Two FGF-receptor homologues of Drosophila: one is expressed in mesodermal primordium in early embryos

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SUMMARY

The fibroblast growth factor (FGF)/receptor system is thought to mediate various developmental events in vertebrates. We examined molecular structures and expression of DFR1 and DFR2, two Drosophila genes closely related to vertebrate FGF-receptor genes. DFR1 and DFR2 proteins contain two and five immunoglobulin-like domains, respectively, in the extracellular region, and a split tyrosine kinase domain in the intracellular region. In early embryos, DFR1 RNA expression, requiring both twist and snail proteins, is specific to mesodermal primordium and invaginated mesodermal cells. At later stages, putative muscle precursor cells and cells in the central nervous system (CNS) express DFR1. DFR2 expression occurs in endodermal precursor cells, CNS midline cells and certain ectodermal cells such as those of trachea and salivary duct. FGF-receptor homologues in Drosophila would thus appear essential for generation of mesodermal and endodermal layers, invaginations of various types of cells, and CNS formation.

Key words: fibroblast growth factor, Drosophila melanogaster, tyrosine kinase, immunoglobulin superfamily, mesoderm, central nervous system

INTRODUCTION

Fibroblast growth factors (FGFs) constitute a growth factor family of at least seven members (Burgess and Maciag, 1989). In mammals, FGFs are not only capable of inducing cell proliferation and chemotaxis (Burgess and Maciag, 1989), but also are potent inducers of angiogenesis (formation of new blood vessel; Cross and Dexter, 1991). Response to FGFs is mediated by high-affinity cell-surface receptors (FGF-Rs) through activation of intracellular tyrosine kinase (Lee et al., 1989). FGF-Rs are widely distributed in various embryonic cells in mammals so that mammalian embryogenesis may require pleiotropic functions of FGFs (Wanaka et al., 1991).

The FGF/FGF-R signaling system has also been shown quite likely essential for mesoderm formation in Xenopus (Jessell and Melton, 1992; Whitman and Melton, 1992). Most, if not all, FGFs can mimic the mesoderm-inducing activity of vegetal pole cells in the early blastula and, hence, may possibly be natural inducers of mesoderm formation (Slack et al., 1987; Kimelman et al., 1988). An injection of mRNA encoding a dominant-negative form of FGF-R into embryos has been demonstrated to cause significant deformation in mesodermal structures of tadpoles (Amaya et al., 1991).

Molecular analysis of FGF-Rs in chicken, humans and Xenopus has indicated that FGF-Rs constitute a family of receptor-tyrosine kinases (Lee et al., 1989; Ruta et al., 1989; Seno et al., 1991; Friesel et al., 1991). Each FGF-R molecule has more than two extracellular immunoglobulin-like domains (Ig-domains) as is also the case for receptors of the platelet-derived growth factor (PDGF) and colony stimulating factor-1 (CSF-1; Williams, 1988). However, unlike receptors for PDGF and CSF-1, receptors for FGFs possess in common additional characteristics such as a relatively long juxtamembrane region, kinase catalytic sequences split by a short stretch of amino acids and a short carboxyl terminal tail.

Understanding the physiological roles of the FGF/FGF-R signaling system may be facilitated by clarification of the roles of homologous genes in Drosophila, in which both genetic and developmental analyses are applicable. In a previous experiment (Shishido et al., 1991), we searched for genomic DNA fragments of Drosophila, encoding novel tyrosine kinases, by polymerase chain reaction (PCR). Two of the seven DNA fragments isolated (dtk1 and dtk2) were found partly to encode polypeptides highly homologous in amino acid sequence to vertebrate FGF-Rs. Using a different approach, Glazer and Shilo (1991) independently identified a Drosophila FGF-R homologue (DFGF-R). Unlike our dtk1 and dtk2 products, the D-FGFR protein appeared to have a long substitution at or near subdomain VIII, a tyrosine kinase subdomain conserved in all tyrosine kinases so far examined (Hanks et al., 1988).

For clarification of the above and further extension of our analysis, complete nucleotide sequences of the coding regions of dtk1 and dtk2 genes were determined and their expression patterns in embryos were examined. dtk1 and
dk2, respectively, were found to be *Drosophila* FGF-R homologues having two and five Ig-domains. *dkl* expression was specific to mesoderm in early embryos, while *dk2*, seemingly corresponding to *DFGF-R*, was expressed in endodermal precursors.

