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Expression of FGF receptor gene in rat development

AKIO WANAKA[1], JEFFREY MILBRANDT[2,3] and EUGENE M. JOHNSON, Jr[1,*]

Departments of [1]Pharmacology, [2]Pathology and [3]Internal Medicine, Washington University School of Medicine, 660 South Euclid Avenue, St Louis Missouri 63110, USA

* Author for correspondence

Summary

We examined the expression of FGF-receptor (FGF-R) mRNA during rat development with *in situ* hybridization histochemistry. Embryonic tissues (E9, E12, E14, E17) and postnatal neural tissues (P1, P7, P14, adult) were examined. We detected significant levels of FGF-R mRNA in various tissues at different developmental stages. As postulated by previous studies using other methods, FGF-R gene expression was observed primarily in mesoderm- and neuroectoderm-derived tissues. In the nervous system, the pattern of gene expression was developmentally regulated; in embryos, FGF-R mRNA was mainly detected in the ependymal layer of the central nervous system (CNS). Postnatally, FGF-R transcripts were observed in specific neuronal populations, such as hippocampal neurons. FGF-R mRNA was also found in sensory systems such as trigeminal and dorsal root ganglia in late stage embryos; however, FGF-R mRNA decreased in the postnatal period. FGF-R mRNA expression was modulated in the developing retina: FGF-R messages were observed in the pigment epithelium and neuroblast layer at embryonic stages; in the postnatal period, they were found in the ganglion cell and inner granular layer. In non-neuronal embryonic tissues, a wide variety of organs expressed FGF-R message. Particularly, the prevertebral column, bone, kidney and skin showed high levels of expression. These observations reinforce the idea that FGF exerts effects on the development of various tissues.

Key words: FGF receptor, gene expression, rat development, nervous system, mesenchyme.

Introduction

Increasing evidence suggests that peptide growth factors have crucial roles in development (for review, see Whitman and Melton, 1989; Davidson, 1990). Among multiple peptide growth factors, evidence suggested that FGF is involved in various developmental events, including mesoderm induction (Slack *et al.* 1987; Kimelman and Kirschner, 1987), induction of homeobox genes and establishment of anteroposterior polarity (Ruiz i Altaba and Melton, 1989), neuronal differentiation and survival (Morrison *et al.* 1986; Walicke, 1988; Hatten *et al.* 1988) and angiogenesis (for review, see Folkman and Klagsbrun, 1988). The presence of FGF and FGF mRNA in developing organs has been examined in several ways, including fibroblast mitogenesis assay (Caday *et al.* 1990), immunoprecipitation (Seed *et al.* 1988), immunocytochemistry (Gonzalez *et al.* 1990) and Northern blot analysis (Hébert *et al.* 1990). Recently, Gonzalez *et al.* (1990) reported the detailed localization of basic FGF in the 18-day rat fetus by immunohistochemistry. Widespread distribution of bFGF in rat embryos implies multiple functions in developing organs. To understand further these roles in development, it is crucial to identify FGF-responsive cells (ie., FGF receptor-bearing cells) and to determine the temporal profile of receptor expression. Studies have examined FGF-receptor expression in selected tissues or organs with receptor cross-linking (Walicke *et al.* 1989; Olwin and Hauschka, 1990); however, the detailed localization of FGF-R mRNA has not been reported.

Since the purification and cloning of chicken bFGF receptor was reported (Lee *et al.* 1989), several laboratories have succeeded in isolating FGF receptor cDNAs (Reid *et al.* 1990; Saffran *et al.* 1990; Mansukhani *et al.* 1990). It was suggested that FGF-R gene belongs to a family which contains multiple tyrosine-kinase receptors (Reid *et al.* 1990). This study revealed that these family members have highly conserved tyrosine kinase domains and relatively unconserved extracellular domains. We have also obtained a partial cDNA for rat FGF receptor and employed this cDNA to localize FGF receptor mRNA in the mature CNS by *in situ* hybridization (Wanaka *et al.* 1990). In the present study, we have extended our investigation to developing rats with special emphasis on the nervous system.

From the earliest stage (E9) that we examined, a widespread distribution of FGF-R mRNA was observed in various embryonic tissues. Although the

intensity of signal varies among different tissues, almost all the organs, with the possible exception of liver, contained FGF-R transcripts. Strong signals were located in the mesoderm-derived tissues, such as, the perichondrium, metanephros, prevertebral column and myotome. In the nervous system, FGF-R mRNA was found in both the central and peripheral nervous systems; however, the temporal patterns of expression are different in each tissue. These results suggest that FGF has biological functions in various developing organs.

Materials and methods

Animals and tissue processing

Timed-pregnant rats (E9, E12, E14, E17), and postnatal day 1, 7, 14 and 10-week-old rats were purchased from Sasco (Omaha, NE). Embryos were removed from uterus and immersed in 4% paraformaldehyde buffered with 0.1 M sodium phosphate overnight. Embryos were rinsed with 0.01 M phosphate-buffered saline twice for 30 min each and dehydrated by using ascending concentrations of ethanol (30%, 50%, 70%, 70%, 85%, 95%, 100%, 100% for 30 min each). After dehydration, they were cleared with toluene twice and immersed in molten paraffin. Embryos were embedded in paraffin; 10 μm sections were cut on a rotary microtome and then mounted on 3-aminopropyl-triethoxysilane-treated slides. These slides were subjected to *in situ* hybridization (see below). Postnatal rats were perfused with 4% paraformaldehyde buffered with 0.1 M sodium phosphate. Tissues (brain, spinal cord, ganglia and eyes) were dissected and immersed in the same fixative overnight and processed in an identical fashion.

In situ *hybridization histochemistry*

In situ hybridization was performed as previously described by using ^{35}S-labelled anti-sense and sense (control) riboprobes for rat bFGF receptor (Wanaka *et al.* 1990) except that paraffin sections were employed in the present study instead of frozen sections. These probes were transcribed from the cDNA template extending from nucleotide 957 to nucleotide 1257 (Nucleotide numbers are according to Reid *et al.* 1990; Wanaka *et al.* 1990). This cDNA fragment largely consists of the extracellular and transmembrane domains that are relatively unconserved among members of FGF-R family. This fragment does not overlap the tyrosine kinase domain. Specificity of the signal was checked as described previously (Wanaka *et al.* 1990; also see Figs 2C, D and 4C, D). Most sections were exposed for 2–3 weeks after emulsion coating. As discussed in our previous report (Wanaka *et al.* 1990), our probe can recognize the two forms of FGF-R mRNA described by Reid *et al.* (1990). Taking into account the previous studies (Neufeld and Gospodarowicz, 1986; Saffran *et al.* 1990), it is likely that acidic and basic FGF share the same receptor, so that we referred to our clone as FGF-R cDNA (Wanaka *et al.* 1990). Recently, other members of the FGF-R family, such as K-*sam* (Hattori *et al.* 1990) and Cek-2, -3 (Pasquale, 1990) were identified. When we compared our probe sequence with these clones, we found relatively weak homology between them (58%, 61% and 78% nucleotide sequence homologies between our probe sequence and corresponding regions of K-*sam*, Cek-2 and Cek-3, respectively). Since we employed high stringency conditions for hybridization and washing (Wanaka *et al.* 1990), it is highly unlikely that our probe recognizes rat homologues of these family members.

Results

FGF-R mRNA expression in the developing nervous system

Brain

In the embryonic central nervous system, FGF-R transcripts were predominantly located within the ependymal layer, which contains mitotic precursor cells (Figs 1 and 2). From the earliest stage (E9) that we examined, FGF-R mRNA was found throughout the central nervous system (from telencephalon to caudal neural tube) (Fig. 1A and B). At a later stage (E12), receptor message was still confined to the ependymal layer and was not found in the mantle or marginal layers (Fig. 1C and D). This suggests that FGF exerts effects primarily on the mitotic undifferentiated precursor cells of the CNS in the early embryo. In the E14 embryo, the primordial hippocampal cortex begins to express FGF-R messages in addition to the ependymal layer. As the embryo grows, expression in the primordial hippocampal cortex becomes prominent. In the E17 embryo, we detected intense labelling in primordial hippocampal cortex and moderate signals in the mantle layer of the telencephalon (Fig. 2A and B). The survival promoting effects of FGF are most potent in the primary hippocampal culture among various CNS regions (Walicke, 1988) and the hippocampal formation is one of the areas that showed robust labelling for FGF-R mRNA in the adult CNS (Wanaka *et al.* 1990). In the mesencephalon, a group of cells also expressed FGF-R transcripts and relatively diffuse labelling was also found in the metencephalon (not shown). Although it was not possible to identify these cells absolutely, a comparison with the expression pattern in a later stage (P14, Fig. 2H) and in our previous study of adult brain suggests that they are mesencephalic cholinergic neurons and neurons of the deep cerebellar nuclei. In the postnatal day-7 (P7) rat brain, the pattern of FGF-R mRNA expression is similar to that of the adult CNS (Wanaka *et al.* 1990). However, areas such as cerebellar granule cells (Fig. 2E), express less discernible signal for FGF-R mRNA than those of adult CNS (Wanaka *et al.* 1990). Interestingly, in the P7 cerebellum, hybridization signals were primarily detected in the internal granule cell layer but not in the external granule cell layer that contains mitotic precursors for granule cells (Fig. 2E). This pattern persisted in the P14 cerebellum; however, the intensity of signals in the inner granular cell layer became stronger than that of P7 cerebellum (Fig. 2E and F). The expression pattern of P14 rat brain is almost identical to that of adult brain. For example, in the P14 brainstem, neurons in the locus coeruleus and trigeminal motor nucleus that were not strongly positive at P7 stage were clearly labelled (Fig. 2G and H).

