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The role of selectins in *Drosophila* eye and bristle development

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SUMMARY

Mutations in the *furrowed* (*fw*) gene of *Drosophila* result in defects in the development of the eye and mechanosensory bristles. The eyes are reduced in size, have furrows or crevices in the retina, and show a disturbed patterning of ommatidia. In addition, the ommatidia have an altered morphology and often contain abnormal numbers of cells. The bristles show altered structure and polarity, and are occasionally duplicated or missing. These results suggest that the product of the *fw* gene is involved in cell determination events within sensory organs. The *fw* gene has been cloned and shown to encode a protein homologous to vertebrate selectins. Like selectins, Fw contains a lectin-binding domain, ten complement binding repeats, and a transmembrane domain. The Fw protein is expressed in the larval imaginal discs where it might mediate carbohydrate-protein interactions necessary for proper development of a subset of adult sensory organs.

Key words: selectin, eye, bristle, *furrowed*, peripheral nervous system, *Drosophila*

INTRODUCTION

The adult peripheral nervous system of *Drosophila* is composed of sensory and motor components. The external sensory organs of the adult fly are established during larval and pupal development, and are patterned over the entire body of the fly to detect environmental stimuli. Mutations in the *furrowed* gene result in defects in a subset of external sensory organs, including the compound eye and the mechanosensory bristles. Although the affected sense organs differ in structure and organization, the compound eye and bristles share a common requirement for neural versus epidermal cell determination during morphogenesis that involves complex cell-cell interactions. Cell adhesion receptors play an important role in cell-cell communication, by providing both the mechanical attachment and the transfer of information from cell to cell.

Most cell adhesion receptors, like integrins and the immunoglobulin superfamily, mediate intercellular communication via specific protein-protein interactions. Both integrins and immunoglobulin superfamily members have been shown to be involved in neural development in *Drosophila*. For example, mutations in the integrin genes of *Drosophila*, *myospheroid* and *multiple edematous wings* disrupt eye morphogenesis as well as muscle attachment and wing morphogenesis (Brower and Jaffe, 1989; Zusman et al., 1990; Brower et al., 1995). Additionally, immunoglobulin superfamily members neuroglian, fasciclin II and III, function to guide growing axons during neural development in insects (Bastiani et al., 1987; Bieber et al., 1989; Harrelson and Goodman, 1988). Cell adhesion molecules have also been found to mediate cell-cell communication in the nervous system via protein-carbohydrate interactions. For example, myelin-associated glycoprotein has been shown to mediate cell adhesion in the nervous system by binding glycans on the surface of neurons (Kelm, 1994). Another important cell adhesion receptor family, the selectins, mediate cell adhesion via protein-carbohydrate interactions (Bevilacqua et al., 1991), however this class of proteins has not been implicated to date in the development of the nervous system in vertebrates. Selectins have only been found to mediate leukocyte-endothelial cell interactions in the immune system of mammals where they function to recruit circulating leukocytes to areas of inflammation, and to the peripheral lymph node for initiation of the immune response (Springer, 1990; Lasky, 1992; Paulson, 1992; Bevilacqua and Nelson, 1993).

Selectins are integral transmembrane proteins composed of three extracellular domains: an amino-terminal carbohydrate recognition or lectin domain, an epidermal growth factor (EGF)-like domain, and a variable number of consensus repeats found in complement binding proteins, followed by a transmembrane domain and a short cytoplasmic tail at the carboxy terminus. Three family members have been identified to date that differ in the number of complement binding repeats: L-selectin has two repeats, E-selectin has six repeats, and P-selectin has nine repeats (Laski et al., 1989; Bevilacqua et al., 1989; Johnston et al., 1989). The lectin domain is homologous to the family of C-type lectins, which are calcium-dependent carbohydrate binding proteins (Drickamer, 1988). This domain has been demonstrated to mediate protein-carbohydrate interaction necessary for cell adhesion via recognition of complex carbohydrate determinants found in several glycoproteins and glycolipids (Varki, 1994; Rosen, and Bertozzi, 1994). The function and role of the EGF domain and complement binding repeats in mediating cell adhesion have not been fully characterized, but they seem to participate in ligand recognition and protein binding respectively (Watson et al.,

1991; Kansas et al., 1994). The cytoplasmic domain is also suspected to have a role in regulating cell adhesion by controlling cytoskeletal interactions and/or receptor avidity (Kansas et al., 1993).

Until now, selectins have not been identified in invertebrates or found to have a role in any developmental process. Since protein-carbohydrate interactions have been shown to take place during development, especially in the nervous system, selectins may mediate these interactions and play important roles in these processes. Here we present results indicating that a selectin cell adhesion molecule exists in *Drosophila* and it is encoded by the *furrowed* gene. The results suggest that this selectin family member may have an important role in mediating cell adhesion and cell-cell interactions during the development of the fly.

MATERIALS AND METHODS

Fly stocks and P element-mediated transformation

Flies were reared on standard molasses/yeast/cornmeal medium at 22.5°C. The *Drosophila melanogaster* alleles fw^{1} and fw^{34e} were obtained from the Bowling Green Stock Center, whereas fw^{U2} and fw^{U3} were obtained from P. Georgiev (Institute of Gene Biology, Moscow, Russia). Embryos were collected at 24 hour intervals and raised at 22.5°C until reaching the appropriate developmental stage to obtain RNA for northern analysis. The RNA sample representing the third instar larvae consists of animals from 72-144 hours after egg laying, whereas the early-mid pupae RNA sample consists of animals from 145-217 hours after laying and the mid-late pupae RNA sample consists of animals from 220-268 hours after laying. For germ line transformation, a 12.7 kb wild-type genomic fragment was clone into the *Not*I site of the CaSpeR 2 vector (Pirrotta et al., 1985) which contains the w^{+} reporter gene. The DNA was injected into Δ2-3 preblastoderm embryos at a concentration of 600 μg/ml. The surviving embryos were reared at 18°C and then mated to $y^{1}w^{67}$ flies. The progeny were screened for w^{+} individuals.

Isolation of nucleic acids and preparation of polyclonal antibodies

DNA manipulations were carried out by standard procedures (Sambrook et al., 1989; Ausubel et al., 1993). Sequencing of genomic DNA and cDNA isolates utilized the dideoxy chain termination method (Sanger et al., 1977). DNA amplification by the polymerase chain reaction was carried out by standard procedures (Innis et al., 1988). Total RNA was prepared using either the SDS-phenol technique (Spradling and Mahowald, 1979) or the RNAzol B method (CinnaScientific, Inc.). Poly(A)$^{+}$ mRNA was then purified by oligo-dT cellulose chromatography. For northern analysis, poly(A)$^{+}$ mRNA was electropheresed in a 1.2% or 1.0% agarose/formaldehyde (6%) gel in MOPS buffer, and transferred to a nytran membrane. Membranes were hybridized overnight at 42°C in a solution of 5× SSCP, 5× Denhardt's, 50% formamide, 1% sarkosyl, 100 μg/ml carrier DNA, and 10% dextran sulfate, then washed for 30-40 minutes at 65°C in 0.2× SSC/0.1%SDS, followed by 15 minutes at room temperature in 0.1× SSC/0.1% SDS. cDNAs were isolated by standard procedures from various libraries; cDNA 13B was isolated from an oligo dT-primed pupal gt10 library (Poole et al., 1985). The B cDNA was obtained from a different oligo dT-primed pupal gt 10 library (Hoover et al., 1993), and the F and 95E cDNAs were obtained from a random-primed pupal library constructed in lambda zap (Hoover et al., 1993). The 12-24 cDNA was obtained from a plasmid-based cDNA library constructed from embryo RNA (Brown et al., 1989).

For preparation of antibodies against Fw proteins, a PCR product from the 13B cDNA was cloned into the pGEX2T expression vector (Pharmacia). The construct was then transformed and expressed in *E. coli DH5α*. Protein expression was induced with 0.001 μM IPTG for 2 hours at 37°C, the fusion protein was electrophoresed on a 7.5% polyacrylamide gel. A gel slice containing the fusion protein was then ground into a fine powder, dissolved in 600 μl of PBS, and injected into rabbits for polyclonal antibody production.

In situ hybridization and immunochemistry

Whole mount in situ hybridization experiments were conducted following the standard protocol of Lehmann and Tautz (1994) and immunolocalization experiments were conducted following the protocol of Patel (1994). Larvae were dissected in PBS, fixed in 4% paraformaldehyde for 20 minutes, and washed in PBT+N (PBS with 0.1% Triton X-100 plus 5% serum). The dissected larvae were then incubated overnight at 4°C in PBT+N with the primary antiserum diluted 1/1000, washed for several hours in PBT at room temperature followed by a 35 minute wash in PBT+N and then incubated overnight at 4°C in 1/25,000 dilution of the HRP-conjugated secondary goat anti-rabbit antiserum. After several hours of washing in PBT at room temperature, the larvae were incubated in 1× PBS, 0.5 mg/ml diaminobenzidene (DAB) for 5 minutes (Patel, 1994). *lacZ* expression in the dissected larval brains was assayed by X-gal staining (Bellen et al., 1989).

