corresponding dark field on the right.

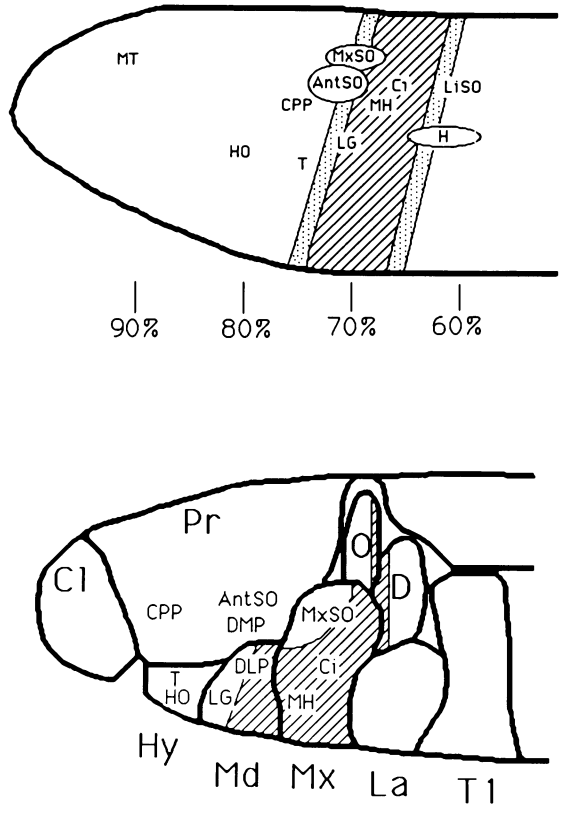

Fig. 8. (Top) A schematic view of the anterior tip of a cellular blastoderm stage embryo. The measurements are per cent lengths from the posterior pole. The location of the primordia of larval cuticular and sensory elements are mapped as in Jürgens *et al.* (1986). The cross-hatched region indicates the cells expressing *Dfd* transcripts at this stage, with the dotted flanks indicating the uncertainty in our measurements. AntSO = antennal sense organ, Ci = cirri, CPP = cephalopharyngeal plates, H = H-piece, HO = hypopharyngeal organ, LiSO = labial sense organ, LG = lateralgraten, MH = mouth hooks, MT = median tooth, MxSO = maxillary sense organ, T = T bar. **(Bottom)** A schematic view of an extended germ band stage embryo. The locations of the primordia of larval cuticular and sensory elements are mapped as in Jürgens *et al.* (1986). The cross-hatched regions indicate ectodermal cells expressing *Dfd* transcripts at this stage. Cl = clypeolabral lobe, D = dorsal ridge, DLP = dorso-lateral papilla, DMP = dorso-medial papilla, Hy = hypopharyngeal lobe, La = labial lobe, Md = mandibular lobe, Mx = maxillary lobe, O = optic lobe, Pr = procephalic lobe, T1 = first thoracic segment.

including the maxillary palp, antennae and eye facets. Figure 7 shows that some *Dfd* transcripts appear in the anterior-lateral and medial regions of the eye-antennal disc. These regions are fated to give rise to posterior and lateral regions of the ventral aspect of the head capsule including the rostral membrane, and perhaps the maxillary palp, ptilinum and proximal segments of the antennae (Bryant, 1978). Most *Dfd* transcripts, however, accumulate in portions of the peripodal membrane of the eye-antennal disc which have not been mapped (Figure 7G and H). Therefore, it is not possible, at this time, to give a complete description of the adult structures arising from cells expressing *Dfd*.

Discussion

Our analysis of the expression patterns of *Dfd* transcripts indicates that the major site of expression throughout embryonic development is in cells of the maxillary and mandibular segments of the head. If the prevailing beliefs about the segmental composition of the *Drosophila* head are correct, with the clypeolabrum as

the first or most anterior, and the labial as the sixth and most posterior segment, these would correspond to the fourth and fifth metameres of the head (Snodgrass, 1935; Ferris, 1950; Rempel, 1975; Struhl, 1981; Jürgens *et al.*, 1986). While expression is generally localized within these segments, particularly during early stages of embryogenesis, there is considerable fine structure variation in the spatial limits of expression over the course of development. These results support the hypothesis that *Dfd*, like other homeotic selector genes, functions in the establishment and maintenance of segment (or parasegment) identity during embryonic development.

At the earliest stages of *Dfd* expression, transcripts appear in one circumferential band of approximately six cells in width. If the metameric primordia of head segments occupy the same cellular space as do the primordia of the thoracic and abdominal segments, then this expression would correspond to 1.5−2 segment primordia (Lohs-Schardin *et al.*, 1979). At the extended germ band stage, after the head segments and body parasegments have formed, epidermal expression of *Dfd* is confined to the posterior region of the mandibular lobe and to the posterior-lateral and ventral regions of the maxillary lobe. Cells that lie between the dorsal ridge and the optic lobe also accumulate *Dfd* transcripts.