Spinal cord

During development, the expression patterns of FGF-R

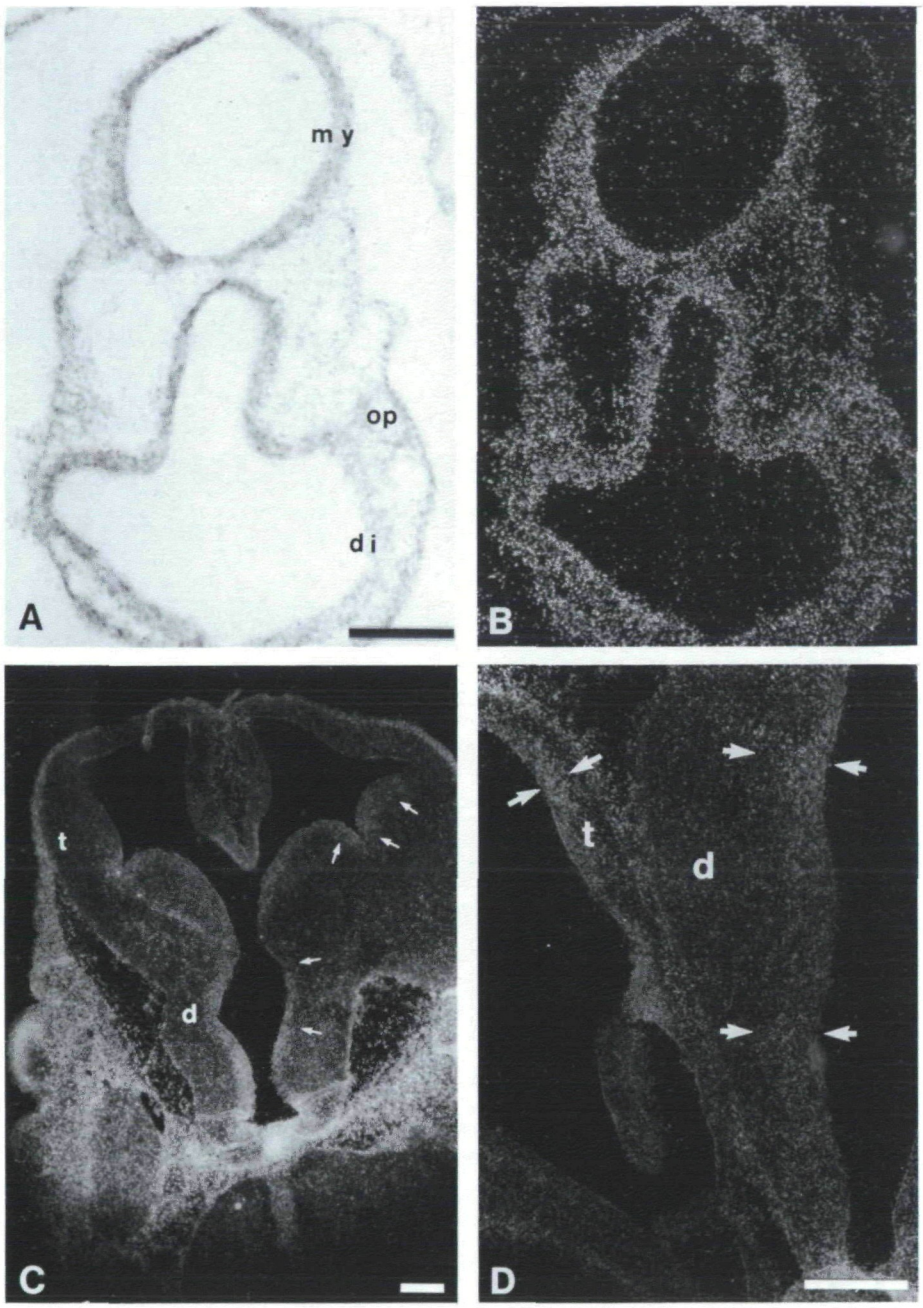

Fig. 1. FGF-R mRNA expression in the E9 and E12 embryonic brains. (A,B) Bright- and dark-field photomicrographs of the transverse section of the E9 embryo. Note the diffuse labelling of the diencephalon (di), myelencephalon (my) and optic vesicle (op). Strong labelling is also observed on the surrounding mesenchymal tissues. Bar: 200 μm. (C) and (D) Dark-field photomicrographs of the transverse section of E12 embryo. Note the labelling in the ependymal layers (marked by arrows) in the telencephalon (t) and the diencephalon (d). Bar: 200 μm.

mRNA in the spinal cord were similar to those in the developing brain; most of the signals were located in the ependymal layer at early stages (E12, E14, Fig. 3A). Later, they were confined to the very narrow layer surrounding the central canal; diffuse, but well above background, labelling was observed in the gray matter of the spinal cord (E17, Fig. 3B). We reported that FGF-R mRNA expression in the adult spinal cord is observed in various types of neurons (Wanaka *et al.* 1990). In particular, we observed intense labelling on the adult motor neurons. In contrast, the FGF-R signals were not prominent on the E17 spinal cord motoneurons (Fig. 3B), but cellular labeling on the spinal cord neurons was detected in the P1 rat (Fig. 3D); and, by P14, spinal cord showed a similar distribution pattern to that observed in the adult (Fig. 3E).

Peripheral nervous system

In the adult rat, we failed to detect FGF-R mRNA in any portion of the peripheral nervous system (ie., sensory, sympathetic or parasympathetic). In contrast to the adult, we found that sensory neurons (dorsal root ganglia and trigeminal ganglia) express FGF-R mRNA during embryogenesis. In the E12 embryo, dorsal root ganglia showed weak, diffuse labelling for FGF-R mRNA (Fig. 3A), while in the E14 dorsal root ganglia (DRG) the labelling increased in intensity, but remained diffuse. The E17 embryo showed distinct