Electron microscopy and histology

Adults were fixed in 4% glutaraldehyde in PBS. The samples were dehydrated through an ethanol series and hexamethyl disilizane (Polyscience), sputter coated with gold-palladium, and viewed in a Jeol JSM35 scanning electron microscope. The adult compound eyes were viewed at 200× and 1300× magnification, and the bristles were viewed at 86× magnification. Adult heads were fixed and embedded in resin following the procedure of Reinke and Zipursky (1988). The heads were sectioned with a glass knife microtome at 2 or 4 μm thick; the sections were mounted on slides and stained with 1% toluidine blue/1% sodium borate solution.

RESULTS

Phenotypic analysis of *furrowed* mutations

The *furrowed* (*fw*) gene has been mapped to the X chromosome at cytological position 11A4 in *Drosophila melanogaster* (Lefevre, 1981). Mutations in this gene result in pleiotropic defects in eye development, with deep furrows or folds in the retina of the adult compound eyes for which the gene was named. The mutants also have defects in the development of the mechanosensory bristles including the microchaetae, macrochaetae, and the bristles surrounding the eye. Additionally, *furrowed* mutations result in a shortened head and scutellum, and reduced viability. Three different *furrowed* alleles, fw^{1}, fw^{U2} and fw^{34e}, were examined for their effects on eye and bristle morphogenesis (Fig. 1). In addition, the phenotype of these alleles was also examined in trans-heterozygous combinations with a small deficiency, *Df(1)KA10*, encompassing the 11A1-10 region of the X chromosome. The range and strength of the phenotypes observed in these flies are more severe than those observed in the homozygous fw^{1}, fw^{U2} and fw^{34e} mutants, suggesting that these alleles are hypomorphs and that they do not represent the null *fw* phenotype. This analysis also suggests that the *furrowed* gene product is necessary for developmental processes other than eye and bristle development. Ten per cent of the fw^{1}/*Df(1)KA10* female progeny have wing defects, such that the wings are not expanded or appear stringy, and have separation of the wing blades (Fig. 2D). The fw^{U2}/*Df(1)KA10* heterozygotes have

reduced viability with only 15% of the heterozygous females eclosing as adults. Additionally, all fw^{U2}/*Df(1)KA10* females that eclose also have severe wing defects. The fw^{34e} mutants mated to *Df(1)KA10* do not produce any viable adult females, indicating that the fw^{34e} allele is haplo-insufficient. The lethal phase in the fw^{34e}/*Df KA10* females occurs in the pupal stages. The analysis of the *furrowed* alleles in *trans* to a deficiency of the *furrowed* gene indicates that *furrowed* is necessary for the viability of the fly, and suggests that the gene is also involved in wing morphogenesis.

Mutations in the *furrowed* gene result in defects in eye development

A scanning electron micrograph of the wild-type eye of *D. melanogaster* shows the normal size, shape, and patterning of the ommatidia (Fig. 1A). Mutations in the *furrowed* gene affect the size, morphology and patterning of ommatidia of the adult eye. The fw^{U2} allele has the most severe defects in eye morphology (Fig. 1B). The eyes are reduced in size, especially in the ventral margin, and show many deep penetrating furrows or crevices suggestive of an effect on the structural integrity of the retina. The ommatidial pattern is severely disorganized and the ommatidia show altered morphology – many loose the typical hexagonal shape and appear to either be flattened amorphic structures with no distinct boundaries or rounded bulbous structures (Fig. 1C). The interommatidial bristles often show altered morphology and spacing, and are occasionally duplicated. The fw^{1} allele displays a similar but more moderate phenotype, whereas fw^{34e} shows very mild defects in eye morphology (data not shown). The disturbances in the patterning and morphology of ommatidia suggest that *furrowed* mutations may affect the recruitment of the cells into the ommatidia in the developing retina.

To examine the internal structure of the adult compound eye, adult heads of *furrowed* mutants were embedded in resin and sectioned. The horizontal cross sections show many defects in the internal structure and organization of the retina in the mutants (Fig. 2A-C). The furrows penetrate the entire depth of the retina reaching the basement membrane which separates the retina from the first optic lobe (Fig. 2B). In the wild-type eye, the ommatidia are organized as straight columns reaching from the corneal lens to the basement membrane. In *furrowed* mutants, however, the ommatidia are disorganized and appear as bent instead of straight columns (Fig. 2A). The ommatidia in the region surrounding the furrows are typically most affected. Ommatidia are often missing in the retinal epithelium producing large gaps or spaces between the columns, and often they are not connected to the corneal lens and/or the basement membrane. These defects are suggestive of a role for the Fw protein in the

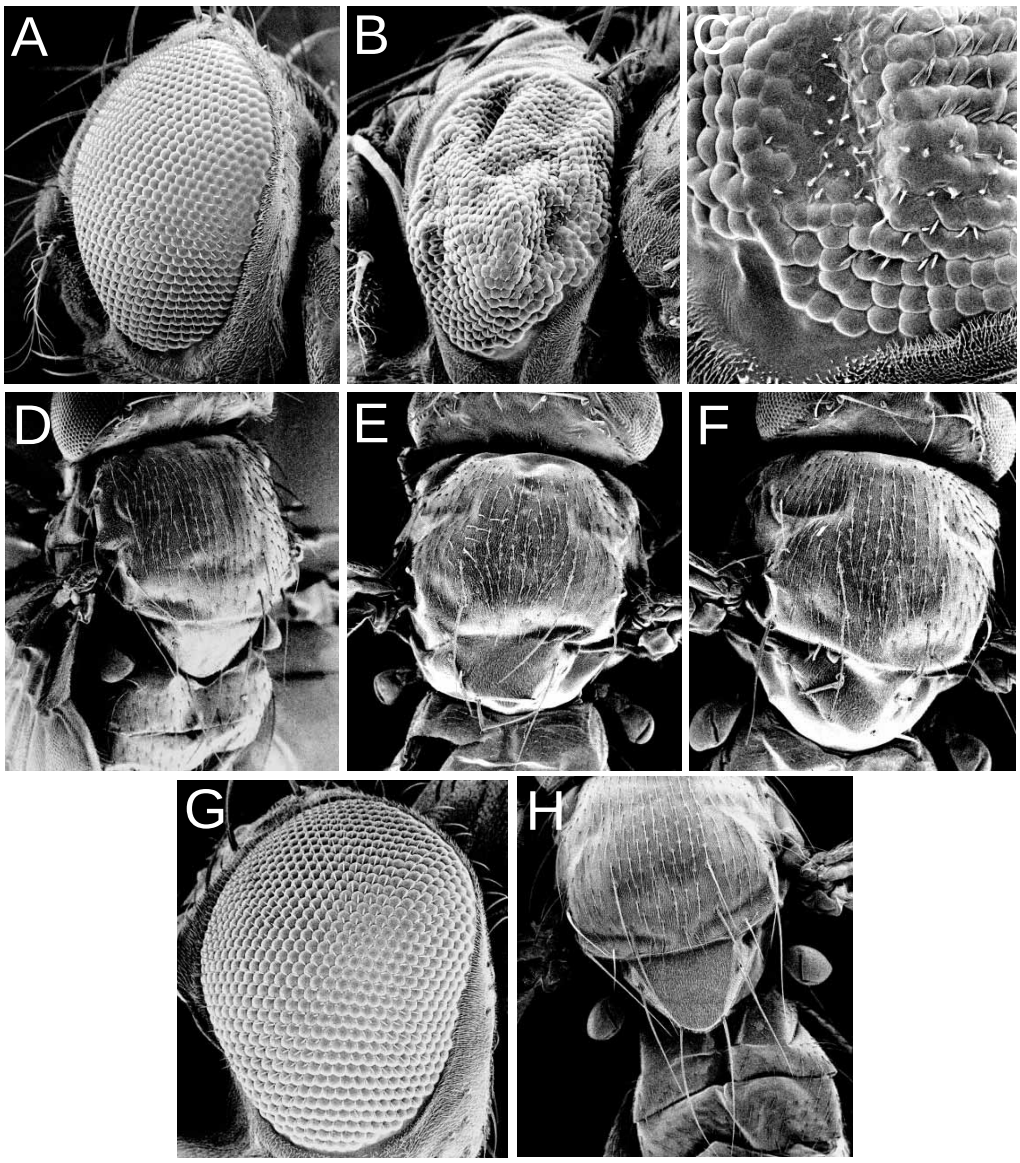

Fig. 1. Description of *furrowed* eye and bristle phenotypes. Scanning electron micrographs showing the adult compound eye and scutellum of various wild-type and mutant strains. (A) Oregon R. (B) fw^{U2}. (C) Close up of the ventral eye region of fw^{U2}. (D) Oregon R. (E) fw^{1}. (F) fw^{U2}. (G) Eye from a strain carrying the fw^{U2} allele and one copy of a P element transposon containing the 12.7 kb fragment encompassing the *fw* locus. (H) Scutellum from a fw^{U2} strain carrying the P element transposon containing the 12.7 kb DNA fragment of the *fw* locus.

establishment and maintenance of the cytoarchitecture of the eye. Additionally, the number of photoreceptors is abnormal, and frequently the spacing between ommatidia is disrupted, suggesting that the cone or pigment cells are also affected. The abnormal internal organization of the ommatidia correlates with altered external morphology and suggests that the Fw protein is involved in cell determination events. Additionally, the loss or altered spacing of the cone and pigment cells could affect the cytoarchitecture of the eye and may explain the furrowing of the retina and the missing or detached ommatidia observed in cross sections.

