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Distinct expression of two *Drosophila* homologs of fibroblast growth factor receptors in imaginal discs

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The expression of two *Drosophila* homologs of FGF receptors (*DRF1* and *DRF2*) in imaginal discs was studied. *DFR1* mRNA was observed in several imaginal discs, whereas *DFR2* mRNA was not detected. *DFR1* expression in the wing and leg discs took place in probable myoblasts in a pattern similar to that of *twist*, a mesodermal gene. The mRNA was also detected in the morphogenetic furrow and its posterior region of the eye disc and around the proliferation center of the brain. These results suggest that *DFR1* is involved in the development of mesodermal and neuronal cells constituting the adult body.

Fibroblast growth factor; Fibroblast growth factor receptor; *Drosophila melanogaster*; Imaginal disc; In situ hybridization

1. INTRODUCTION

Mammalian fibroblast growth factors (FGFs) constitute a family consisting of at least seven members. They are involved in the growth, differentiation, and maintenance of various cells and tissues of mesodermal and ectodermal origin including endothelial cells and neuronal cells (reviewed in [1]). The signals for FGFs are transferred into the cytosol via their specific receptors termed FGF-Rs; these are transmembrane proteins having intracellular tyrosine kinase domains (reviewed in [2]).

Four vertebrate genes have been identified that code for FGF-Rs [2,3], and these are possibly translated into more than ten distinct molecules by alternative splicing [4–6]. Studies on the binding activities of FGF-Rs to FGFs have revealed a complex cross-talking network; for example, FGF-R1 binds equally to acidic and basic FGFs, but FGF-R4 binds preferentially to acidic FGF [7]. Moreover, two FGF-R molecules synthesized from a single FGF-R2 gene by alternative splicing show distinct binding specificities for FGFs [6]. Consequently, although an increasing amount of evidence suggests the importance of FGFs and FGF-Rs in mesodermal and neuronal differentiation, the individual mechanisms for the signalling pathways of each FGFs and FGF-Rs are difficult to define because of the complexity of their molecular natures.

Thus, it would appear to be important to investigate FGFs and FGF-Rs in simpler organisms. In this connection, we recently identified two FGF-R homologs in the *Drosophila melanogaster* genome (*DFR1* and *DFR2*), and showed that *DFR1* is expressed in the mesodermal primordium during early embryonic stages, and in the CNS during late embryonic stages [8]. On the other hand, *DFR2* is expressed in midline precursor cells during early embryonic stages and in tracheal cells from early to late embryonic stages [8,9]. Among various observations presented on *Drosophila* FGF-Rs, *DFR1* expression in the mesodermal primordium is most interesting, because it is apparently related to such observations in vertebrates as that a dominant negative form of FGF-R inhibits normal differentiation of *Xenopus* mesodermal tissues [10].

Here, we present the expression profiles of these two *Drosophila* FGF-R homologs in imaginal discs, and show that *DFR1* mRNA is expressed in probable pre-sursors of muscle cells in the wing and leg discs. In addition, *DFR1* mRNA is detected in the eye disc and brain, suggesting the importance of *DFR1* in neural development.

2. MATERIALS AND METHODS

Whole mount in situ hybridization was carried out essentially as described [8,11]. A digoxygenin-labelled antisense RNA probe for *DFR1* was synthesized by in vitro transcription using T3 RNA polymerase, and fragmented into 100–200 bases. *Drosophila* imaginal discs were dissected from late 3rd instar larvae of the wild type strain Canton-S. The dissected discs were fixed in 4% paraformaldehyde in phosphate-buffered saline containing 0.1% Tween 20 (PBS-Tween) for 5 min at room temperature. The fixed discs were dehydrated in methanol, re-fixed and treated with 75 μg/ml of proteinase K in PBS-Tween for 5 min at room temperature. The proteinase-treated discs were processed to hybridization by the method described previously [8]. Positive hybridization signals were visualized with anti-digoxygenin antibody conjugated with alkaline phosphatase, using NBT and BCIP as substrates. The stained discs were mounted and observed under an Axiophoto optic microscope (Zeiss).