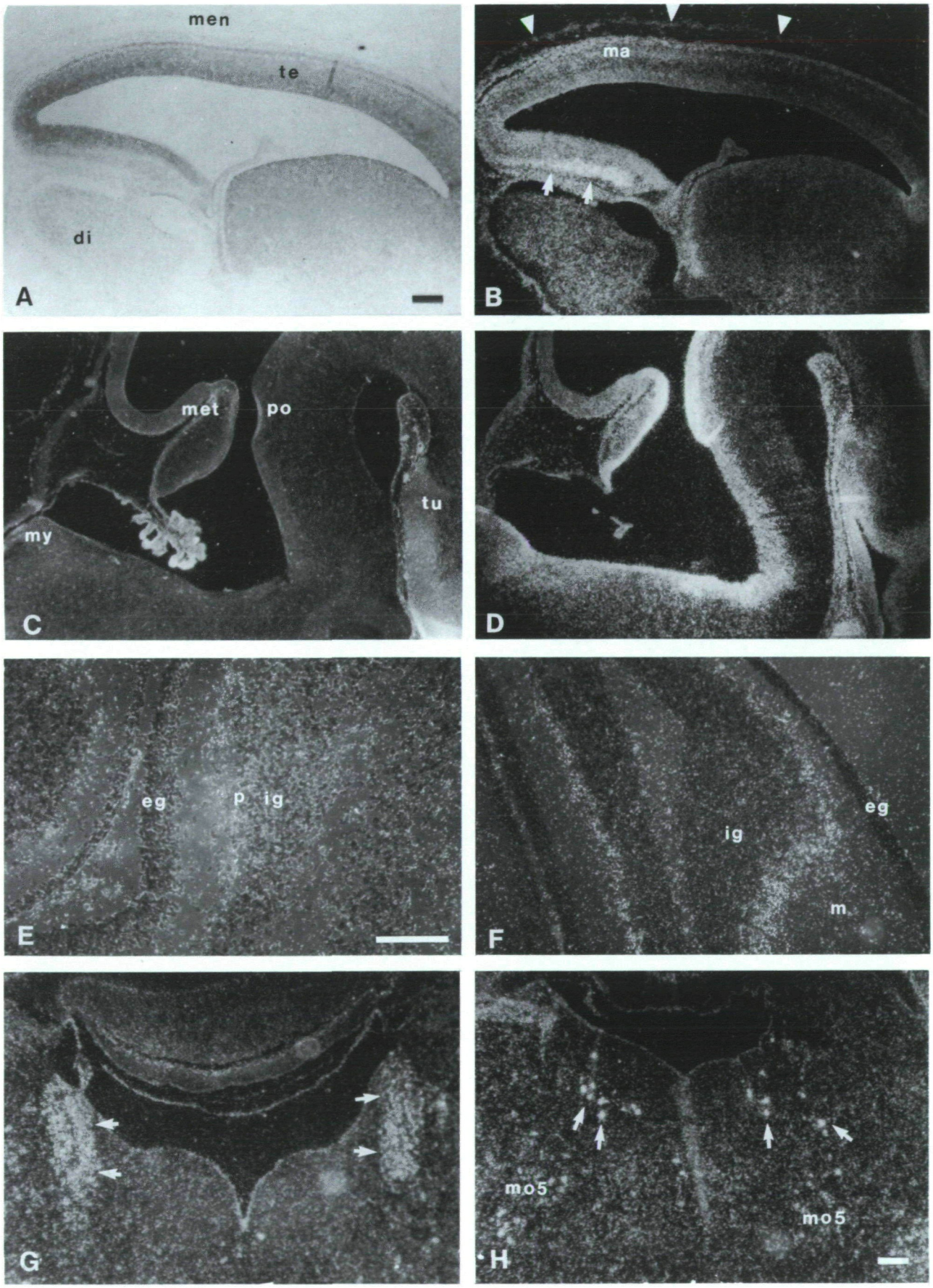
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Fig. 2. FGF-R mRNA expression in the embryonic and postnatal brain. (A,B) Bright- and dark-field photomicrographs of parasagittal section of E17 embryo. The ependymal layer of the telencephalon (te) is labelled. The mantle layer (ma) is also labelled moderately. Intense signal can be seen in the primordial hippocampal cortex (arrows). Note that the meninges (men) are positive for FGF-R mRNA (arrowheads). di; diencephalon. Bar: 200 μm. (C,D) Dark-field photomicrographs of parasagittal section of E17 embryonic brain. C is sense control section. Note the only background level of the signal. D is the section hybridized with antisense control. Note the intense labelling in the ependymal layer. Abbreviations: met; metencephalon, po; pons, tu; tuberculum posterius, my; myelencephalon. Bar: 200 μm. (E) The P7 cerebellum. The inner granule cell layer (ig) shows moderate labelling, whereas the external granule cell layer (eg) is devoid of signals. Note the Purkinje cells (p) are negative for FGF-R mRNA. Bar: 200 μm. (F) The P14 cerebellum. The distribution pattern is similar to that of the P7 cerebellum (E). The labeling of the inner granule cell layer becomes more intense. m; molecular layer. (G,H), The P14 brainstem. The locus coeruleus (arrows in G), the laterodorsal tegmental nucleus (arrows in H) and the trigeminal motor nucleus (mo5) express high level of FGF-R mRNA. Bar in H for E–H: 200 μm.

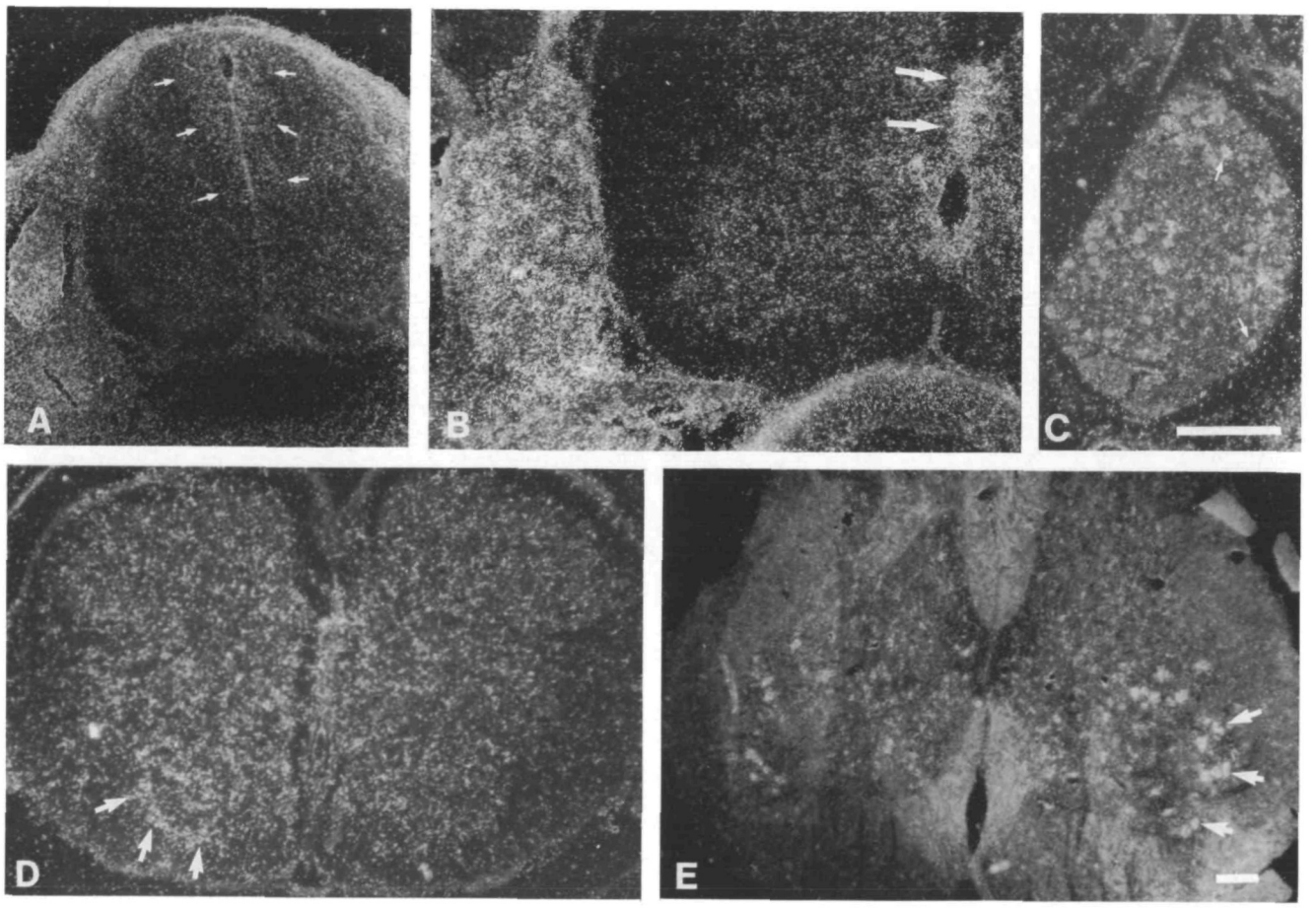

Fig. 3. Expression of FGF-R mRNA in the developing spinal cord, dorsal root ganglia. (A) Transverse section of E12 spinal cord. Note the labelling in the ependymal layer (arrows). The dorsal root ganglion shows moderate and diffuse labeling. (B) Transverse section of the E17 embryo. Intense labeling can be observed in the narrow ependymal layer (arrows) and diffuse labeling is also observed in the gray matter. Note the distinct cellular labelling in the dorsal root ganglion. (C) The P1 dorsal root ganglion. Only slight labelling is seen in some of the neurons (arrows). Bar in C for A–C: 200 μm. (D) The P1 spinal cord. At this stage, ependymal layer fails to show distinct labeling. Note that motor neurons have moderate signals (arrows). (E) The P14 spinal cord. Note the strong cellular labeling on the motor neurons. Bar, in E for D and E: 200 μm.

neuronal labelling for FGF-R mRNA in the DRG (Fig. 3B). The labelling decreased in the P1 DRG (Fig. 3C) and no detectable signal was observed in P7 or P14 DRG, suggesting that FGF-R mRNA is expressed transiently in at least some sensory neurons. The trigeminal ganglia showed a similar developmental pattern (not shown). Sympathetic ganglia and nodose ganglia failed to express FGF-R mRNA in these embryonic stages. Parasympathetic ganglia including ciliary and sphenopalatine ganglia at E17 stage also failed to show any distinct cellular labelling.