Effects of *furrowed* mutations on the development of the lamina

In addition to the defects seen in the retina, the lamina also shows defects in morphology. The lamina in wild-type adults has a smooth convex surface which is tightly associated with the basement membrane of the retina. In all *furrowed* mutants analyzed, the lamina has depressions and appears to be flattened in some regions (Fig. 2A,B). The depressions in the lamina are typically adjacent to penetrating furrows. Additionally, in the mutant the lamina appears to be separated a great distance from the base of the retina (Fig. 2B). This boundary, known as the basement membrane, which provides openings for photoreceptor axons to pass, is not clearly defined in the mutant. Since the basement membrane is made up of the feet of the cone and the pigment cells (Cagan and Ready, 1989), the *furrowed* mutations may affect the development of these epidermal cells. The irregular shape and depressions observed in the adult lamina of the *furrowed* mutants could be either a structural compression of the tissue in the lamina due to stresses exerted from the defective retina, or a loss of neural tissue in the lamina due to a disruption in its innervation by the photoreceptors from the developing retina. To distinguish between these two possibilities, the enhancer trap line A72, which has restricted expression of a reporter gene in the developing anlage of the lamina (Mozer and Benzer, 1994), was used to examine the development of the lamina in *furrowed* mutants. The adult lamina develops from precursor cells which are arranged in a broad crescent shape on each hemisphere of the larval brain. The development of the neurons in the lamina depends on inductive cues from the innervating photoreceptor axons from the developing eye imaginal disc (Selleck and Steller, 1991). The enhancer trap line A72 expresses β-galactosidase only in the laminal precursor cells that have been innervated by the neurons of the eye disc (Fig. 2E). In *furrowed* mutants, the expression is reduced in intensity and in the number of laminal precursor cells staining in the crescent (Fig. 2F). The reduction in staining seen in each mutant correlates well with the severity of eye defects seen in the mutant allele. The fw^{U2} mutant, which has very severe eye defects, has the greatest reduction in expression in both intensity and number of cells staining in the crescent area. The fw^{34e} mutant, which has very mild eye defects, has a slight reduction in expression (data not shown). Thus, *furrowed* mutations cause the loss of neural precursor cells in the developing lamina and the morphological defects observed in the adult lamina might be a consequence of this early loss, although the larval eye imaginal discs do not contain the furrows observed in the adult eye. These results suggest that *furrowed* mutations might disrupt the photoreceptor neurons or proper innervation of the lamina by these cells. Since the direction of photoreceptors from the eye disc to the larval brain

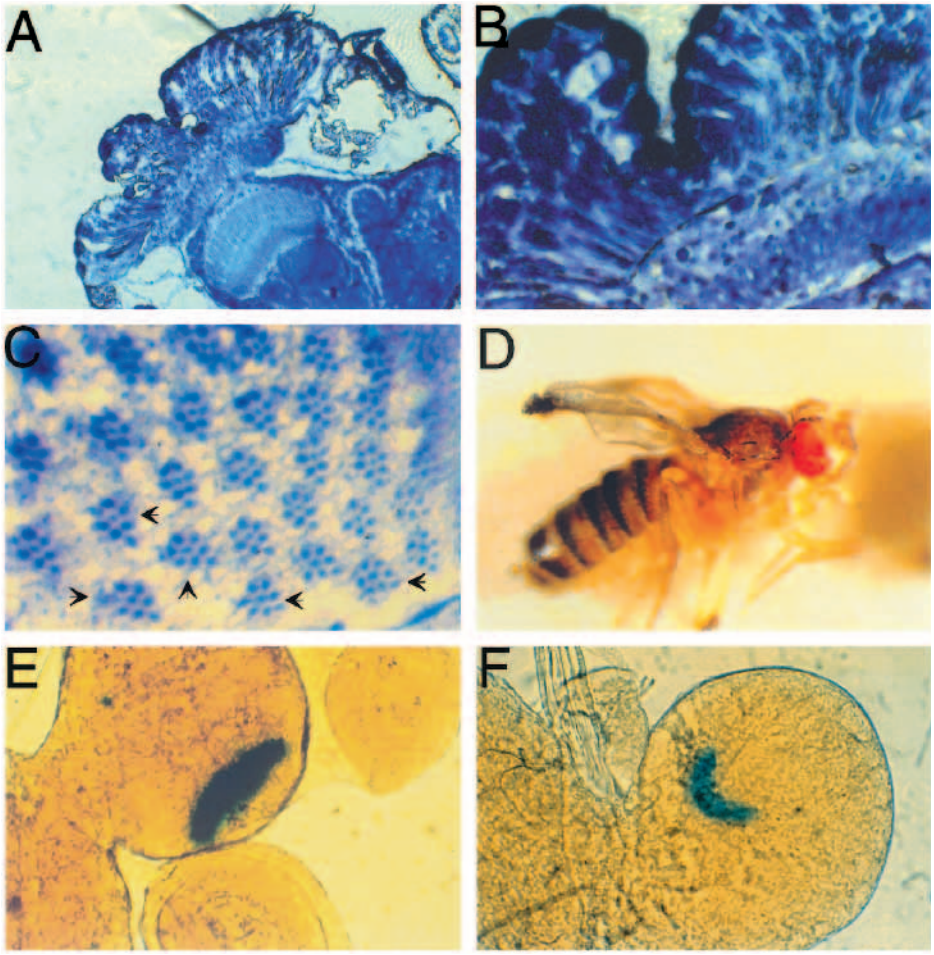

Fig. 2. Analysis of eye and wing defects in *furrowed* mutants. (A,B) Horizontal cross section of adult heads. The section in B shows that the furrow extends to the basement membrane and that the retina and the lamina are separated. (C) Transverse section of the eye showing extra numbers of photoreceptors. (D) Wing defects observed in $fw^{1}/Df(KA10)$ females. (E) Wild-type expression of the β-galactosidase reporter gene in the lamina precursor cells in the enhacer trap line A(72). (F) Brain of a larva carrying the f^{U2} allele shows dramatically reduced expression of the same enhancer trap line.

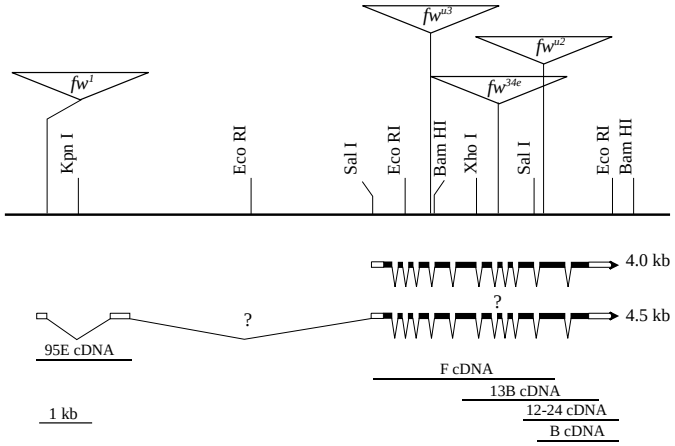

Fig. 3. Organization of the *furrowed* locus. The upper part of the figure indicates the insertion sites of transposable elements responsible for various *fw* alleles. The location of the fw^{1} insertion was determined by DNA sequence analysis, whereas the insertions responsible for the fw^{U2}, fw^{U3} and fw^{34e} alleles were mapped by Southern blots and their location is only approximate. The lower part of the figure indicates the intron/exon structure of the two *fw* transcripts. The question marks in the second intron and the 10th exon of the 4.5 kb transcript indicate that the precise structure of the intron/exon boundary in these regions has not been determined. The structure of various cDNAs analyzed in these studies is indicated in the lower part of the figure.

is dependent upon ommatidial patterning in the retina, the disorganization of the ommatidia in the mutant, i.e. loss of epidermal cells, could affect the routing of the photoreceptor axons to the optic lobes (Cagan and Ready, 1989; Meinertzhagen and Hanson, 1993; Wolf and Ready, 1993).

Mutations in the *furrowed* gene disrupt bristle development

Mutations in the *furrowed* gene also affect the development of mechanosensory bristles on the thorax, the scutellum, and the tissue surrounding the eye. In wild-type adults, the microchaetae on the thorax are in linear rows, whereas the macrochaete are interspersed at regular intervals as pairs on the thorax and scutellum (Fig. 1D). All *furrowed* mutants analyzed showed defects in morphology, orientation and patterning of bristles, some of them suggestive of defects in cell determination events.