**MATERIALS AND METHODS**

**Fly strains**

Canton-S (wild type) was obtained from Y. Hotta (University of Tokyo). Fly strains with twist and snail were obtained from C. Nüsslein-Volhard (Max Plank Institute, Tübingen). Strain *Df(1)260-1* was obtained from UMEÅ Stock Center. Strain *sr16/TM1* (De La Pompa et al., 1989) and a *Dmyd-lacZ* transformant line, 14.1(II) (Paterson et al., 1991) were obtained from A. Ferrus (Instituto Cajal, Madrid) and W. J. Gehring (University of Basel), respectively.

**Molecular analyses**

Genomic DNA and cDNA clones were isolated from our genomic DNA and pupal cDNA libraries, respectively (Kojima et al., 1991). Nucleotide sequences were determined as described previously (Emori et al., 1985). All other procedures for molecular analyses were essentially as described by Sambrook et al. (1989).

**In situ hybridization to whole embryos**

Fixation and pretreatment of embryos were performed as described by Tautz and Pfeifle (1989). RNA probes were prepared as follows. cDNA inserts in cFR4-6 (*DFR1*), cFR5-1 (*DFR2*) and PCR-amplified twist genomic DNA (Thissie et al., 1988) were cloned into Bluescript plasmid vectors (Stratagene). Purified plasmid DNA was linearized by a suitable restriction enzyme and used as a template. Antisense RNA was prepared by in vitro transcription using digoxigenin-11-UTP (Boehringer Mannheim) and T7 or T3 RNA polymerase. The reaction was carried out according to the manufacturer’s standard protocol. After fragmentation into an average size of 150 nucleotides by incubation at 60°C in a buffer containing 40 mM NaHCO₃, 60 mM Na₂CO₃, and 50 mM DTT, RNA was used as a probe for hybridization.

Hybridization was carried out essentially as described by Yokouchi et al. (1991). Embryos were incubated overnight at 50°C in a solution containing 20 mM Tris-HCl (pH 8.0), 2.5 mM EDTA (pH 8.0), 1× Denhardt’s solution (0.02% BSA, 0.02% Ficoll, 0.02% polyvinylpyrrolidone), 300 mM NaCl, 1 mg/ml *Escherichia coli* tRNA, 50% formamide, 10% dextran sulfate with a truncated poly(A) tail. The analysis of genomic sequences indicated the in-frame termination codon to be located at nucleotide positions 56 to 54 and no additional in-frame initiation codon to be present within the region from −1 to −53. Regions with the first and second ATG codons (nucleotide positions, 61-67 and 106-112, respectively) matched the *Drosophila* consensus sequence for the translational initiation (Cavener, 1987). However, only the second ATG codon may possibly be functional, since, as in the case of vertebrate FGF-R genes, it is directly followed by a nucleotide sequence coding for a hydrophobic stretch of 22 amino acids capable of serving as a signal peptide. The expected cleavage site of the putative signal peptide may be between Ala-19 and Val-20 (von Heijne, 1983). The second hydrophobic stretch was detected in the middle of the ORF (amino acid positions, 295-318), presumably representing the transmembrane domain. Thus, the extracellular and intracellular domains of the *DFR1* protein were assigned to amino acid residues 20-294 and 319-714, respectively.

**The crosses**

To examine *DFR1* expression in *A5-C*− embryos, crosses were made with *Df(1)260-1/+* females derived from *Df(1)260-1/FM4* and wild-type flies, and wild-type males. Consistent aberrations observed in one quarter of the embryos were noted as mutant phenotypes. P(*Dmyd-lacZ*)/+; *sr16/+* flies were obtained by crossing flies homozygous for the P insertion (on the second chromosome) and *sr16/TM1* flies. P(*Dmyd-lacZ*)/+; *sr16/+* flies were crossed to each other and the resultant embryos (progeny) were stained with anti-β-galactosidase antibody as described (Higashijima et al., 1992). Consistent aberrations observed in one quarter of the *lacZ*-expressing embryos were noted as mutant phenotypes.