At two stages for which detailed fate maps have been prepared (Figure 8, Jürgens *et al.*, 1986), *Dfd* transcripts are found in regions that are fated to give rise to larval cuticular and sensory structures that are missing in Dfd^- embryos. Structures arising from the region of blastoderm expression include the mouth hooks, H-piece and lateralgraten (ventral, anterior extensions of the cephalopharyngeal plates) as well as the maxillary and antennal sense organs (Figure 8A). Most, but not all of these structures are missing in Dfd^- animals; the maxillary sense organ develops but is missing two papillae (the dorso-lateral and dorso-medial papillae, DLP and DMP, respectively). The antennal sense organ forms but not in its normal location. Mapping the *Dfd* expression pattern onto a structural fate map of an extended germ-band stage embryo provides a possible explanation for the development of these sense organs in the absence of a functional *Dfd* allele. The antennal sense organ maps in the procephalic lobe while the maxillary sense organ maps to the dorso-anterior region of the maxillary lobe. *Dfd* is not expressed in either of these regions at this stage. Therefore, at the extended germ band stage, there is an excellent correspondence between the regions of *Dfd* expression and regions fated to give rise to mouth hooks, cirri and other structures eliminated in Dfd^- mutants.

The pattern of *Dfd* expression late in embryogenesis correlates well with the structures affected in the Dfd^- terminal phenotype. Just before hatching, *Dfd* is expressed laterally within the atrium as well as at the opening of the atrial cavity and within the subesophageal ganglion (Figure 6K−M). The homeotic phenotype observed in Dfd^- embryos is due to the replacement of atrial cuticular structures by cephalopharyngeal plates. These duplicated structures derive from the procephalic lobe and are of unknown segmental provenance (Regulski *et al.*, 1987; Jürgens *et al.*, 1986).

Other notable elements in the early pattern of *Dfd* expression include the lack of tissue- or cell-type specificity, and, like other homeotic and segmentation genes, its spatial and metameric specificity. During gastrulation, *Dfd* transcripts accumulate in the primordia for ectodermal, mesodermal and neural tissues. Both ectodermal and neural expression persists into mid-to-late stages of embryogenesis, and mesodermal labeling is detected well into the germ band stage. Therefore the Dfd^- phenotype and the specificity of wild-type *Dfd* expression fits the predicted pattern for a homeotic selector gene, that is, *Dfd* is expressed in a regional, not cell-type specific, manner throughout embryogenesis, several different structures deriving from the region of normal *Dfd* expression are eliminated in Dfd^- embryos, and finally, elimination of *Dfd* function causes pattern duplications (Lewis, 1978; Kaufman *et al.*, 1980; Akam, 1983; Levine *et al.*, 1983; Regulski *et al.*, 1987).

The persistent expression of *Dfd* in specific regions of the eye-antennal (head) imaginal disc supports the notion that *Dfd* is necessary for the proper morphogenesis of ventral-posterior and ventral-lateral regions of the adult head. In more primitive insects these regions of the adult head would give rise to characterisetic appendages of the mandibular and maxillary segments (Snodgrass, 1935; Ferris, 1950; Rempel, 1975). The association of the *Dfd* function with maxillary and mandibular lobes in the embryo and larvae, and the regions of *Dfd* expression in the head disc, suggest that this expression may correspond to the remnants of maxillary and mandibular segments which some authors had supposed were lost in the organization of higher insect heads. Therefore, even though the evolution of *Drosophila* has resulted in the elimination of maxillary and mandibular appendages, with the fusion and disappearance of segmental boundaries in the head, there may still be molecular and cell lineage boundaries that manifest, to some extent, the missing morphological boundaries.

During mid-to-late stages of embryogenesis, *Dfd* transcripts accumulate in a discrete group of cells at the anterior boundary of the dorsal ridge. These cells are then apparently incorporated into the frontal sac where they take up a dorso-lateral position. If this dorso-lateral frontal sac expression does correspond to the primordia of a portion of the head-disc, then the early expression of *Dfd* in the dorsal ridge region may represent a molecular correlate of previously described genetic and morphological determinative events which result in setting aside imaginal tissue at this stage (Janning, 1978). In addition, *Dfd* may play a role in the specification of other imaginal tissues, as we have evidence that *Dfd* is expressed in a discrete region of the genital disc.

While the overall spatial pattern of *Dfd* expression resembles that of other homeotic selectors such as *Antennapedia* (*Antp*) or *Ultrabithorax* (*Ubx*), in that expression is limited to a few segments (Levine *et al.*, 1983; Akam and Martinez-Arias, 1985), some details differ. For example, the expression and function of the *Ubx* gene in the epidermis has been shown to be in the parasegmental frame during embryonic development (Hayes *et al.*, 1984; Akam and Martinez-Arias, 1985; Sanchez-Herrero *et al.*, 1985). *Dfd* transcripts, however, accumulate in a segmental frame in the epidermis of the extended germ band stage, which is the most favorable stage for visualization of the parasegmental lobes of the embryo. The posterior boundary of *Dfd* epidermal expression at this time and throughout embryonic development lies at the posterior edge of the maxillary lobe, which, as measured by *engrailed* expression (DiNardo *et al.*, 1985), is the posterior boundary of the maxillary segment primordia. A similar segmental phasing for *Sex combs reduced* expression has been observed in the head epidermis (Mahaffey and Kaufman, personal communication). The neural and mesodermal expression patterns of *Dfd* do appear out of phase with segmental boundaries, and therefore may fit into a parasegmental frame.