The fw^{1} and fw^{34e} mutants show severe defects in the morphology of the macrochaetae, with the bristle shafts shortened, gnarled or bent (Fig. 1E,F). Often bristles have altered polarity or orientation such that the shaft does not point to the posterior as in wild type (Fig. 1E). The defects observed in the structure and orientation of the bristle shafts suggest that *furrowed* mutations disturb cell-cell interactions in the epidermis which are needed to establish proper cytoskeletal architecture, since both polarity and extrusion of the shaft are determined by the cytoskeleton (Gubb and Garcia-Bellido, 1982). Patterning of the bristles is also disturbed in *furrowed* mutants. The fw^{34e} allele often produces mispatterning of microchaetae with bristles irregularly spaced on the thorax, and large bald patches where bristles are missing (Fig. 1F). Additionally, *furrowed* mutants frequently show duplications of macrochaetae, both shaft and socket cells, on the scutellum and lower thorax. The duplications and the loss of bristles, as well as the patterning defects of bristles on the epidermis, suggest that the *furrowed* gene is involved in cell determination events within sensory organ precursor cells. Since the bristle shaft, an epidermal cell, has altered morphology and orientation, and it is often duplicated or lost along with the socket, *furrowed* mutations appear to affect both the differentiation and determination of epidermal cell fates within the mechanosensory bristle.

Molecular characterization of the *furrowed* gene

Analysis of deficiencies, inversions, and translocations of the X chromosome localized the *furrowed* gene to cytological position 11A4. *Df(1)RC29*, *Df(1)M13*, *Df(1)KA10*, and *Df(1)HF368* display a *furrowed* phenotype and have breakpoints within the 11A4 region; additionally, *In(1)GEM224* has a breakpoint in this region and uncovers the *furrowed* phenotype (Lindsley and Zimm, 1992). The region deleted in these deficiencies, or altered by the inversion, spans approximately 40 kilobases represented in four phage clones, 320, 348, K3, and C8, that were provided to us by K. Konrad, T. Goralski, and J. L. Marsh (Goralski, 1985). Southern analyses reveal that four spontaneously derived *furrowed* alleles fw^{1}, fw^{34e}, fw^{U2}, and fw^{U3}, show alterations within a 12 kb region contained in the K3 phage clone (data not shown). The restriction map in Fig. 3 shows the location and nature of the genomic alterations caused by the insertions of various transposable elements within a 12 kb region of genomic DNA. DNA sequence analysis indicates that the fw^{1} mutation is due to the insertion of the 412 retrotransposon at nucleotide 880 (see below for a description of the sequence of the *fw* locus).

Northern analyses reveal two transcripts expressed from the genomic region altered in *furrowed* mutants

DNA fragments from the 12 kb genomic region altered in *furrowed* mutants were used as probes in northern analyses to examine if any transcripts are encoded by this region. Northern analyses using the 4.5 kb *Eco*RI genomic fragment from the K3 phage clone as a probe, which is altered by DNA insertions in the fw^{34e}, fw^{U2} and fw^{U3} mutations, recognizes 4.5 and 4.0 kb transcripts. The expression of these two RNAs is altered in the fw^{1} and fw^{34e} mutants, supporting the idea that these two transcripts are encoded by the *furrowed* gene (Fig. 4A). Both the 4.5 kb and 4.0 kb transcripts are expressed in wild-type pupae, where they accumulate at similar levels, whereas only the 4.5 kb transcript can be detected in wild-type adults. In fw^{1} mutant pupae, the 4.0 kb transcript is expressed at relatively wild-type levels whereas the 4.5 kb transcript is drastically reduced in expression. In fw^{1} adults, the 4.5 kb transcript, which should be expressed at this stage, is not transcribed at detectable levels; the expression of the 4.0 kb transcript is seen as an aberrant smear, probably due to the insertion of the 412 retrotransposon within the first intron of the *fw* gene in this mutant allele. In fw^{34e} pupae, the 4.5 kb transcript is expressed at equal or slightly reduced level relative to wild-type, whereas the 4.0 kb transcript is not expressed at detectable levels. In the fw^{34e} adults, the 4.5 kb transcript is equal or slightly reduced in the level of expression relative to wild type. Expression of *furrowed*-encoded RNAs in the fw^{U2} and fw^{U3} mutations was not examined. The developmental expression of

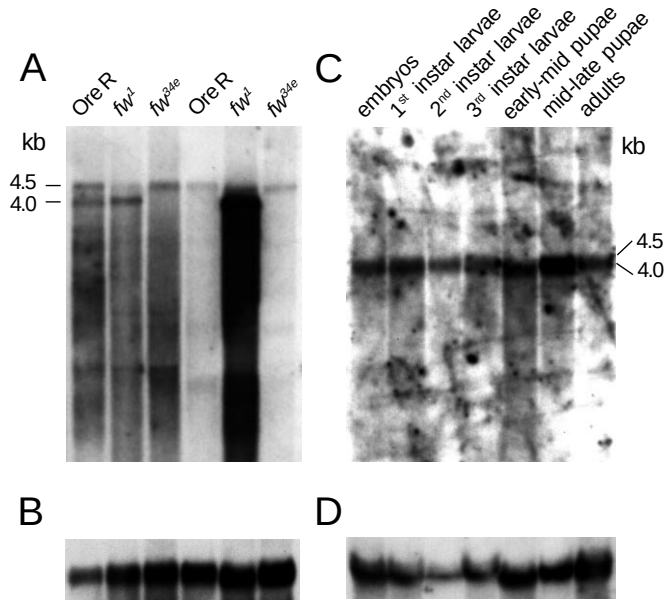

Fig. 4. Expression of *furrowed* RNAs in wild type and in mutants with various alleles. (A) 10 μg poly(A)$^+$ RNA from pupal and adult stages of Oregon R, *fw*^{1}, and *fw*^{34e} were electrophoresed in a 1.2% agarose-formaldehyde gel, transferred to a Nytran membrane and hybridized with the 4.5 kb *Eco*RI fragment shown in Fig. 3. (B) Same blot dehybridized and re-probed with the *Dras2* gene to measure the amount of RNA loaded on each lane (Mozer et al., 1985). (C) Expression of *furrowed* RNAs during various stages of *Drosophila* development. 15 μg of poly(A)$^+$ RNA were subjected to northern analysis as described in A. (D) The same filter was stripped and rehybridized with the *Dras2* gene.

the two RNAs was examined using the 4.5 kb *Eco*RI genomic fragment as a probe. Northern analysis reveals that the *furrowed* gene is expressed in all developmental stages (Fig. 4B).

Germ line transformation with a 12.7 kb genomic fragment rescues the *furrowed* mutant phenotype and defines the *furrowed* gene boundaries

To confirm that the region containing alterations, as observed by both Southern and northern analyses, encompasses the *furrowed* gene, a 12.7 kb DNA fragments from the region was injected into preblastoderm embryos to generate transgenic flies using P element-mediated germ line transformation. Four transgenic lines were generated and tested for the ability to rescue the eye and bristle defects in three *furrowed* mutant alleles, *fw*^{1}, *fw*^{34e}, and *fw*^{U2}. All defects in eye and bristle morphogenesis are restored to wild type in all three *furrowed* mutant backgrounds with one copy of the transgene (Fig. 1G,H). Thus, the 12.7 kb transgene is able to completely rescue all eye and bristle defects in the three mutant alleles tested, confirming that the *furrowed* gene is contained within the 12.7 kb of genomic DNA and encodes the 4.5 kb and 4.0 kb RNAs expressed throughout development.

Analysis of the DNA sequence of the *furrowed* gene and mRNAs

Sequence analysis of the genomic DNA and the corresponding cDNAs was initiated to determine the nature of the *furrowed* gene and its transcripts. The sequence of the 12.7 kb genomic DNA used in the transgenic construct that rescues the *furrowed* mutant phenotype was determined and has been deposited in GenBank with accession number U70770. Several cDNA clones (designated 95E, F, 13B, 12-24 and B; see Fig. 3) were isolated from various cDNA libraries using fragments of genomic DNA from phage K3 as hybridization probes, and sequenced. The organization of the exons and introns was determined by aligning the sequence of the cDNAs with the corresponding sequence in the genomic DNA (Fig. 3). Several of the cDNA clones overlap in sequence to give a composite cDNA of 3.8 kb in length. All cDNA clones making up the 3.8 kb composite mRNA recognize both the 4.5 and the 4.0 kb transcripts, indicating that the two transcripts are very similar and contain common exons. The sequence and translation of the composite mRNA is shown in Fig. 5. The genomic region proximal to the 5′ end of the composite cDNA contains promoter elements needed for the start of transcription including a CAAT box and TATA box, suggesting that the 3.8 kb composite transcript should represent the full length 4.0 kb mRNA. The difference in size between the 3.8 kb composite transcript and the estimated size of the 4.0 kb mRNA from northern analyses is probably due to poly-adenylation of the mRNA.