**RESULTS**

**Molecular cloning of two *Drosophila* FGF-R gene homologues**

Using *dkl* and *dk2* fragments as probes, genomic DNA clones were isolated. cDNA clones were isolated using suitable genomic DNA probes. Since, as described below, polypeptides encoded by cDNA inserts are very similar in sequence and organization to vertebrate FGF-Rs, *dkl* and *dk2*, respectively, were renamed *DFR1* and *DFR2* (*Drosophila* FGF-receptor homologues 1 and 2). In situ hybridization to polytene chromosomes showed *DFR1* and *DFR2* to be mapped at 90D-E and 70C, respectively (data not shown).

**DFR1 encodes a *Drosophila* FGF-receptor having two Ig-domains**

The complete nucleotide sequence of the cDNA insert in cFR4-6 and its relevant genomic regions were determined (Fig. 1B). As schematically shown in Fig. 1A, the *DFR1* gene included no intron. The cFR4-6 insert contains a single long open reading frame (ORF) of 2251 bp, followed by 523 bp of the 3′ non-coding sequence with a truncated poly(A) tail. The analysis of genomic sequences indicated the in-frame termination codon to be located at nucleotide positions −56 to −54 and no additional in-frame initiation codon to be present within the region from −1 to −53. Regions with the first and second ATG codons (nucleotide positions, 61-67 and 106-112, respectively) matched the *Drosophila* consensus sequence for the translational initiation (Cavener, 1987). However, only the second ATG codon may possibly be functional, since, as in the case of vertebrate FGF-R genes, it is directly followed by a nucleotide sequence coding for a hydrophobic stretch of 22 amino acids capable of serving as a signal peptide. The expected cleavage site of the putative signal peptide may be between Ala-19 and Val-20 (von Heijne, 1983). The second hydrophobic stretch was detected in the middle of the ORF (amino acid positions, 295-318), presumably representing the transmembrane domain. Thus, the extracellular and intracellular domains of the *DFR1* protein were assigned to amino acid residues 20-294 and 319-714, respectively.

As in the case of vertebrate FGF-Rs (Lee et al., 1989), the intracellular region contains a relatively long juxtamembrane domain (77 amino acids), split tyrosine kinase catalytic sequence (278 amino acids) and a short carboxyl terminal tail (41 amino acids). All 11 tyrosine kinase subdomains (I to XI; Hanks et al., 1988) were found to be conserved. The *DFR1* kinase insert (19 amino acids) is slightly
Two Drosophila FGF-R homologues longer than counterparts in vertebrate FGF-Rs (14 amino acids). Except for the kinase insert, the overall sequence homology of the kinase domain between DFR1 and vertebrate FGF-Rs was estimated as about 60%. Homologies with other types of vertebrate tyrosine kinases were approximately 40%. The carboxyl terminal tail of DFR1 includes a tripeptide sequence, Tyr-Leu-Asp, which may provide the tyrosine phosphorylation site supposedly required for binding of SH2 of phosphoinositide-specific phospholipaseC-γ (Mohammadi et al., 1991).

Fig. 1. (A) Restriction map of the DFR1 genomic region and organization of the DFR1 transcription unit. The DFR1 exon is boxed. The hatched region represents a possible coding region. The solid bar shows the region used as a probe for cDNA screening. B. BanHI; E, EcoRI; H, HindIII; S, SalI. (B) Nucleotide sequence of the DFR1 gene and amino acid sequence of its putative translation product. Lower-case letters shows the amino acid sequence between the first and second methionines. The thick underline shows the putative signal peptide. Eleven potential N-glycosylation sites (N-X-S/T) are shaded. Conserved Cys residues of Ig-domain are circled. The acidic region is enclosed by an open box. The shaded box indicates the predicted transmembrane region. The tyrosine kinase catalytic sequence is enclosed by a polygon. The kinase insertion sequence is shown by a thin underline. Conserved Tyr residue in the C-terminal tail is indicated by a triangle. The putative polyadenylation signal is shown by a wavy line and truncated poly(A), by a dotted line. The region corresponding to the dtk1 sequence is labeled by a broken underline.
The extracellular domains of FGF-Rs are, in general, characterized by a series of Ig-domains, each containing two Cys residues, possibly connected by a disulfide bond, in an interval of several scores of residues capable of constituting β-sheet structures (Williams and Barclay, 1988). The consensus sequence for the Ig-domains is Vx(I/L)xC(8x-12x)W(20x-50x)DxGxYxC (x=arbitrary amino acid). As shown in Figs 2 and 3, the DFR1 protein contains two Ig-domains. (Most vertebrate FGF-Rs have two or three Ig-domains (Lee et al., 1989; Ruta et al., 1989; Seno et al., 1991).) As in the case of vertebrate FGF-Rs, DFR1 extracellular domains contain a short stretch of acidic amino acids (Fig. 3). Locations of two potential N-glycosylation sites were found conserved between DFR1 and the flg, one of human FGF-Rs (Ruta et al., 1989).