Retina

The developing retina was also found to express FGF-R

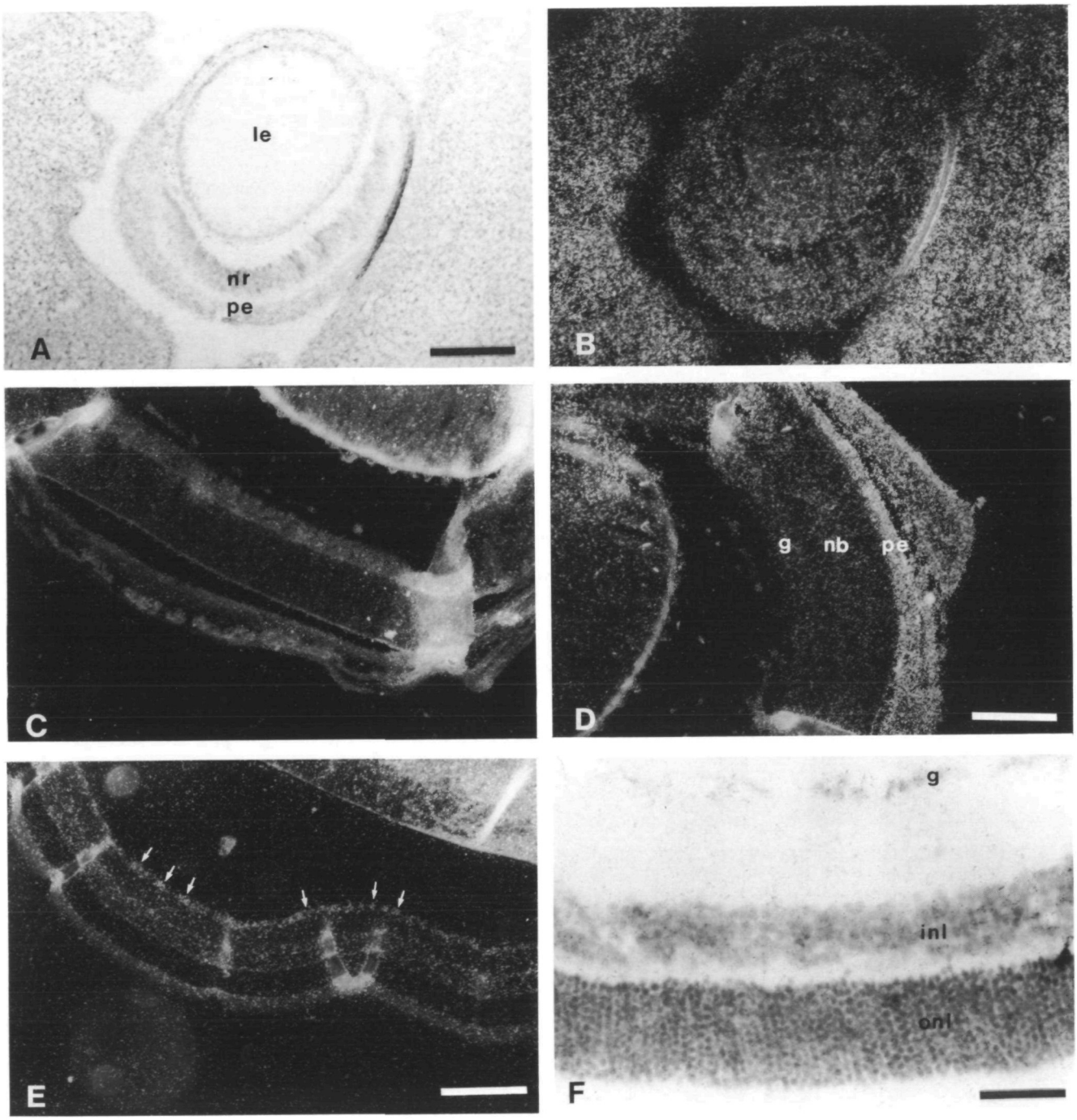

Fig. 4. FGF-R mRNA expression in the developing eye. (A) and (B) Bright- and dark-field photomicrographs of E12 embryonic eye. Note the diffuse labelling on the pigment epithelium (pe), neural retina (nr) and lens (le). Bar, in A also for B: 200 μm. (C,D) Dark-field photomicrographs of the P1 eye. (C) Sense control section of the P1 eye. Note the pigment epithelium and neural retina have only background level of silver grains. (D) P1 eye section. Silver grain accumulation can be detected on the pigment epithelium (pe), neuroblast layer (nb) and ganglion cell layer (g). Bar in D also for C: 200 μm. (E) adult retina. Note the labelling on the ganglion cells (arrows). Bar: 200 μm. (F) Bright-field photomicrograph of adult retina. The inner nuclear layer (inl) is labelled. onl; The outer nuclear layer. Bar: 50 μm.

mRNA. As shown in Fig. 4A, B, and D, the pigment epithelium and neural retina of E12 and P1 embryos expressed FGF-R mRNA. It has been reported that aFGF can affect the newborn rat photoreceptor cells and promote their survival (Hicks *et al.* 1988) and that retinal pigment epithelium releases and binds aFGF (Plouet *et al.* 1988). FGF also exerts effects on process outgrowth of retinal ganglion cells (Lipton *et al.* 1988). Recently, work from our laboratory demonstrated that intraocularly injected bFGF is internalized in the adult

retinal ganglion cells and anterogradely transported to the optic tectum (Ferguson *et al.* 1990). Therefore, we also investigated the expression of FGF-R mRNA in the postnatal retina. In the P1 retina, in addition to the neuroblast layer, the ganglion cell layer is also labelled moderately (Fig. 4D). From the P7 to the adult, the pattern of expression was similar; we found FGF-R messages in the ganglion cell layer and the inner nuclear layer (Fig. 4E and F, adult).

Inner ear and pituitary

During the development of the inner ear, we observed FGF-R transcripts in the sensory regions including the ampullae (Fig. 5A and B), utricle (Fig. 5C and D) and cochlea, in addition to the surrounding mesenchyme and chondrocytes (Fig. 5A–D). At the E12 stage, the infundibulum was labelled strongly, whereas Rathke's cyst was less prominent for FGF-R mRNA. In later stages (E14, E17), strong labelling was observed in the posterior lobe, whereas the intermediate and anterior lobes were weakly positive for FGF-R mRNA (Fig. 5E and F, E14 and E17, respectively).

FGF-R mRNA expression in non-neuronal embryonic tissues

In general, as suggested by previous studies (Liu and Nicoll, 1988; Gonzalez *et al.* 1990), FGF-R mRNA is expressed in many developing organs, particularly mesoderm-derived tissues (Figs 6–8). However, the intensity and the pattern of expression varied as a function of development. It should be noted that liver was the only organ in which we failed to detect FGF-R mRNA at any embryonic stage. We summarize the pattern of FGF-R mRNA expression in the different organs separately in the following sections.

Bone, cartilage and skin

In the developing embryo, strong labelling was observed in the prevertebral column, perichondrium and osteoblasts (Fig. 6). The prevertebral column, which is clearly positive for FGF-R mRNA, contains chondrocytes that are actively dividing and synthesizing hyaline matrix (Fig. 6A). At later stages, when the ossification centers have formed, FGF-R mRNA is mainly observed in the perichondrium and is found neither in the ossification center nor in the hypertrophied chondrocytes (Fig. 6B). In more differentiated bone, osteoblasts were strongly labelled (Fig. 6C and D), whereas osteoclasts, which have large cell bodies and multiple nuclei, were devoid of FGF-R mRNA (not shown).

The facial primordia were some of the most strongly labelled tissues in the developing embryo. As shown in Fig. 6E and F, nasofacial cartilage, maxilla, mandible and tongue were intensely labeled.

There was intense labeling in the limb, particularly in the distal part of the limb. Fig. 7A and B show E17-hindfoot section. Robust and diffuse labelling can be seen in the mesenchymal tissues. The most intense signal was observed in the compact mesenchyme beneath the epidermis and in the perichondrium. As mentioned previously, ossification centers were negative for FGF-R mRNA.

Fig. 7C and D show the facial skin region of E17 embryo. As observed in the hindfoot section (Fig. 7A and B), the compact mesenchyme was intensely labelled. The whisker follicles also showed robust labelling (Fig. 7C and D).