Northern analyses suggest that the 4.5 kb transcript differs from the 4.0 kb RNA by the presence of additional exons in the 5′ region. This conclusion is based on the fact that the *Kpn*I-*Sal*I fragment located on the left of the *fw* locus hybridizes only to the 4.5 kb transcript. The 4.5 kb transcript appears to have two unique exons at the 5′ end of the mRNA, represented in the 95E cDNA clone isolated from a random hexamer primed mid-late pupae cDNA library using the 3.2 kb *Kpn*I/*Eco*RI genomic fragment as a probe. The two exons represented in the 95E cDNA are the transcribed, untranslated region in the 4.5 kb mRNA. A transcription start site and promoter elements, the CCAAT box and TATA box, are present at bp 600 in the genomic sequence proximal to the 5′ end of the two exons, suggesting that the 4.5 kb transcript is expressed from an alternative promoter. Northern analyses indicate that no additional transcribed sequences are present in the region between the second exon of the 4.5 kb RNA and the first exon of the 4.0 kb transcript; in addition, sequences containing the first exon of the 4.0 kb transcript are also present in the 4.5 kb RNA. These results suggest that the 4.5 kb RNA has the structure represented in Fig. 3, although the exact pattern of splicing between exons 2 and 3 has not been determined; this uncertainty has been indicated by a question mark in the intron between exons 2 and 3 in the structure of the 4.5 kb transcript in Fig. 3.

Molecular characterization of the *fw*^{1} mutation, which affects the expression of the 4.5 kb transcript, shows that the 412 retrotransposon has inserted into an intron specific to the 4.5 kb transcript (Fig. 3). The 412 element has inserted at base pair 880 in the same transcriptional orientation as the *furrowed* gene. Since the *fw*^{1} mutation only affects the expression of the 4.5 kb transcript and not the expression of the 4.0 kb transcript (Fig. 4A), the insertion in the intron may affect the proper splicing or stability of the 4.5 kb mRNA. Southern analysis of the *fw*^{34e} allele indicates the presence of insertion sequences in the central region of the furrowed transcript (Fig. 3). Since this mutation affects the expression of the 4.0 kb transcript but does

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ATGTGGCCATTCAGCTGGTGGGTGTGGAGGGCAGCCTCAGTTTGTGCGAGGTGGAGACCTTCAGCCAACGACGAGTTCTCCGTGGATCGA 90
METTrpProPheSerTrpTrpValTrpArgAlaAlaSerValCysAlaArgTrpArgProSerAlaAsnAspGluPheSerValAspArg

TGCCTGAGTCCGAAAATCGGTGTGGACACTGTGGTAACGACATTCTCGAAAACCTGCTACGACTTTCATATCACAAAAGGAGAAAGCTTC 180
CysLeuSerProLysIleGlyValAspThrValValThrThrPheSerLysThrCysTyrAspPheHisIleThrLysGlyGluSerPhe

GACAAGGCGCAAGCCATTTGCAAGCAAACTGGTGGCGACTTGGTACACGACTTTCGTGGCGCCACCAGTTCGTACATCTTGTCCGAGCTG 270
AspLysAlaGlnAlaIleCysLysGlnThrGlyGlyAspLeuValHisAspPheArgGlyAlaThrSerSerTyrIleLeuSerGluLeu

GAACGCCGGAAGAGCGAACTGAAACCGCAGCTTGTGTGGATTGGCGCCCAAAAGGAGCCGGGCATTACATCGCGCACCTGGAAATGGGTG 360
GluArgArgLysSerGluLeuLysProGlnLeuValTrpIleGlyAlaGlnLysGluProGlyIleThrSerArgThrTrpLysTrpVal

AACGGCGATGTGGTTCAAAAGCCAACATGGGGCAAGGACCAGCCGAACAACTACAATGGCGAGCAGAATTGCGTGGTCCTCGATGGAGGT 450
AsnGlyAspValValGlnLysProThrTrpGlyLysAspGlnProAsnAsnTyrAsnGlyGluGlnAsnCysValValLeuAspGlyGly

CGCAATTGGCTGTGGAATGATGTGGGCTGCAATCTGGACTATTTGCACTTCATCTGCCAGCACTCGCCATTGTCTTGCGGCTCGCCGGAT 540
ArgAsnTrpLeuTrpAsnAspValGlyCysAsnLeuAspTyrLeuHisPheIleCysGlnHisSerProLeuSerCysGlySerProAsp

GCCCAGCAAAACACCACTGTCATGGGCAAAAAGTTCACTCTGGGCGAGAAGATTCAGTACACCTGTCCCAAGGGCCATTCGCTGCTCGGT 630
AlaGlnGlnAsnThrThrValMETGlyLysLysPheThrLeuGlyGluLysIleGlnTyrThrCysProLysGlyHisSerLeuLeuGly

CAGACGGAACGGGAGTGTAGACTTGATGGCACTTGGAGTGGCTCCTCGCCGACCTGCAAATATGTGGACTGCGGCAGTCTGCCGGAGCTG 720
GlnThrGluArgGluCysArgLeuAspGlyThrTrpSerGlySerSerProThrCysLysTyrValAspCysGlySerLeuProGluLeu

AAGTTTGGATCCATCCACATGTCGGAGGAGCGGACTAGCTTCGGCGTGGTGGCCACATACAGTTGCCACGAGAACTACACGCTAATCGGG 810
LysPheGlySerIleHisMETSerGluGluArgThrSerPheGlyValValAlaThrTyrSerCysHisGluAsnTyrThrLeuIleGly

AATGAGAATCGCACGTGCGCCATGGACGGCTGGAGCGGCAAGCAGCCGGAATGCCTGGTGGACTGGTGTCCAGATCCGCAGCCCATAGCC 900
AsnGluAsnArgThrCysAlaMETAspGlyTrpSerGlyLysGlnProGluCysLeuValAspTrpCysProAspProGlnProIleAla

GGTGGCGATGTGCGATTCAATGACAAGCGTGCCGGTTCCACGGCCACGTATTTTTGTGAGCCGGGCTATGTGCTAGTTGGCGAGGCGATC 990
GlyGlyAspValArgPheAsnAspLysArgAlaGlySerThrAlaThrTyrPheCysGluProGlyTyrValLeuValGlyGluAlaIle

ATCTCGTCGCGGTTTGGGCGGCGAGTGGTCCAGCAAGACGCCCTCGTGCAGATTCGTCGATTGTGGTGCACCGGCACGACCAAATCGTGGC 1080
IleSerCysGlyLeuGlyGlyGluTrpSerSerLysThrProSerCysArgPheValAspCysGlyAlaProAlaArgProAsnArgGly

ATTGCCATCCTGCTGAACGGCACCACCACCGTCAATTCGGTGGTTAAATACGAGTGCGACGAGGATCATTGGCTGGACGGGCAGTCGGAG 1170
IleAlaIleLeuLeuAsnGlyThrThrThrValAsnSerValValLysTyrGluCysAspGluAspHisTrpLeuAspGlyGlnSerGlu

CTCTATTGCACCCGGGAAGGGAAGTGGTCGGGTGAGGCGCCCGTCTGTGAACTGGTCACCTGCGAGACGCCATCGGTGCCATCCGGTTCG 1260
LeuTyrCysThrArgGluGlyLysTrpSerGlyGluAlaProValCysGluLeuValThrCysGluThrProSerValProSerGlySer

TTTGTCATTGGCTACGATTACAATGTCCATTCGAAGATCCAGTACAACTGTGATCCCGGTCACATTATGGACGGGACTCCGGTGCTCGAG 1350
PheValIleGlyTyrAspTyrAsnValHisSerLysIleGlnTyrAsnCysAspProGlyHisIleMETAspGlyThrProValLeuGlu

TGCCTGGACTCCGGGGAATGGAGTGCCGATGCGCCCTACTGCGAATATATCGACTGCGGCACAATCCTGCCCATTCCCTATGGCAGCCAC 1440
CysLeuAspSerGlyGluTrpSerAlaAspAlaProTyrCysGluTyrIleAspCysGlyThrIleLeuProIleProTyrGlySerHis

AAGTACGTGACGAATACCACCTATGTGGGCTCCGAAGTGGGCTTCAGCTGCTCCCAATCGCACAAACTGAGCGGAGTCCTCAAGCGCACG 1530
LysTyrValThrAsnThrThrTyrValGlySerGluValGlyPheSerCysSerGlnSerHisLysLeuSerGlyValLeuLysArgThr

TGCCTGGAATCGGCTGTCTGGAGCGATGCGTCGGCCAAGTGCGAGGAGATTCGCTGTCCGGAACCGAAACTGCCTGCGCACAGTCTGCTC 1620
CysLeuGluSerAlaValTrpSerAspAlaSerAlaLysCysGluGluIleArgCysProGluProLysLeuProAlaHisSerLeuLeu