Using a human FGF-R cDNA (Seno et al., 1991) as a probe, Southern blot analysis of the total genomic DNA of Drosophila was carried out. DFR1-derived fragments were found to be hybridized in all lanes under a less stringent condition (data not shown). From the above, the DFR1 gene would appear quite likely to be a counterpart of vertebrate FGF-Rs in Drosophila.

DFR2 is another type of FGF-R gene homologue encoding a polypeptide with five Ig-domains

We next determined the nucleotide sequences of the cDNA insert in cFR5-1 and its relevant genomic regions. As schematically shown in Fig. 4A, the DFR2 gene had an intron 85 bp long. The DFR2 cDNA contained a single long ORF followed by 33 bp non-coding sequence. Since neither possible signal peptide sequence nor probable translational initiation codon was found to be coded for by the cDNA, we sought to determine further 5′ sequences using a DFR2 genomic clone. Fig. 4B shows the composite nucleotide sequence thus obtained, which contains a 3159 bp long ORF following an in-frame termination codon of nucleotide positions, 121-123.

19 amino acids following the first methionine (amino acid positions, 2-20) may serve as a signal peptide, being highly hydrophobic. The second hydrophobic stretch, including 25 amino acids (amino acid positions, 601-625) may represent the transmembrane region, since it is followed by a putative tyrosine kinase sequence as described below.

As noted for DFR1, the intracellular region of DFR2 contained a split tyrosine kinase catalytic domain (Figs 3, 4B), highly homologous to those of vertebrate FGF-Rs. Homology between DFR1 and DFR2 kinase domains was 79%. The Tyr-Leu-Asp sequence was found near the carboxyl terminus of DFR2. The DFR2 extracellular region included five Ig-domains, of which two were found in the region flanked by the acidic domain and transmembrane domain (Figs 2, 3). Three and four of nine N-glycosylation sites of DFR2 were conserved in both DFR1 and flg, respectively (Fig. 3).

DFR2 is virtually identical in nucleotide sequence to DFGF-R, whose sequence has been reported by Glazer and Shilo (1991). However, their original DFGF-R sequence appears to include some defects in two regions. One base deletion in an C stretch of DFGF-R (nucleotide positions, 865-868 in Fig. 4B) disrupted the coding region for the extracellular domain. Nucleotide changes in the region from 2850 to 2892 (see Fig. 4B) results in loss of the invariant glutamic acid of subdomain VIII of DFGF-R.
Fig. 4. (A) Restriction map of the DFR2 genomic region and organization of the DFR2 transcription unit. (B) Nucleotide sequence of the DFR2 gene and amino acid sequence of its putative translation product. The arrowhead indicates the location of an 85 bp intron. All symbols and abbreviations are the same as in Fig. 1.
Specific expression of *DFR1* in prospective mesoderm during early embryogenesis

The spatial and temporal expression of *DFR1* RNA was examined by in situ hybridization of whole embryos. The expression of *DFR1* RNA was first detected at the cellular blastoderm stage (stage 5; Campos-Ortega and Hartenstein, 1985), about 150 minutes following egg fertilization, just before the onset of gastrulation. The ventral surface of the embryo, 16-17 cells wide and from 10 to 85% egg length, was hybridized with the *DFR1* probe (Fig. 5A,B,D). This region of *DFR1*-expressing cells corresponds to mesodermal primordium, which eventually participates in the ventral furrow formation. Note that at stages 6-7 (about 3 hours after egg fertilization), all *DFR1*-expressing cells invaginated to form mesoderm (Fig. 5C,E). Fig. 5F shows the staining pattern of an embryo at stage 8 (3 hours 30 minutes; stage for germ-band extension). The anterior part of the *DFR1*-positive region appears to be separate from the main body in forming the cephalic mesoderm. This is most probably due to invagination of midgut primordium (see an arrowhead labeled am in Fig. 5F). At stage 9 (about 4 hours), the *DFR1*-positive region began to segment (Fig. 5G). These findings indicate the expression of *DFR1* to be mesoderm-specific at least in early embryos.