Excretory system and digestive tract

At 12 days gestation, both the collecting duct and the metanephric mesenchyme were labelled diffusely. At later stages, signals are mainly observed in the cells of the collecting duct and its surrounding concentrated mesenchyme (Fig. 8A and B, E14). It is of interest that cells that have not concentrated around the collecting duct express less FGF-R mRNA. This pattern can be seen clearly in the E17 metanephros; FGF-R transcripts were primarily observed on the collecting ducts that are located in the perimeter of the kidney and were less expressed in the interior portion (Fig. 8C and D). This finding suggests that the target cells of FGF in the developing kidney are differentiating mesenchymal cells and that the level of receptor message decreases when these cells mature, as with the interior part of the E17 metanephros (Fig. 8C and D).

In the digestive tract, the strongest signals were detected in the smooth muscle layer of esophagus and less intense signals were observed in the muscle layer of the lower digestive tract. The labeling of villous epithelium was less intense than that of the muscle layer (Fig. 8E and F). This pattern was similar from the esophagus to the lower digestive tract.

Other tissues

The skeletal muscle continuously expressed high levels of FGF transcript throughout all embryonic stages (E9 to E17) (Fig. 6A, E12). The intensity of the labelling was generally much greater than that of the smooth muscles of digestive tract or blood vessels. The heart was also labelled; however, its labelling was diffuse and moderate throughout the embryonic stages that we examined (Fig. 9B). The lung was diffusely labeled in E12 to E17 embryos (Fig. 9A and C). The gonads and salivary glands are also labelled moderately (not shown). As shown in Fig. 8A and B, the adrenal gland did not show strong labelling and adrenal medulla had less signal than adrenal cortex.

No detectable FGF-R transcripts were observed in the liver in any of the embryonic stages that we examined (Figs 6B and 9C). Consistent with this finding, no immunoreactivity for bFGF was detected in the 18-day rat fetal liver (Gonzalez *et al.* 1990).

Discussion

FGF-R mRNA expression in the developing nervous system

We have demonstrated that FGF-R mRNA is expressed primarily in the ependymal zone of the embryonic brain (E12–E17), although some specific regions in later

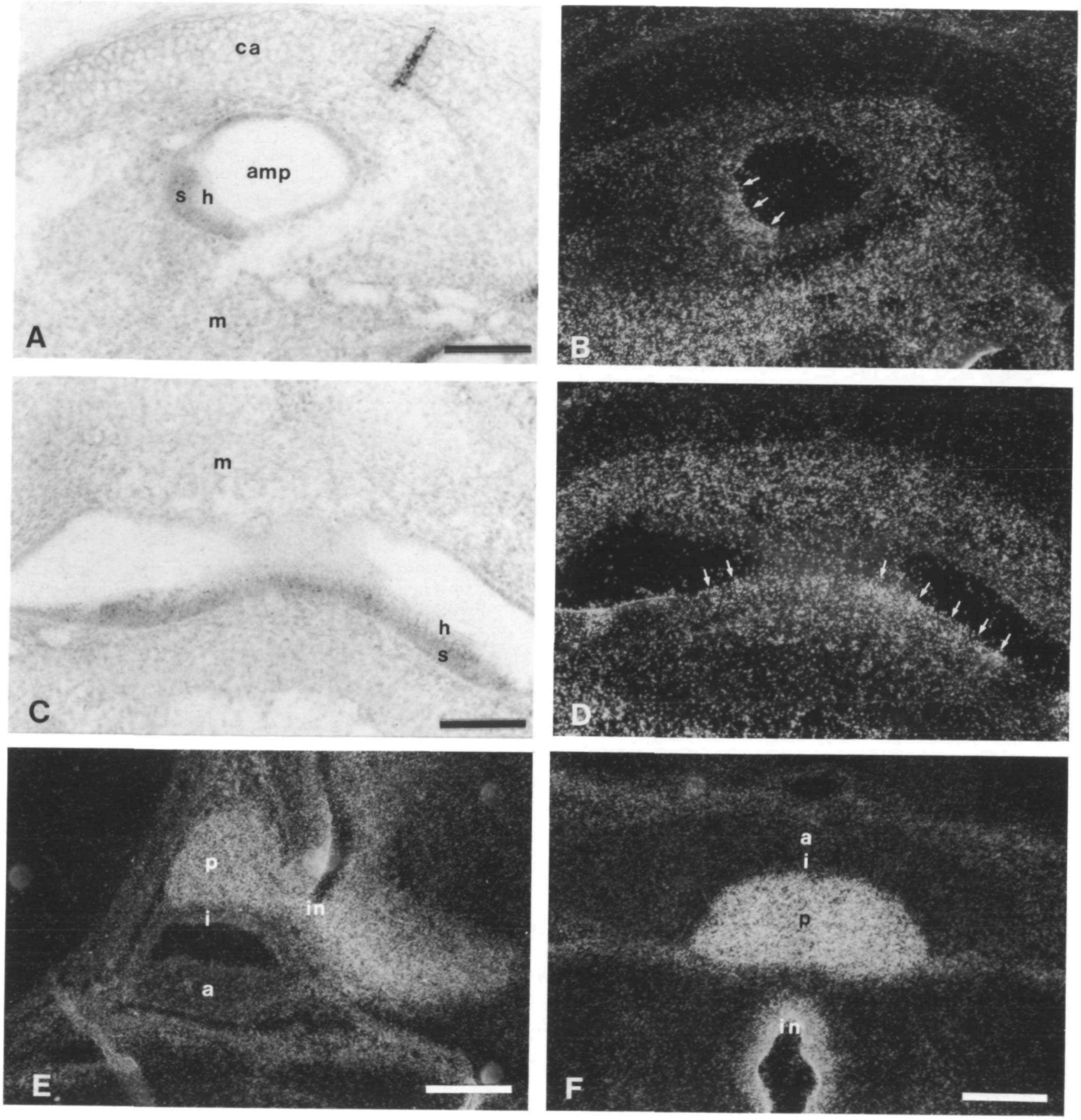

Fig. 5. FGF-R mRNA expression in the developing inner ear and pituitary. (A,B) Bright- and dark-field photomicrographs of the ampullae of the E17 embryo's inner ear. Note the signals on the hair cells and supporting cells (arrows). (C,D) Bright- and dark-field photomicrographs of the utricle (E17). Silver grain accumulation can be seen in the sensory region (arrows). (E) Dark-field photomicrograph of the parasagittal section of E14 brain. Intense labelling is observed in the infundibulum and posterior lobe. (F) Dark-field photomicrograph of the transverse section of E17 brain. Note the strong signal in the posterior lobe of pituitary. Abbreviations: ca; cartilage, amp; ampullae, h; hair cell layer, s; supporting cell layer, m; mesenchyme, a; anterior lobe of pituitary, i; intermediate lobe of pituitary, p; posterior lobe of pituitary, in; infundibulum. Bars: 200 μm.

stages (E17) were also positive for FGF-R mRNA. FGF has shown significant neurotrophic activity when tested in primary neuronal cultures from various regions of the embryonic brain (Morrison *et al.* 1987; Walicke, 1988; Hatten *et al.* 1988). Walicke demonstrated that FGF can promote the survival of neurons from almost all E18 brain regions with the maximal response in the hippocampal culture (Walicke, 1988). The broadness of this trophic effect could be partly explained by the widespread distribution of FGF-R transcripts in the