TCCGTCACCGGAAATGATCGCATGTATGGAAGGACACTTATCCGCACCTCCGAATCCTCGCAGAACACAGCTCAAACCTATAGGATTGGA 1710
SerValThrGlyAsnAspArgMETTyrGlyArgThrLeuIleArgThrSerGluSerSerGlnAsnThrAlaGlnThrTyrArgIleGly

GCACTGGCCAAGTACCGATGCGAACGGGGCTACAAGATGGTGGGCGAGGCACTGGCCACCTGCACGGATAGTGGACAATGGAGCGGCACC 1800
AlaLeuAlaLysTyrArgCysGluArgGlyTyrLysMETValGlyGluAlaLeuAlaThrCysThrAspSerGlyGlnTrpSerGlyThr

ATTCCGGAATGTGTGTATGTGGAGTGCGGTGCTCCGGAGGGCATCAACAATGGCAAGGTGGTCCTGGCCACAAATGCCACGTACTACGGC 1890
IleProGluCysValTyrValGluCysGlyAlaProGluGlyIleAsnAsnGlyLysValValLeuAlaThrAsnAlaThrTyrTyrGly

GCCGCTGTCCTCTATGAGTGCAATGTGAACTTCAAGCTAAACGGAGTATCCCGACGCCTGTGCACGGAGCACGGCAACTGGAGCCACGAG 1980
AlaAlaValLeuTyrGluCysAsnValAsnPheLysLeuAsnGlyValSerArgArgLeuCysThrGluHisGlyAsnTrpSerHisGlu

GCACCGGAATGCGTGGAGGTGGTCTGCGATACGCCCAACATCAATGAGAATCTGATCGTGGAGGCGGGTCCGCGTGCTGTGGGCTCCGTG 2070
AlaProGluCysValGluValValCysAspThrProAsnIleAsnGluAsnLeuIleValGluAlaGlyProArgAlaValGlySerVal

GCCACCTTCAAGTGCGCCAAGGGCAGGATCATGATGGGAAACGATACGCGTGTGTGTCAGAAAAATGGCAAATGGACTGGCAAGAGTCCC 2160
AlaThrPheLysCysAlaLysGlyArgIleMETMETGlyAsnAspThrArgValCysGlnLysAsnGlyLysTrpThrGlyLysSerPro

ACCTGCAGACCCGTCGACTGTGGTCGTCCTCTGGCCATCGAGAATGGTCGCGTGATTGTGGTCAACGATTCCACGTTGTACGGCGGATCG 2250
ThrCysArgProValAspCysGlyArgProLeuAlaIleGluAsnGlyArgValIleValValAsnAspSerThrLeuTyrGlyGlySer

GCGGAGTATCACTGCATACCGAACTACAATCGGATTGGCCAGTATTTGCGCAAGTGCACCGAGGATGGAGCCTGGAGCGGCAAGCAGCCG 2340
AlaGluTyrHisCysIleProAsnTyrAsnArgIleGlyGlnTyrLeuArgLysCysThrGluAspGlyAlaTrpSerGlyLysGlnPro

CGCTGCGAATTGGCCACGGCGGAGGGTCAGGAGACTTCGGAGCTGGGCACGGGCGTTGGCATTGGTGCCACCGTGATCGTCGCCCTGCTG 2430
ArgCysGluLeuAlaThrAlaGluGlyGlnGluThrSerGluLeuGlyThrGlyValGlyIleGlyAlaThrValIleValAlaLeuLeu

GTGATCTTTGGTCTGATCTTCCTATATCGCAACAAGGCGAGACCCGCCAAAAATACGGAGAATGTGCAGGCGGCGGAGACGAAGGACGAA 2520
ValIlePheGlyLeuIlePheLeuTyrArgAsnLysAlaArgProAlaLysAsnThrGluAsnValGlnAlaAlaGluThrLysAspGlu

CGCAATGCCGCCGTAATGTCCTACTCGACGCTGGAGGCCAACAATCGGATGCACATGGACAACAATCCGTCGGCGACGTTCAACACGTTC 2610
ArgAsnAlaAlaValMETSerTyrSerThrLeuGluAlaAsnAsnArgMETHisMETAspAsnAsnProSerAlaThrPheAsnThrPhe

CATGGCGGAGCCGGCGGAAGGAGCAACGGCGGCGGTAATCCGGATGCAAACAGCAATGGTGAAAGACTGAACAACAATCGGTCGGAAAAC 2700
HisGlyGlyAlaGlyGlyArgSerAsnGlyGlyGlyAsnProAspAlaAsnSerAsnGlyGluArgLeuAsnAsnAsnArgSerGluAsn

ATTTACGATCAAATACCAAACGAGCAATTCTACGACGCTCCCTATGAGATGCGCACCAATGATGAGGTTTACGAACCGGAACCGGTGGCC 2790
IleTyrAspGlnIleProAsnGluGlnPheTyrAspAlaProTyrGluMETArgThrAsnAspGluValTyrGluProGluProValAla

AGTAATGTCATCACCATCAATGGATTTCCGTCAGATAGAGATCGATATCTATATCGATATCGATATCTATATCAATACAACTTTGTGTTG 2880
SerAsnValIleThrIleAsnGlyPheProSerAspArgAspArgTyrLeuTyrArgTyrArgTyrLeuTyrGlnTyrAsnPheValLeu

ATATCGAAATCCAAAGGGATGGCAGAATTAATTGACAAACGTTAA                                              2925
IleSerLysSerLysGlyMETAlaGluLeuIleAspLysArg---
```

Fig. 5. Structure of the *furrowed* 4.0 kb RNA and its encoded protein. Residues of the lectin domain located in the amino-terminal region of the protein are boxed. The 10 complement-like repeats are located immediately adjacent to each other and are indicated by alternate thin and thick lines to differentiate between adjacent repeats. The transmembrane domain in the carboxy-terminal region of the protein is indicated by bold letters.

not affect the 4.5 kb mRNA (Fig. 4A), this result suggests that some of the exons present in the 4.0 kb RNA might not be present in the 4.5 kb transcript. If the site of insertion of the sequences responsible for the *fw*^{34e} allele corresponds to intron sequences in the 4.5 kb RNA, these sequences might be spliced out, thus explaining the normal levels of this RNA in mutants with the *fw*^{34e} allele. Northern analysis using probes containing various pairs of exons between exons 4 and 12 recognize both transcripts (data not shown); however, the high degree of similarity between these exons (see below) might result in recognition of both transcripts even if one of them lacks some of the exons. All cDNAs isolated that contain the central region of the *furrowed* gene show the same exon structure, suggesting that both transcripts might be identical in this region. Therefore, northern and sequence analysis support the conclusion that the two *furrowed* RNAs are transcribed from different promoters, differ in the 5′ transcribed untranslated region and encode very similar or identical proteins.

Analysis of the putative Fw protein sequence reveals homology to the selectin family of cell adhesion receptors

Comparison of the deduced amino acid sequence from the 4.0 kb transcript with protein sequence databases reveals sequence homologies to a number of interesting domains found in several proteins (Fig. 5). The amino terminus, a region homologous to the C-type lectin domain, comprises 122 amino acids encoded by the first three exons. C-type lectin domains are calcium dependent, carbohydrate recognition domains found in many carbohydrate binding proteins (Drickamer, 1988). Northern analysis shows that the three exons encoding the lectin domain are also present in the 4.5 kb transcript. The alignment of the putative Fw lectin domain with the C-type lectin consensus sequence and with the lectin domains of several carbohydrate binding proteins is shown in Fig. 6A. Most of the conserved amino acids, including those necessary for carbohydrate and calcium binding, are conserved, such that the overall identity of the putative Fw lectin domain to the consensus sequence is 70%; this variation in homology is very similar to that observed for other functional lectin domains. The putative Fw lectin, like the lectin domain in selectins, is missing one of the two calcium binding sites (Springer, 1990).

A second domain identified in the Fw protein is homologous to the complement binding repeats, which are units of 50-70 amino acids found in many proteins that function in the complement immune response of mammals (Klickstein et al., 1987; Fearon and Ahearn, 1989). The putative Fw protein has ten complement binding repeats encoded by exons 4 through 11 (Fig. 5). Each repeat comprises 58 or 60 amino acids, except the seventh repeat which has 86 residues (Fig. 6B). The ten repeats have all invariant cysteines conserved, and most of the other highly conserved residues found in the mammalian consensus sequence for complement binding repeats. Interestingly, most complement binding repeats are encoded within

A