**DFR1** expression in somatic mesoderm and other tissues

The invaginated mesodermal cells mainly differentiate into somatic and visceral muscles (Campos-Ortega and Hartenstein, 1985). *DFR1* expression in somatic mesoderm is described in the following.

*DFR1* expression dramatically changes at stage 11 (6-7 hours), when germ-band fully extends and intersegmental furrows become much more discernible. As shown in Fig. 6A, the overall expression of *DFR1* in the mesoderm is virtually extinct and, instead, a small number of cells strongly expressing *DFR1* emerge in the ventrolateral region of each segment. Since such *DFR1*-positive cells were found in all of 50 embryos derived from *As-C+/+* parents, the *achaete-scute* gene products may not be required for their presence. Thus, these *DFR1*-positive cells are suggested not to be neural cells. During germ-band retraction (stage 12), isolated *DFR1*-positive cells increase in number and scatter in the entire region of the somatic mesoderm (Fig. 6B).

At late stage12 (about 8 hours), three lows of *DFR1*-positive cells are distinguishable in bodywall (Fig. 6C). These cells appear to be precursor cells for ventral (v), pleural (p) or dorsal (d) groups of muscles. When the germ-band has completely retracted, cell fusions to produce the muscle pattern finish (Bate, 1991). As expected, at early stage 13 (9.5 hours), vs 2-4 (muscles 26,27,25; ventral group; Crossley, 1978) and pet 1-3 (muscles 21-23; pleural group) were clearly *DFR1*-positive (Fig. 6D). Thus, the majority of *DFR1*-positive cells appear to eventually differentiate into muscle.

*DFR1* expression also occurred in tissues other than somatic mesoderm. In the middle of stage 12, mid-ventral, *DFR1*-positive cells were detected (Fig. 6E). They may be ventral nerve cord cells, since their presence requires the *achaete-scute* gene products (Fig. 6F). *DFR1* expression in CNS persists at least until stage 16 (13 hours; Fig. 6H). Cells surrounding the hindgut and foregut were noted to be *DFR1*-positive at stage 12-13 (Fig. 6G).
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DFR2 expression in invaginating endodermal and ectodermal cells

The expression of DFR2 RNA is first observed in primordia of anterior midgut and posterior midgut in the beginning of germ-band extension (stages 6-7, about 3 hours; Fig. 7A). This expression becomes much more prominent at stage 8 (about 3.5 hours), when anterior and posterior midguts invaginate as shown in Fig. 7B. Other expression patterns of DFR2 RNA are essentially identical to those described by Glazer and Shilo in the case of DFGF-R (1991). At stages 8, DFR2-positive regions 1-2 cells wide become discernible (Fig. 7C). They appear to correspond to CNS midline cells and/or their precursors. At stage 10 (about 5 hours), cells contributing to tracheal pit formation begin to express DFR2 RNA (Fig. 7D). At stage 13, DFR2-positive signal can also be observed in cells corresponding to the presumptive salivary duct and salivary tubes (data not shown). These observations suggest that DFR2 is involved in the formation of the endodermal layer (midgut), and development of mesectoderm and tubular organs derived from ectoderm.

DFR1 expression in gastrulation-defective mutants, twist and snail

Embryos of null mutations in twist or snail fail to form a normal ventral furrow; and cannot form mesodermal cell layer (Nüsslein-Volhard et al., 1984; St. Johnston and Nüsslein-Volhard, 1992; Leptin, 1991). Examination was made of the distribution of DFR1 RNA in twist− and snail− embryos. A quarter of progeny derived from either twist+/ or snail+/ parents was found negative to the DFR1 probe (255 and 179 embryos were examined, respectively; Fig. 8A,B,D). The morphology of DFR1-negative embryos was abnormal, showing twist− or snail− phenotypes (Fig. 8B,D). Thus, concomitant expression of twist and snail gene products may be required for mesoderm-specific DFR1 expression in early embryos. At later stages, a small number of DFR1-positive cells were detected in the ventral region.
of twist<sup>-</sup> and snail<sup>-</sup> embryos (Fig. 8C,E). From their positions and size, these cells appeared to be neuronal cells.