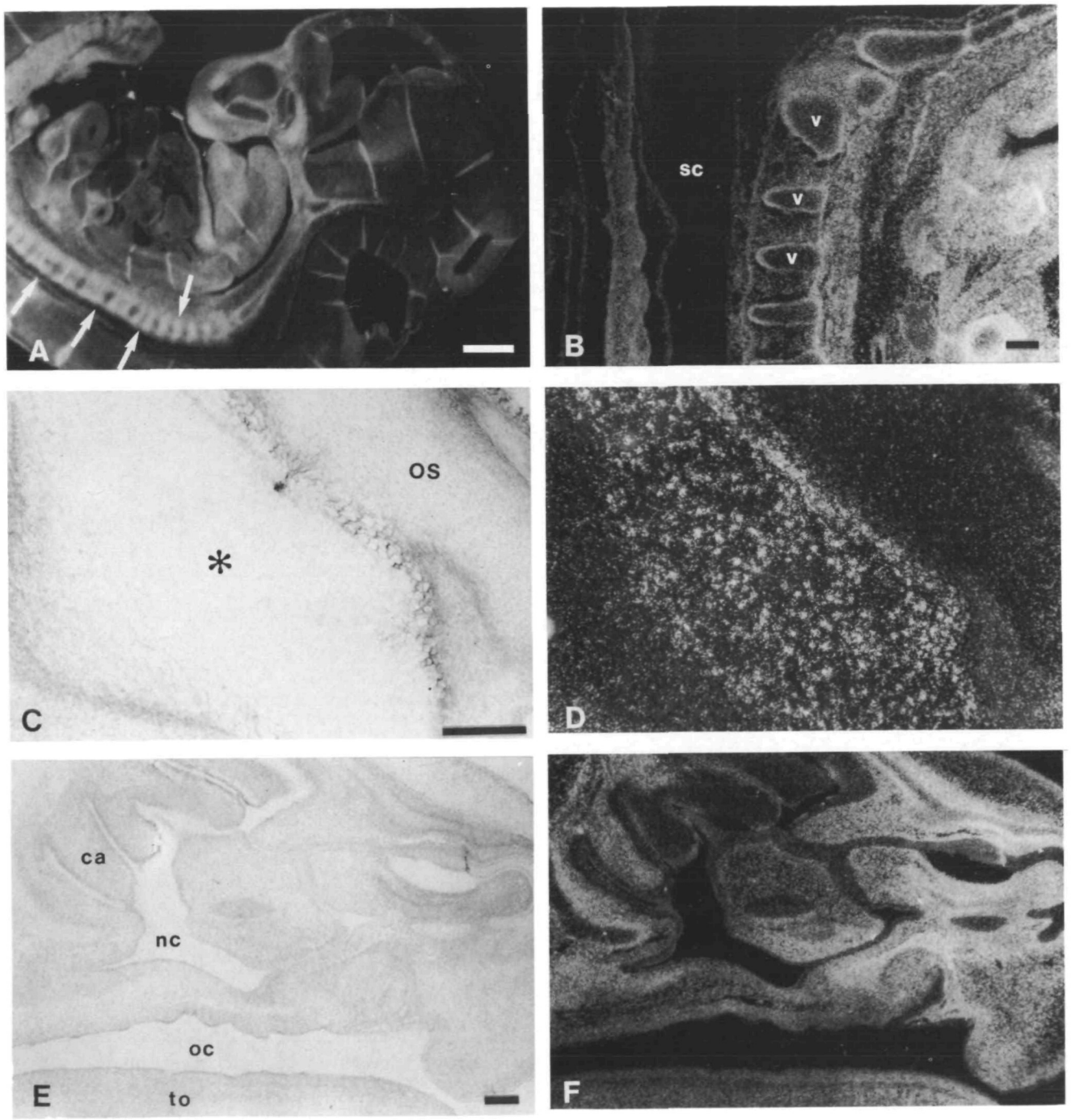

Fig. 6. FGF-R mRNA expression in the developing skeletal tissues. (A) Dark-field photomicrograph of the parasagittal section of E12 embryo. Note the strong signal in the prevertebral column (arrows). Intense labelling can also be seen in the facial region. Bar: 1 mm. (B) Dark- field photomicrograph of the parasagittal section of E17 embryo. Perichondrium surrounding vertebrae (v) shows intense labelling. Note the strong signals in the muscle mesenchyme surrounding vertebral column. sc; spinal cord. Bar: 200 μm. (C,D) Bright- and dark-field photomicrographs of E17 bone section. Silver grain accumulation is observed on the osteoblasts that are distributed in the absorbed region of differentiating bone (asterisk). os; ossification center. Bar: 200 μm. (E,F) Bright- and dark-field photomicrographs of the parasagittal section of facial region of E17 embryo. Note the intense labelling in the mesenchymal tissues of the facial primordia. ca; nasal cartilage, nc; nasal cavity, oc; oral cavity, to; tongue. Bar: 200 μm.

ependymal layer observed in this study. We also observed that primordial hippocampal cortex expressed a high level of receptor message; this is consistent with the maximal response in the hippocampal culture. As suggested previously (Davies, 1988), part of the trophic effects may be ascribed to promotion of mitosis of the

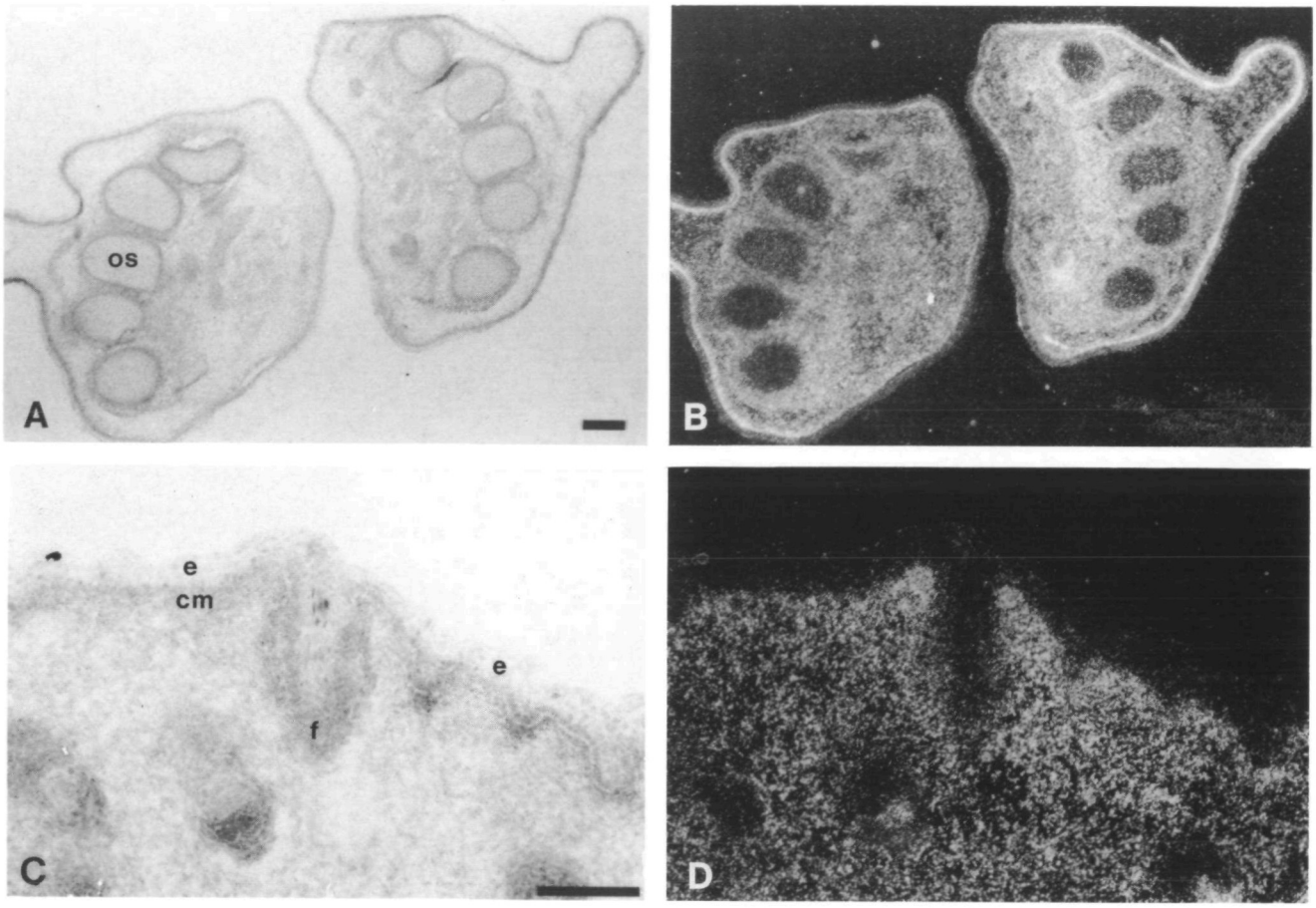

Fig. 7. FGF-R mRNA expression in the limb and skin. (A,B) Bright- and dark-field photomicrographs of the hindfoot section (E17). Note the weak labelling in the ossification centers (os). Bar: 200 μm. (C,D) Bright-field (C) and dark-field (D) photomicrographs of the skin of the facial region (E17). Strong labelling is observed in the compact mesenchyme (cm) and whisker follicle (f). Note the absence of the labelling in the epidermis (e). Bars: 200 μm.

dividing precursor cells. In fact, it has been reported that FGF stimulates the proliferation of rat neuronal precursor cells in culture (Gensburger *et al.* 1987; Murphy *et al.* 1990). Previously, we have shown that FGF-R mRNA is widely distributed in the mature CNS (Wanaka *et al.* 1990). However, there was some apparent inconsistency between our results and *in vitro* culture studies (Walicke, 1988); for example, Walicke identified trophic effects in thalamic culture, whereas we failed to detect FGF receptor mRNA in specific thalamic neurons (Wanaka *et al.* 1990). These inconsistencies could be explained by an ability of FGF to promote neuronal differentiation in these areas since FGF-R mRNA is expressed in the ependymal layer of the diencephalon, as shown in the Fig. 1C and D. Taken together, these findings suggest that multiple mechanisms (e.g. direct action on specific postmitotic neurons, differentiation of precursor cells in the ependymal cell layer, indirect effects *via* glial elements) may underlie the 'trophic effect' of the FGF in primary neuronal culture.