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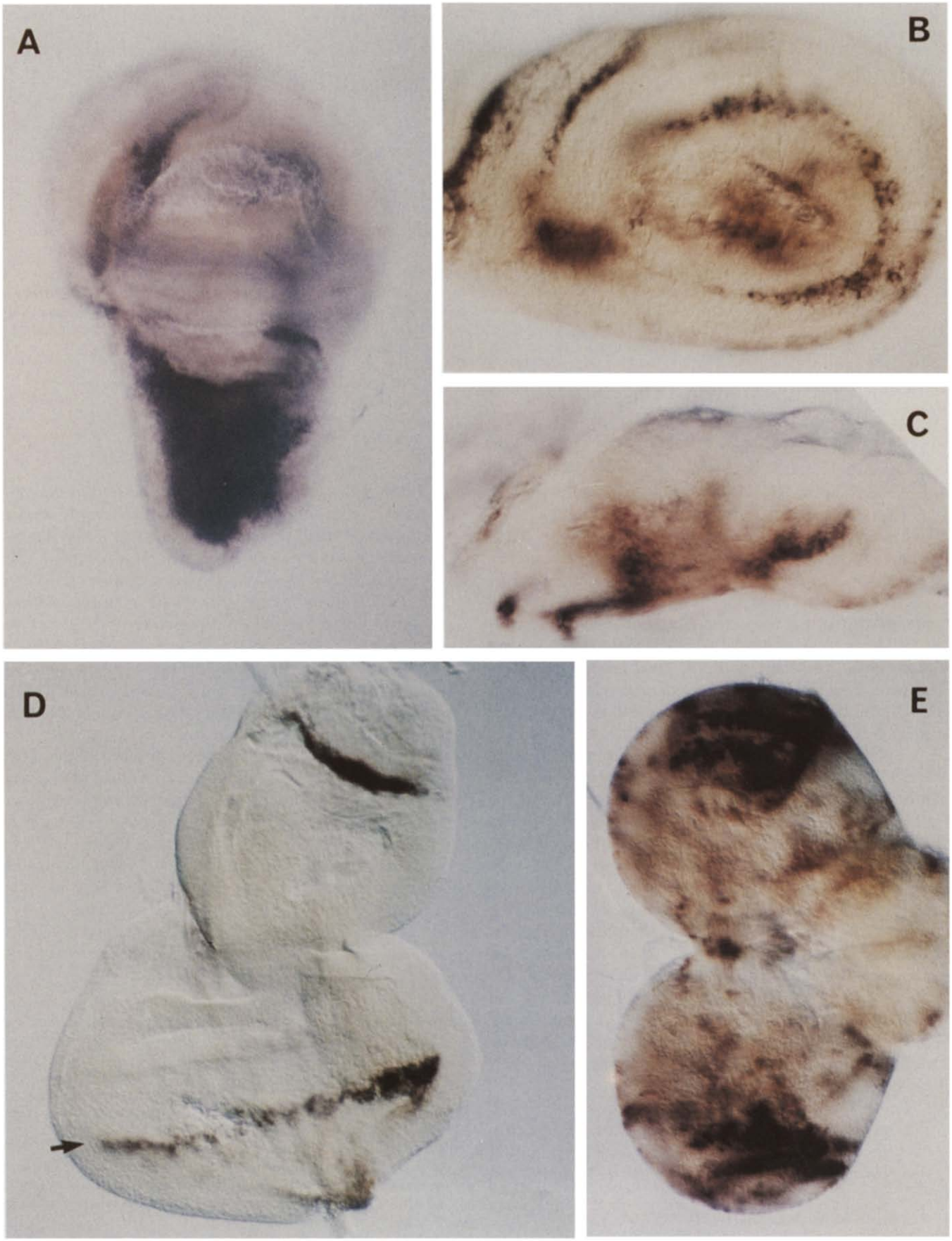
A
B
C
D
E

3. RESULTS

DFR1 mRNA was clearly detected in various imaginal discs and the brain (Fig. 1). In the wing disc, the strongest signal was observed in the notum, which contains mesodermal cells developing into flight muscles [12–15], but no signal was detected in the wing primordium. In the leg disc, the distinct expression was observed in circular or whirlpool-shaped regions (Fig. 1B). Positive cells were located in the adepithelial regions of the basal section, as can be seen in the lateral view (Fig. 1C). These *DFR1*-positive regions are similar in location and timing to those recently reported for *twist* expression [15,16]; thus, they probably correspond to muscle precursor cells (myoblasts).

Besides the wing and leg discs containing mesodermal cells, the eye–antenna disc was also shown to express *DFR1* mRNA (Fig. 1D). In the eye disc, positive signals were detected in the morphogenetic furrow and its posterior region, where neuronal differentiation is starting and has already started, respectively [17]. However, the positive signals are located in the basal region and do not seem to correspond to differentiated photoreceptor neurons positioned in the apical regions. In the brain, *DFR1* expression was clearly observed around the proliferation center (Fig. 1E), suggesting possible roles for *DFR1* in the CNS.

On the other hand, no *DFR2* mRNA could be detected in any of the imaginal discs, as exemplified in Fig. 2. This is not due to the experimental conditions, because significant expression was detected in the connecting tissues of the discs, probably in the trachea (Fig. 2).