**DFR1 may be required not for gastrulation but for the formation of somatic mesoderm**

During in situ hybridization experiments, we noticed that the DFR1 gene is located within a short deletion of sr16 at the 90D-E region (De La Pompa et al., 1989; Bellen et al., 1992). This deletion was estimated to be 70-300 kb in length; it is known to include at least two genes couch potato (cpo) and stripe (sr). cpo is apparently unrelated to DFR1 (Bellen et al., 1992). We first examined the gastrulation of 3-5 hour embryos derived from sr16/+ parents, by following twist RNA expression in presumptive mesodermal cells (Thisse et al., 1988). No abnormal invagination was observed in more than 100 embryos at least until stage 8. Germ-band-extending embryos from the same parents were also hybridized with a mixture of DFR1 and DFR2 probes. DFR1<sup>-</sup>-DFR2<sup>+</sup> embryos should correspond to those homozygous for sr16. As shown in Fig. 9A, sr16/sr16 embryos were essentially normal in DFR2 expression in midline cells. In contrast, aberrant DFR2-expression was observed in twist<sup>-</sup> embryos defective in gastrulation (Fig. 9B). Although we do not know possible morphological changes in 5- to 7-hour-old DFR1-defective embryos, it should thus be possible for normal gastrulation to occur without the DFR1 gene product.

To examine the muscle formation in sr16 embryos, we utilized P[Dmyd-lacZ], by which Dmyd expression can be

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**Fig. 7.** DFR2 expression in wild-type embryos. Whole embryos were hybridized with DFR2 probe. (A,B,D) Lateral views; (C) a ventral view. (A) Stages 6-7 embryo. am, anterior midgut; pm, posterior midgut. (B,C) Stage 8 embryos. Note that DFR2 is expressed in ventral midline (vl). (D) Stage 11 embryo. Tracheal pits (tp) are labeled with DFR2 probe.

**Fig. 8.** DFR1 expression pattern in gastrulation-defective mutant embryos. Embryos were hybridized with the DFR1 probe. (A,B,D) Lateral views; (C,E) ventral views. (A) Wild-type embryo at stage 8 (3.5 hour). (B) Germ-band-extending twist<sup>ID96</sup> embryo. (C) Germ-band-retracting twist<sup>ID96</sup> embryo. (D) Germ-band-extending snail<sup>hid</sup> embryo. (E) Germ-band-retracting snail<sup>hid</sup> embryo. Note that, in twist<sup>-</sup> or snail<sup>-</sup> mutant embryos, DFR1 is not expressed at early stages (B,D), but a small number of DFR1-expressing, presumably neural cells can be seen in ventral region at later stages (C, E).
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Drosophila FGF-R homologues detected as lacZ gene expression (Paterson et al., 1991). Dmyd, also called nautilus, is a Drosophila homologue of the vertebrate MyoD-family genes, specifically expressed in a subset of muscle precursors (Michelson et al., 1990; Paterson et al., 1991). The effect of sr16 on Dmyd expression was examined. At stages 12-13, the number of Dmyd-expressing cells was found extensively reduced, particularly, in the dorsal region (Fig. 9C-F). At later stages, reduction of muscle fiber content was evident under a microscope with Nomarski optics (data not shown). These results show DFR1 possibly to be required for the formation or extension of muscle precursor cells.

DISCUSSION

The Drosophila genome includes two homologues of mammalian FGF-R genes, DFR1 and DFR2. Their expression dynamically changes during embryogenesis. At earlier stages, DFR1 is expressed in mesodermal primordium, and DFR2 in the primordia of the anterior and posterior midguts. At later stages, DFR1 RNA is expressed in developing tissues derived from mesoderm and neuroectoderm, including muscle precursors and CNS. DFR2 RNA appears to be expressed in those tissues derived from ectoderm and mesectoderm, including the trachea, salivary tubes and CNS.