```
                                        *                                   *
Consensus       -CY-YS-----W--A----C-----HL--------I-S-EEQ-YL-H---------WIGI-D-----G
                  F F                                    FV

furrowed        TCYDFHITKGESFDKAQAICKQTGGDLVHDFRGATILSELERRKSELKPQLV----WIGAQKEP---G  64
mLHRC           WTYHYSEKPMNWENARKF-CKQNYTDLVA------IQNKREIEYLENTLPKSPYYYWIGIRKI----G  54
ELAM-1          WSYNTSTEAMTYDEASAY-CQQRYTHLVA------IQNKEEIEYLNSILSYSPSYYWIGIRKVNNV--  54
raMBP           KFFVTNHERMPFSKVKAL-CSE-LRGTVA------IPKNAEENKAIQEVAKTSA--FLGITDEVTE-G  174
raMBPC          KYFMSSVRRMPLNRAKAL-CSELQ-GTVAT------PRNAEENRAIQNVAKDVA--FLGITDQRTENV  179
barnacle        WASTY---QVRWNDAQLA-CQTVHPGALAT-----IQSQLENAFISETVSNNRL--WIGLNDIDLE-G  69
Sacrophaga      EYLIETELKYNWHQAWHE-CARHDQQLVTI-ESADKNNAIIDLVKRVVGKSHNL--WLGGNDEYSSRG  64

                                    *    *        *            * *  *        *
Consensus             W-WV-GT---------NW----P-N----EDCV----------G-WND--C--------CE

furrowed        ITSRTWKWVNGDVVQKPT----WGKDQPNNYNGEQNCVLDGGRN---WLWNDVGCNLDYLHFICQ    123
mLHRC           KM---WTWV-GTNKTLTKEAENWGAGEPNNKKSKEDCVEIYIKRERDSGKWNDDACHKRKAA-LCY  118
ELAM-1          -----WVWV-GTQKPLTEEAKNWAPGEPMMRQKDEDCVEIYIKREKDVGMWNDDACSKKKLA-LCY  118
raMBP           Q----FMYV--TGGRLT--YSNWKKDEPNDHGSGEDCVTI-----VDNGLWNDISCQASHTA-VC-  229
raMBPC          -----FEDLTGNRVR----YTNWNEGEPNNVGSGENCVVL-----LTNGKWNDVPCSDSFLV-VCE  235
barnacle        H----YVWSNGEATDF----TYWSSNNPNNWEN-QDCGVVNYD--TVTGQWDDDDCNKNKNF-LCK  128
Sacrophaga      RPFF-FFWS-PTGQAFS--FAYWSENNPDNYKHQEHCVHIW--DTKPLYQWNDNDCNVKMGY-IC-  123