Neurons in the hippocampus and pontine cholinergic neurons, which showed the highest level of FGF-R transcripts in the mature CNS, expressed a significant amount of FGF-R mRNA during the late embryonic stages, whereas other regions start expressing in the early postnatal period. These findings suggest that there may be regional developmental differences in the establishment of the cellular responsiveness to FGF. This also warrants further analysis with immunocytochemistry using anti-FGF-R antibody to examine the protein level of FGF-R. The cerebellum showed a somewhat different expression pattern of FGF-R mRNA as compared to other regions. The external granule cell layer that contains mitotic precursors for granule cells was not labelled. These cells only express receptor mRNA in the postmitotic state after migration to the inner granule cell layer. This pattern is opposite to that seen with NGF receptor mRNA which is expressed in the external granule cell layer but not the inner granule cell layer (Wanaka and Johnson, 1990). These findings suggest that FGF and NGF may influence independent cell populations to regulate cerebellar development. We currently do not have any explanation for the difference in FGF-R mRNA expression of precursor cells in the external granule cell layer as compared to other embryonic brain regions. This difference may be due to the dissimilar nature of

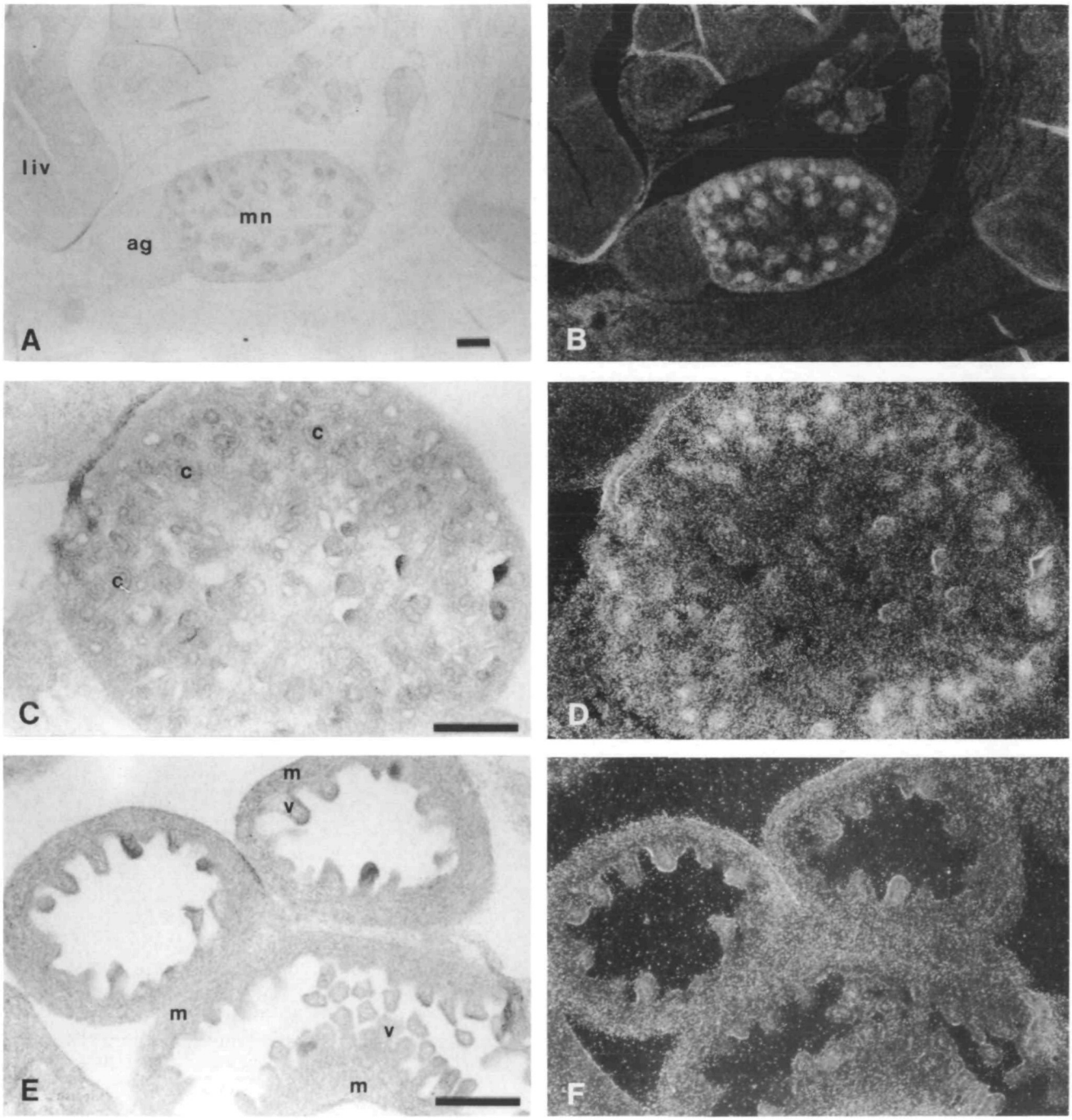

Fig. 8. FGF-R mRNA expression in the excretory and digestive systems. (A,B) Bright- and dark-field photomicrographs of the parasagittal section of the E14 embryo. Metanephros shows (mn) strong labelling. Note the weak labelling in the adrenal gland (ad). liv; liver. (C,D) Bright- and dark-field photomicrographs of the E17 metanephros. Note the intense labelling on the collecting ducts (c) and surrounding mesenchyme. (E,F) Bright- and dark-field photomicrographs of the E17 intestine. Moderate to strong labelling is observed in the muscle layer (m). Intestinal villi (v) also shows moderate labeling. Bars: 200 μm.

these precursor cells; precursor cells in the external granule cell layer largely differentiate into granule neurons, whereas precursor cells in the other brain regions differentiate into both neurons and glial cells.

Immunocytochemical analysis revealed that the E18 rat central nervous system contains bFGF in the neuron-rich areas of the telencephalon (Gonzalez *et al.* 1990). Taken together with the present findings, it is conceivable that FGF exerts its effects in an autocrine or a paracrine manner in the developing CNS. In this