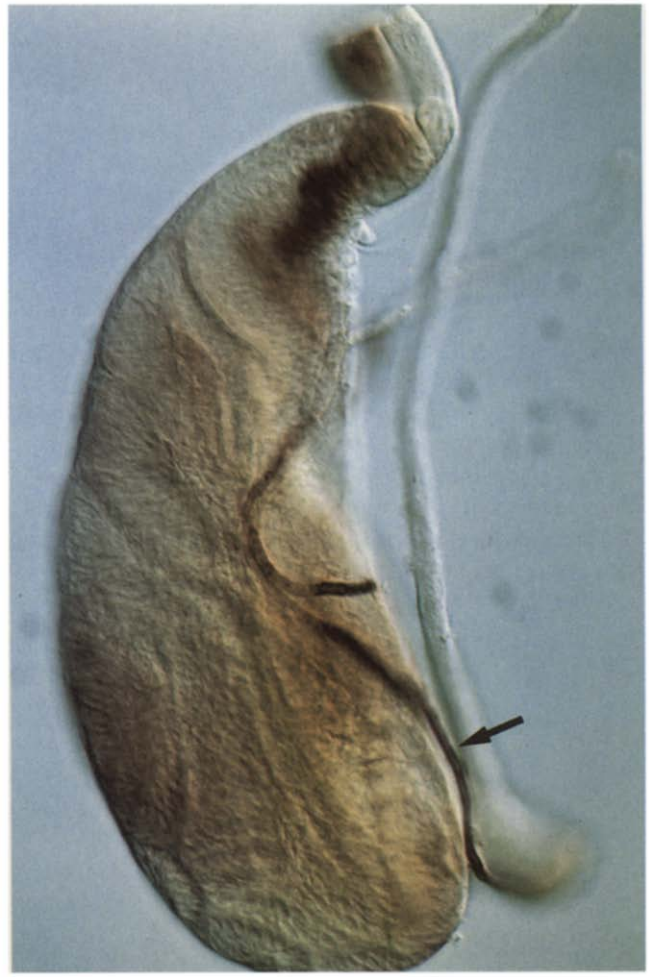

Fig. 2. The absence of *DFR2* mRNA in *Drosophila* imaginal disc. In the leg disc, no significant signal is observed. Note that the connecting tissue, probable tracheal tissue, is stained (arrow).

4. DISCUSSION

We have shown here that *DFR1*, a homolog of vertebrate FGF receptors, is expressed during the development of *Drosophila* imaginal discs, especially in probable myoblasts and CNS cells. These expression profiles during the larval and possibly pupal stages are comparable in cell-type specificity to those in the embryonic stage [8]. This means that the same *DFR1* gene should be involved in the differentiation of mesodermal tissues and CNS during both the embryonic and metamorphic stages of the insect.

The development and differentiation of the mesodermal cells that constitute the adult body are less understood than for epidermal and neuronal cells. Recently, the expression of *twist*, a mesoderm-identifying gene encoding an embryonic transcription factor [18], was revealed to possibly identify adult muscle cells as well [15,16]. The observation that the expression profile of *DFR1* in the wing and leg discs is highly related to that of *twist* [15,16] is very intriguing and suggestive about the development of adult mesodermal tissues such as muscles. It is thus plausible that similar mechanisms may work in the development of mesodermal cells in imaginal discs as operate in embryos in which *twist* plays a key role in mesodermal development by regulating various genes.

DFR1 expression in neuronal cells should also be important. The expression around the proliferation cen-

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Fig. 1. Expression of *DFR1* mRNA in *Drosophila* imaginal discs and brain. In situ hybridization was carried out using the digoxygenin-labeled antisense RNA probe for *DFR1*. In the wing disc (A), the notum is strongly stained. In the leg disc (B,C), a specific profile of positive signals is observed in adepithelial cells of the basal section (C, lateral view, apical is up). The eye–antenna disc also expresses *DFR1* mRNA (D). Note that positive signals are seen in the morphogenetic furrow (arrow) and its posterior region of the eye disc. The brain also shows *DFR1* expression around the proliferation center (E). Anterior is left.

ter of the brain indicates the possible function of *DFR1* in neurogenesis. This may be related to the postulated functions of vertebrate FGF-Rs in neuronal cells (reviewed in [2]). On the other hand, the morphogenetic furrow of the eye disc, where the neuronal identity begins to be determined, was positive to the *DFR1* probe; however, only the basal cells containing mainly undifferentiated cells were stained in the posterior region of the furrow, and the apical region containing already identified photoreceptor neurons was not stained. This may indicate that *DFR1* is involved in the very early differentiation of photoreceptor neurons before their movement to the apical surface [17], or that the *DFR1* expression is not directly linked to the neuronal development.

Taken together, *DFR1* can be considered as a possible counterpart of vertebrate FGF-Rs and to play roles during mesodermal and neuronal development in both larval and adult bodies. However, further functional analyses using genetic approaches are needed.

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