B

C--P-----G----------Y-------Y-C--G--L-G-----C---G-WS---P-C-VV
                    F       F                          I

CGSPDAQQNTTVMGKKFTLGEKIQ----YTCPKGHSLLGQTERECRLDGTWSGSSPTCKYVD
CGSLPELKFGSIHMSEERTSFGVVAT--YSCHENYTLIGNENRTCAMDG-WSGKQPEC-LVD
CPDPQPIAGGDVRFNDKRAGSTAT----YFCEPGYVLVGEAIISCGLGGEWSSKTPSCRFVD
CGAPARPNRGIAILLNGTTTVNSVVK--YECDEDHWLDGQSELYCTREGKWSGEAPVCELVT
CETPSVPS-GSFVIGYD---YNVHSKIQYNCDPGHIMDGTPVLECLDSGEWSADAPYCEYID
CGTILPIPYGSHKYVTNTT-YVGSEVG-FSCSQSHKLSGVLKRTCLESAVWSDASAKCEEIR
CPEPKLPAHSLLSVTGNDRMYGRTLN21-YRCERGYKMVGEALATCTDSGQWSGTIPECVYVE
CGAPEGINNGKVVLATNATYYGAAVL--YECNVNFKLNGVSRRLCTEHGNWSHEAPECVEVV
CDTPNINENLIVEAGPRAVGSVAT----FKCAKGRIMMGNDTRVCQKNGKWTGKSPTCRPVD
CGRPLAIENGRVIVVNDSTLYGGSAE--YHCIPNYNRIGQYLRKCTEDGAWSGKQPRCELAT
```

Fig. 6. Similarities between the Fw protein and homologous proteins in other organisms. (A) Alignment of the putative Fw lectin domain with the consensus sequence (Drickamer and McCreary, 1987) and with other lectin domains. mLHRc is the lectin domain of mouse L-selectin (Siegelman and Weissman, 1989). ELAM-1 is the lectin domain of human E-selectin (Bevilacqua et al., 1989), raMBP is the lectin domain of the rat mannose binding protein (Drickamer and McCreary, 1987), raMBPC is the lectin domain of rat mannose binding protein C (Drickamer et al., 1986), barnacle indicates acorn barnacle lectin domain, and *Sacrophaga* is the lectin domain from the flesh fly. The asterisk (*) denotes the invariant residues in the consensus sequence. The first calcium binding site is missing in the putative Fw lectin domain since residue 61 is K instead of D, although the G at residue 64 is conserved. The second calcium binding site is present at residues 86 (D) and 89(N). The carbohydrate recognition sequence of the Fw protein is QP*N at residues 87-89. (B) Alignment of the ten putative Fw complement binding repeats with the consensus sequence for complement binding proteins in mammals. The consensus sequence for repeats found in complement binding proteins is presented in the first line (Klicktenstein et al., 1987). The ten repeats of Fw are aligned with the consensus sequence. All the invariant cysteines are conserved in each of the ten repeats, as well as most of the other amino acids.

one exon with the exception of the third and seventh repeats which are encoded by two exons (Fig. 5). The Fw complement binding repeats show strong homology to those of the mammalian selectin family of cell adhesion receptors (30-40%), with the strongest homology to the human P-selectin (Johnston et al., 1990). This degree of homology is similar to that seen between selectin family members in mice and humans. The tenth complement binding repeat is followed by a putative transmembrane domain (Fig. 5). This domain comprises 24 highly hydrophobic residues and is followed by 130 amino acids at the carboxy terminus. This cytoplasmic tail does not have homology to any protein domain in the databank of protein sequences.

The organization of the Fw protein, with an amino-terminal lectin domain followed by ten complement binding repeats and a transmembrane domain, is very similar to that found in the selectin family of cell adhesion receptors. The Fw protein is most similar to P-selectin, which has nine complement repeats. However, Fw lacks the EGF-like domain found in all other selectin family members; instead, the EGF domain is replaced by an additional complement binding repeat. The structural similarity and the high degree of homology to selectins at the amino acid level suggests that the *furrowed* gene product is a member of the selectin cell adhesion family. The *furrowed* mutant phenotype suggests that the putative Fw selectin protein may function as a cell adhesion receptor during the development of the adult compound eye and mechanosensory bristles. It is therefore of interest to examine the expression of *furrowed* in larval tissues, such as the eye and wing imaginal discs, that give rise to the adult peripheral nervous system.

Analysis of *furrowed* expression in larvae

A single stranded RNA anti-sense probe that recognizes both the 4.5 kb and the 4.0 kb transcripts in northern analyses was used for RNA in situ hybridization to examine the expression pattern of the *furrowed* gene in the imaginal discs of third instar larvae. The single stranded sense control does not result in any staining (data not shown). *furrowed* is expressed in all imaginal discs in first, second and third instar larvae. Fig. 7A,C,E shows the expression of *furrowed* RNAs in the eye, wing, leg, and haltere imaginal discs, respectively, of third instar larvae. In the eye disc, the transcripts are present in all cells but are more abundant in the cells of the dorsal and ventral margins (Fig. 7A). In the wing disc, *furrowed* is expressed more abundantly in the cells of the prospective dorsal and ventral wing surfaces, the ventral pleura, and the notum (Fig. 7C). It is expressed in all cells of the leg, the haltere (Fig. 7E), the antennal, the labial, the clypeo-labral, the humeral, and the genital discs. *furrowed* is also expressed in portions of the digestive system, the gastric caecae, and the proventriculus, and is expressed in low levels in the larval brain (data not shown). Although the *furrowed* gene has broad expression in the imaginal cells, the gene is not expressed in other larval tissue such as the salivary glands, fat bodies, the Malphighian tubules, or the cuticle (data not shown).

In order to investigate the distribution of the Fw protein, polyclonal antibodies were generated in rabbits. A fusion protein was designed to contain six of the ten complement binding repeats. This protein corresponds to the probe used in the *in situ* hybridization, but does not contain the putative transmembrane and cytoplasmic tail domains. The polyclonal antibodies generated against this protein should recognize the product(s) of both transcripts. The rabbit anti-serum recognizes a protein of the predicted 110 kDa size for the Fw protein in wild-type flies on western blots (data not shown). Control

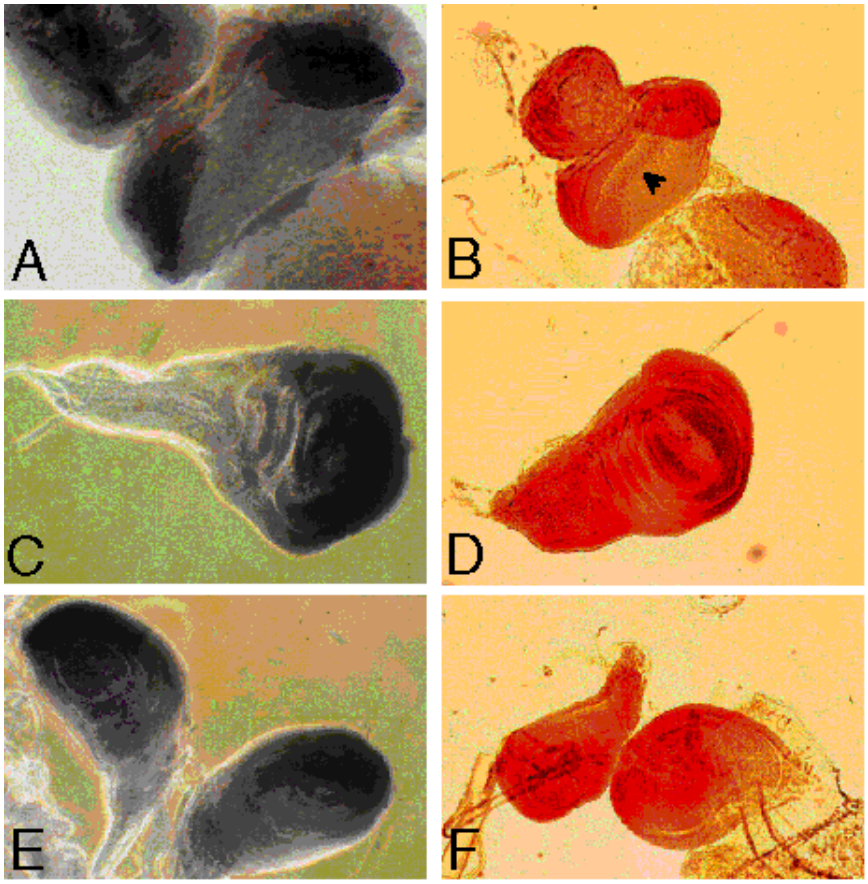

Fig. 7. Expression of *furrowed* RNA and protein in larvae. The imaginal discs of wild-type (Oregon R) third instar larvae are shown. The imaginal discs in A (eye disc), C (wing disc), and E (leg and haltere) were hybridized in situ with a digoxigenin-labeled single-stranded antisense probe to *furrowed* mRNA represented in the 13B cDNA clone, and show the wild-type expression of the two *furrowed* RNAs. The imaginal discs in B (eye disc and larval brain), D (wing disc), and F (leg and haltere) were incubated with polyclonal antisera against the *furrowed* protein, and shows the wild-type distribution of Fw protein. *furrowed* has broad expression in all imaginal discs, but appears to be more abundant in the dorsal and ventral regions of the eye and wing discs. Additionally, the protein appears to be abundant in the morphogenetic furrow of the eye disc (marked by an arrow).

experiments with the pre-immune serum and the secondary antibodies alone (no primary antibody) do not recognize the 110 kDa Fw protein in western analysis and do not show staining in any larval tissues (data not shown). Therefore, the staining seen with the anti-serum generated against the Fw product is specific for the this protein. The anti-serum was then used to examine the cellular distribution of the Fw protein in larvae. The Fw product is localized in all imaginal discs of first, second, and third instar larvae. Fig. 7B,D,F shows Fw protein localization in the eye, wing, leg, and haltere discs of third instar larvae. The Fw protein is localized in all cells of the imaginal discs in agreement with the distribution of *fw* transcripts. In the eye discs, the protein is more abundant in the cells of the dorsal and ventral margins, and in the morphogenetic furrow (Fig. 7B). In the wing imaginal discs, the Fw protein is more abundant in the cells of the prospective dorsal and ventral wing surfaces, the ventral pleura, and the notum (Fig. 7D). The Fw protein is localized in all cells of the leg and haltere discs (Fig. 7F), as well as the antennal (Fig. 7B), the labial, the clypeo-labral, the humeral, and the genital discs. The Fw protein also localizes to the brain and to portions of the digestive system, the proventriculus, and the gastric caecae. Thus, immunolocalization reveals that the Fw protein distribution in the larval imaginal discs is identical to the distribution of the transcripts. The broad distribution of the Fw protein in many cells of all imaginal discs is reminiscent of the protein expression seen for the integrin cell adhesion receptor family, especially the β integrin encoded by *l(1)myospheroid* (Brower et al., 1985) and for Notch (Kidd et al., 1989; Johansen et al., 1989), suggesting that the putative Fw protein could be functioning as a cell adhesion receptor during the development of the fly.

DISCUSSION

Molecular analyses of the *furrowed* gene in *D. melanogaster* have shown that the gene is contained in 12.7 kb of genomic DNA, and encodes two transcripts which are expressed in most developmental stages. The two RNAs encode a putative transmembrane protein that has strong homology to the selectin family of cell adhesion receptors previously identified only in vertebrates. The transcripts and the putative selectin protein have a broad distribution in all imaginal discs, which is similar to the expression pattern seen for other cell adhesion molecules such as the integrins and Notch. Analysis of spontaneous mutations in the *furrowed* gene shows that changes in *furrowed* transcription result in defects in the morphology and patterning of the ommatidia and mechanosensory bristles, as well as defects in cell determination in both structures manifested by the duplications and/or loss of epidermal cells in these sensory organs.

Most of the cell adhesion receptor families such as the integrins, super-immunoglobulins, and cadherins, that function in concert with selectins during cell adhesion events in vertebrates have also been identified in *Drosophila* and found to be involve in many developmental processes. The finding of a *Drosophila* selectin is the first demonstration that these proteins function outside the immune system of mammals. Additionally, the discovery of a selectin family member in *Drosophila* provides a model system for future genetic analysis and dissection of the role of the various structural domains in the selectin protein during cell adhesion. Selectins have been shown to mediate cell adhesion in vertebrates through protein-carbohydrate interactions. The lectin domain mediates these interactions by binding a carbohydrate ligand, typically a complex carbohydrate structure, on the surface of other cells. The putative lectin domain in the *Drosophila* selectin has very strong homology to other functional lectin domains. Interestingly, the putative Fw lectin domain is most similar to the lectin domain of selectins, since both domains have only one of the two calcium binding sites. However, the carbohydrate recognition region of the putative Fw lectin domain (QPN) is a hybrid between the recognition sequence found in mannose-binding proteins (EPN) which is specific for mannose, and the recognition sequence found in other lectin domains which are specific for galactose (QPD) (M. Quesenberry and Y. C. Lee, personal communication). The carbohydrate recognition sequence of the putative Fw lectin domain suggests that this protein may recognize both galactose and mannose, increasing the number of potential carbohydrate ligands and making a variety of highly glycosylated proteins potential candidates that could interact with Fw.

Since protein-carbohydrate recognition is less specific than protein-protein recognition, it is possible that the other domains of selectins provide specificity to the ligand recognition by binding protein determinants. The complement binding repeats in the selectins of vertebrates have an important role during cell adhesion since deletion of the repeats result in the loss of adherence (Watson et al., 1991). The identification of the putative Fw selectin in *Drosophila* now provides a model genetic system to determine the role of the complement binding repeats in cell adhesion. The broad expression of the *furrowed* product, and the broad carbohydrate specificity hinted at by the recognition sequence suggest that the complement binding repeats may have a role in determining ligand specificity. The putative Fw selectin protein in *Drosophila*, however, lacks an EGF repeat. Perhaps the presence of the EGF domain in vertebrates suggests that the selectin cell adhesion family evolved the additional domain for specific recognition or function in the immune system. Kansas et al. (1993) have proposed that the cytoplasmic domain regulates cell adhesion via interaction with the cytoskeleton. The nature of the phenotype in *furrowed* mutants suggests that the cytoplasmic domain of the Fw protein may interact with the cytoskeleton or associated proteins, since the cytoskeletal architecture has been proposed to play a role in determining the polarity of the epidermis and extrusion of the bristle shaft (Gubb and Garcia-Bellido, 1982).

Based on the phenotype, gene expression, and genetic interactions, we can speculate on how the putative selectin protein functions during development of the fly. The mutant phenotype in the adult peripheral nervous system indicates that the putative selectin protein has two functions during bristle and eye development. The defects in patterning and in cell determination of the ommatidia and bristles in the *furrowed* mutants indicate that the putative Fw selectin is directly or indirectly involved in neural cell determination. The defects in morphology of the compound eye and bristles, as well as defects in bristle polarity, indicate that the putative Fw selectin might mediate the cell-cell adhesion interactions necessary for proper morphogenesis of these structures. This could take place by

directly providing mechanical cell-cell adherence and/or directing cell-cell communication via interactions with the cytoskeleton or associated proteins. This function is also supported by the defects in wing morphogenesis observed in *furrowed* mutants, suggesting that the putative Fw selectin might function as a cell adhesion receptor during *Drosophila* development. The selectin encoded by the *furrowed* gene has a complex role in establishing proper morphology and cell fates within the developing adult peripheral nervous system. Further characterization of the *furrowed* gene will help in understanding the role of this protein during the development of the fly.

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