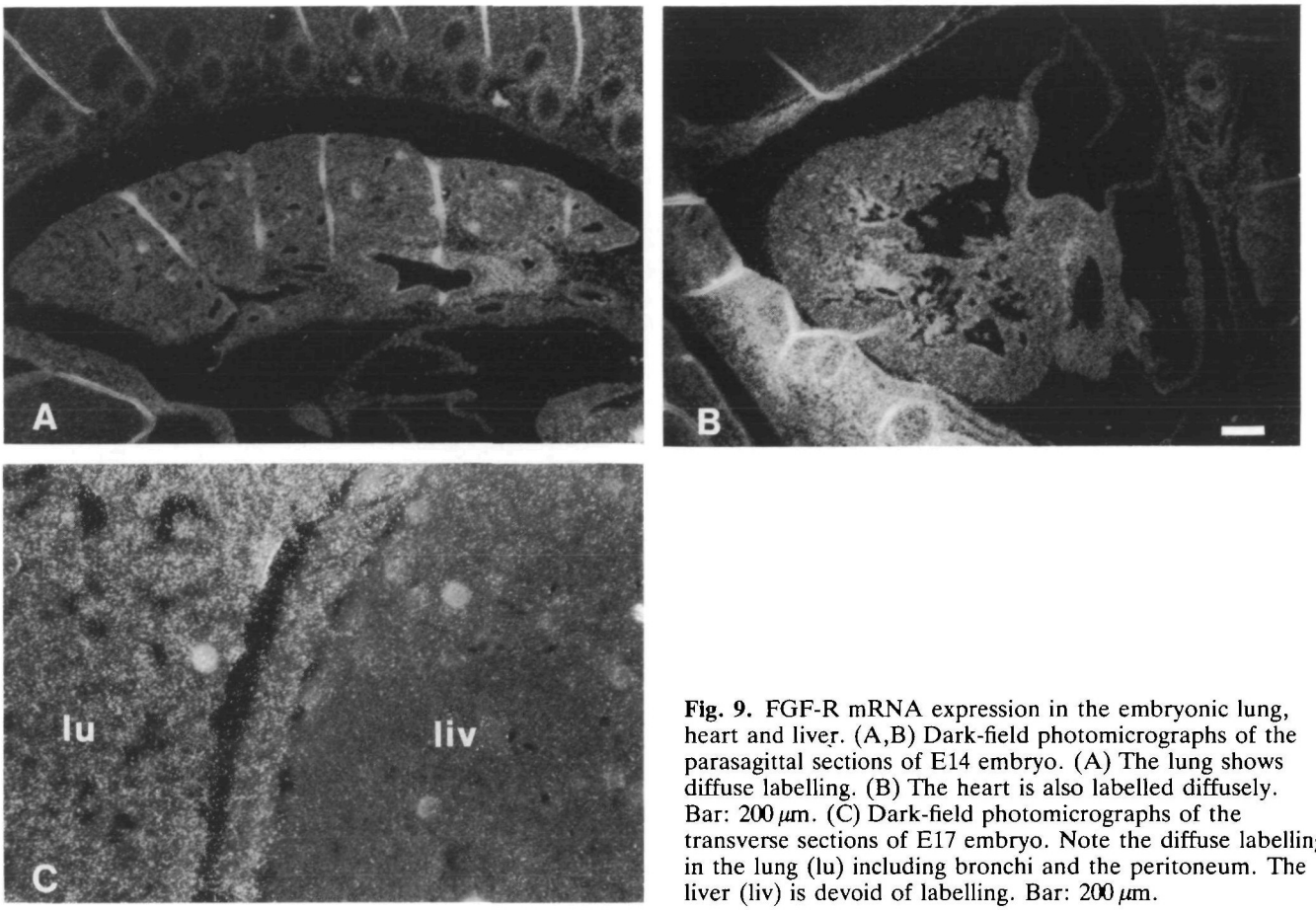

Fig. 9. FGF-R mRNA expression in the embryonic lung, heart and liver. (A,B) Dark-field photomicrographs of the parasagittal sections of E14 embryo. (A) The lung shows diffuse labelling. (B) The heart is also labelled diffusely. Bar: 200 μm. (C) Dark-field photomicrographs of the transverse sections of E17 embryo. Note the diffuse labelling in the lung (lu) including bronchi and the peritoneum. The liver (liv) is devoid of labelling. Bar: 200 μm.

respect, it would be of interest to examine the developmental expression of FGF in the nervous system.

In contrast to the abundance of FGF-R mRNA in the mature spinal cord (Wanaka *et al.* 1990), the embryonic spinal cord failed to show labelling except for the robust labelling in the ependymal layer. By P14, the spinal cord neurons establish the adult labelling patterns (see Results, *Spinal cord*). We also detected transient expression of FGF-R mRNA in the sensory nervous system (Fig. 3), although no trophic actions of FGF on the developing sensory neurons have been identified. In late embryonic stages (E14–birth), these neurons send their processes to and make connections with their central targets (Smith, 1983). It is possible that these transiently expressed receptors may be involved in process outgrowth or synapse formation. In this respect, it will be of interest to study the function of FGF on these late-embryonic-stage neurons by using organotypic cultures treated with FGF or anti-FGF antibodies in combination with a retrograde transport technique.

In the developing retina, we found that the pigment epithelium and neural retina in E12–E17 embryos and the neuroblast and ganglion cell layers in the newborn rat expressed FGF-R mRNA. There is a considerable amount of evidence that FGF has roles in the development of the eye. First, FGF has been isolated from neural retina (Baird *et al.* 1985). Second, the retinal pigment epithelium both synthesizes and responds to FGF (Schweigerer *et al.* 1987; Plouet *et al.* 1988). Third, FGF also promotes process outgrowth of retinal ganglion cells *in vitro* (Lipton *et al.* 1988). Our present findings are consistent with these *in vitro* studies. However, our findings are inconsistent with a recent autoradiographic study that showed [^{125}I]bFGF binding primarily on the outer and inner plexiform layers where synaptic contacts occur (Fayein *et al.* 1990). This inconsistency may be derived from the difference in methodological approaches; *in situ* hybridization detects the cells synthesizing receptor messages, whereas [^{125}I]bFGF binding may preferentially detect the receptor protein expressed at the terminal surfaces. In addition to the embryonic retina, we found that FGF-R mRNA is expressed in the ganglion cell and inner nuclear layers of adult retina. It has been demonstrated that FGF can promote process outgrowth of adult ganglion cells in organotypic culture system (Bähr *et al.* 1989) and that intraocularly injected bFGF is internalized and anterogradely transported by adult ganglion cells in a receptor-mediated manner (Ferguson *et al.* 1990). Moreover, the labelling on the inner nuclear layer suggests that FGF can also influence the cells in the inner nuclear layer, such as bipolar cells,

horizontal cells, amacrine cells or Müller glia. In summary, the present study suggests multiple roles for FGF in different stages of retinal ontogeny.

In the inner ear of the embryo, FGF-R transcripts were detected in the sensory region (ie., hair cells and supporting cells) of the ampullae (Fig. 5A and B), utricle (Fig. 5C and D) and cochlea, in addition to the surrounding mesenchyme. The FGF-related proto-oncogene, *int*-2, was also found in these sensory regions (Wilkinson *et al.* 1989), suggesting that *int*-2 exerts differentiation effects on these developing sensory cells. Our results raise the possibility that FGF may also be involved in inner ear development. Alternatively, although it is not known whether *int*-2 can bind to the FGF receptor, it may be possible that *int*-2 exerts its effects through this FGF receptor. Indeed, recently, it has been shown that CHO cells transfected with the FGF-R gene can interact both with *hst*/K-FGF, a member of the FGF family and with basic FGF (Mansukhani *et al.* 1990).

FGF-R in non-neuronal embryonic tissues

When we compared our present results with the immunocytochemical analysis of bFGF in 18-day rat fetus (Gonzalez *et al.* 1990), we found a good correlation between these studies; most areas that were intensely labeled for FGF-R mRNA also show strong immunoreactivity for bFGF. For example, the compact mesenchyme of the skin and the perichondrium of the developing bone showed strong signal for FGF-R and also exhibit robust immunostaining for bFGF at the same embryonic stage. This overlapping distribution implies an autocrine or a paracrine nature of FGF action, although the precursor of FGF(s) lacks signal sequence and thus FGF(s) are not typical secretory proteins (Blam *et al.* 1988). It is conceivable that these developing tissues undergo excessive remodeling such that FGF could be released from intracellular storage. In addition, FGF has a strong affinity for extracellular matrix, and binding to the extracellular matrix prolongs half-life of FGF (for review, see Gospodarowicz *et al.* 1987).

Comparison with the distribution of other receptor-tyrosine kinase species

The FGF receptor belongs to a family of tyrosine kinase receptors (Lee *et al.* 1989; Reid *et al.* 1990; Saffran *et al.* 1990). This family consists primarily of growth factor receptors, such as EGF, insulin, IGF-1, PDGF and CSF-1 (for review, see Yarden and Ullrich, 1988). In addition to these well-characterized growth factor receptors, several proto-oncogenes, such as c-*kit* (Yarden *et al.* 1987) and *trk*B (Klein *et al.* 1989), are also members of this family. Although ligands for these putative receptor-tyrosine kinases have not yet been identified, recent reports reveal the widespread distributions of c-*kit* transcripts (Orr-Urtreger *et al.* 1990) and of *trk*B mRNA (Klein *et al.* 1990) in developing embryos, suggesting that these putative receptors are involved in embryogenesis. The distribution patterns of *trk*B (Klein *et al.* 1990) and c-*kit* transcripts (Orr-Urtreger *et al.* 1990) are significantly different from that of FGF-R mRNA; FGF-R mRNA is more broadly detected than these tyrosine-kinase receptors. It is likely that growth factors are differentially expressed in a tissue-specific manner and thereby, regulate the complex pattern of development.

During the initial reviewing process of this paper, one report on FGF-R mRNA expression in the developing chicken nervous system was published (Heuer *et al.* 1990). They demonstrated the localization of FGF-R mRNA in the ependymal layer of the chick embryo and these results are consistent with our present data.

In summary, the present localization of FGF-R mRNA in the developing nervous system revealed that there are basically two phases for their expression; First, FGF-R mRNA is broadly expressed in the mitotic precursor cell layer of the embryo, and subsequently, FGF-R mRNA appear in specific populations of postnatal neurons. These findings may warrant a reconsideration of the broad 'trophic effect' of FGF in culture systems which may represent effects on precursor populations rather than effects on postmitotic neurons. Also the further examination of the physiological functions and pharmacological effects of FGF on mature neuronal populations where receptor message is expressed (Wanaka *et al.* 1990) is warranted. The widespread distribution of FGF-R mRNA in the various embryonic organs and its similarity to that of FGF protein (Gonzalez *et al.* 1990) strongly suggest that FGF may influence the development of these organs in paracrine and/or autocrine manner(s).

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