Structural and evolutional relationship between Drosophila and vertebrate FGF-Rs

A comparison of the amino acid sequences of kinase domains indicated DFR1 to have about 80% and 60% homology with DFR2 and any member of vertebrate FGF-Rs, respectively. DFR1 and DFR2 genes may thus possibly be derivatives of a common ancestral arthropod gene, diverging from an ancient vertebrate FGF-R gene at the time of the major phylogenetic branch leading to arthropods and chordates.

Extracellular regions of Drosophila and vertebrate FGF-Rs are much more diversified than kinase regions. DFR1 and DFR2 have two and five Ig-domains, respectively. (Most members of the vertebrate FGF-R family contain three Ig-domains, while the remaining have two.) DFR1 and DFR2 may recognize different ligands. Our sequence analysis showed the extracellular region flanked by the acidic region and transmembrane domain to be considerably conserved in both sequence and organization. In particular, some N-glycosylation sites were found invariant in position among DFR1, DFR2 and flg protein. (see Fig. 3). This region may possibly be essential for common functions of the FGF-R family members.

Possible roles of DFR1 in mesoderm formation and subsequent differentiation of mesodermal cells

FGFs secreted by non-mesodermal cells may induce mesoderm formation in vertebrates (Jessell and Melton, 1992; Whitman and Melton, 1992) but, so far, nothing has been reported as to the possible involvement of FGF or FGF-like factors in mesoderm formation in Drosophila. Only the nuclear concentration of the dorsal protein is considered important in determining mesodermal fate in Drosophila (Roth et al., 1989; Thiss et al., 1991). Thus, our finding of specific expression of DFR1 in mesodermal cells is of particular importance. Recent molecular-cloning experiments show that other mesodermal genes, which include twist, snail and MyoD, are also shared in common by Drosophila and vertebrates (St. Johnston and Nüsslein-Vol-
hard, 1992; Michelson et al., 1990; Paterson et al., 1991) and, thus, it is reasonable to consider that Drosophila and vertebrates share in common some essential mechanisms underlying mesoderm formation and/or subsequent differentiation of mesodermal cells.

Our results suggest that DFR1 expression is not necessarily mandatory for ventral furrow formation. In its place, DFR1 would rather appear required for patterning of muscle precursor cells (somatic mesoderm), since DFR1 RNA is predominantly expressed in embryonic muscle precursor cells and its absence results in extensive reduction in the number of Dmyd/nau-expressing muscle precursor cells, particularly, in the dorsal region (see Fig. 9D,F). During the early stages of muscle formation, some muscle precursor cells migrate from the ventral to lateral or dorsal region so as to produce the muscle pattern (Dohrman et al., 1991). Although there is no direct evidence to date, muscle patterning is supposed to be specified by the underlying ectoderm (Bate, 1991). The above findings suggest the possible involvement of DFR1 (and maybe its ligand) in cell migration during muscle formation.

The stripe gene included in the sr16 deletion is required for the normal development of adult flight muscles and CNS (De La Pompa et al., 1989). Although, at present, the relation between stripe and DFR1 at the molecular level is unknown, similarity in expression (mutant phenotype) and chromosomal locus suggest that they are genes intimately related, if not identical, to each other.

### Possible involvement of FGF-receptors in CNS formation

In Drosophila, various members of an immunoglobulin superfamily are expressed in developing nervous system; they include fasciclin, neuroglian, receptor-type phosphatases and a Drosophila homologue of mammalian trk (Grenningloh et al., 1990; Yang et al., 1991; Tian et al., 1991; Pulido et al., 1992). Some are homologous in amino acid sequence to vertebrate neural cell adhesion molecules and proteins with Ig-domains may possibly essential for neural development. As seen in Fig. 7C, DFR2 RNA is expressed in most midline cells, which probably play a key role in the formation of axon commissures. DFR1 RNA expression may occur along a pair of longitudinal connectives (see Fig. 6H). Since neurotrophins and FGFs are possibly required for neural cell differentiation in vertebrates (Jessel and Melton, 1992), the above findings imply important functions of DFR1 and DFR2 proteins in CNS formation in Drosophila.

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