As has been shown for other patterning genes of *Drosophila*, the hierarchical relationships among these genes may be dissected by testing for the spatial limits of their expression in a variety of mutant backgrounds. The *Dfd* transcripts, due to their abun-

dance and sharp boundaries of expression, should provide an excellent marker for the primordia of the fourth and fifth head segments, and allow the elucidation of the genetic circuitry necessary to spatially limit *Dfd* expression to one circumferential band of cells in the presumptive head region of the *Drosophila* embryo.

Materials and methods

RNA isolation and Northern analysis

RNA was isolated by a modification of the phenol extraction method of Artavanis-Tsakonas *et al.* (1984). Embryos were collected and dounced, without dechorionation, in a 1:1 mixture of hot (65°C) phenol and extraction buffer (50 mM Na-Citrate, pH 7.0, 0.5% SDS and 0.5% 2-mercaptoethanol). The aqueous layer was re-extracted with phenol followed by two phenol:$CHCl_3$ (1:1) extractions and one $CHCl_3$:isoamyl alcohol (24:1) extraction. The RNA was then stored as an ethanol precipitate until poyl(A)$^+$ selection, which was done exactly as stated in Maniatis *et al.* (1982). Poly(A)$^+$ RNA (10 μg/lane) was electrophoretically fractionated on a 0.7% formaldehyde−agarose gel and transferred without pretreatment to nylon using 20 × SSC. Before blotting, the lane containing *Hin*dIII digested lambda DNA markers was excised and stained overnight in 0.1 × SSC, 0.1 μg/ml ethidium bromide at 4°C. Conditions for pre-hybridization, hybridization and washing were as stated previously (McGinnis *et al.*, 1984a) except that the hybridization solution contained 0.5% SDS and the hybridization was performed at 55°C.

In situ hybridization to tissue sections: paraplast sections of embryos

Embryos of different developmental stages were collected and fixed using the two-phase fixation procedure of Akam and Martinez-Arias (1985), except that DMSO was added to the 4% paraformaldehyde:heptane mixture (to 40% of the aqueous phase), and the embryos were agitated in this fixative for 2 h. After de-vitellinization and rehydration, the embryos were placed in small wire baskets and submerged in the following solutions: 4% paraformaldehyde in PBS for 15 min; PBS for 5 min; followed by 30%, 50%, 70%, 90%, 95% and 100% ethanol for 10 min each; xylene, twice, for 10 min each; xylene at 58°C for 10 min; xylene:paraplast (1:1) for 20 min at 58°C, and finally paraplast (58°C) for 15 min. The embryos were then transferred to fresh paraplast for 10 min and small drops of paraplast with embryos were placed in embedding moulds. After 20 min, the mould was removed from the 58°C incubator and allowed to cool until the surface of the paraplast began to harden. The mould was then filled with paraplast and allowed to harden at room temperature. Blocks were stored at 4°C until sectioning.

Six micron selections were cut and floated onto a drop of water on a poly-L-lysine coated slide which had been equilibrated to 42°C using a slide warmer. After the water had evaporated, the sections were baked overnight at 45°C. Sections were de-waxed and rehydrated through graded solutions of ethanol prior to pretreatment with pronase and acetylation (Hafen *et al.*, 1983). The sections were hybridized overnight at 55°C using a hybridization solution (15−40 μl/slide) that contained: 0.1% BSA, 0.1% polyvinylpyrrolidone, 0.1% Ficoll, 1.67 mg/ml carrier tRNA, 62.5% formamide, 100 mM NaCl, 10 mM Tris−HCl, pH 7.5, 1 mM EDTA, 12.5 mM DTT, 10% dextran sulfate and probe at a concentration of 0.25 ng/μl.

After hybridization, the sections were washed as follows. Coverslips were floated off in a solution of 2 × SSC, 0.1% 2-mercaptoethanol at 50°C. The sections were washed for 30 min in the same solution at 50°C with the addition of 50% formamide. Slides were rinsed briefly in 2 × SSC and then incubated at 37°C in 20 μg/ml boiled RNase A (in 0.5 M NaCl, 10 mM Tris, pH 8.0) for 30 min. The sections were washed at 58°C for 30 min each in 2 × SSC, 50% formamide, 0.1% 2-mercaptoethanol and finally in 0.1 × SSC, 0.1% 2-mercaptoethanol.

The probe used for all *in situ* hybridization was a ^{35}S-labeled riboprobe synthesized from a pSP65 clone containing a full-length *Dfd* cDNA (pcDfd41, Regulski *et al.*, 1986). The probe was reduced to an average size of ~50−180 nucleotides by alkaline hydrolysis (Cox *et al.*, 1984) prior to hybridization.

In situ hybridization to eye-antennal imaginal disc sections

Eye-antennal discs were dissected from climbing third instar larvae and placed immediately in 4% paraformaldehyde for 30 min. The discs were then rinsed for 30 min in PBS. Dehydration, embedding and hybridization was performed as above.

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