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Developmental expression of two murine fibroblast growth factor receptors, *flg* and *bek*

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Summary

Developmental expression of two closely related fibroblast growth factor receptors, *bek* and *flg*, is described from early postimplantation until advanced organogenesis. Transcripts of *bek* and *flg* were first seen in the primitive ectoderm of egg-cylinder-stage embryos. Later, starting with somitogenesis, and then throughout embryogenesis, they were actively transcribed both in the mesoderm and neuroectoderm. *Bek* was expressed also in the surface ectoderm and in various epithelia, whereas *flg* expression was restricted mainly to the mesenchyme. In the limb bud *bek* transcripts displayed a gradient-like distribution and appeared earlier than *flg*. The two receptors, in contrast to their almost identical ligand binding specificity, displayed distinct spatial specificities throughout development, suggesting that developmental localization may contribute to functional specificity. The role of *bek* and *flg* in gastrulation and in epithelial–mesenchymal interactions of organogenesis will be discussed.

Key words: FGF receptors, embryogenesis, *in situ* hybridization.

Introduction

Fibroblast growth factors (FGF) and their receptors (FGF-R) have numerous important biological activities. They contribute to the control of normal and transformed cell growth and they are involved in tumor angiogenesis as well as in atherosclerosis (for reviews, see: Rifkin and Moscatelli, 1989; Folkman and Klagsbrun, 1987; Klagsbrun and Edelman, 1989). They are expressed in the developing embryo, where they are active in mesoderm induction (reviewed by Smith, 1989; Whitman and Melton, 1989). Hence the FGF system is important both for understanding early vertebrate development and for the analysis of medically important processes.

A number of FGF-related and other polypeptide growth factors can induce mesoderm in *Xenopus* animal caps. FGF mainly affects the posterior and ventral mesoderm, where it interacts with homeobox containing genes, whereas activin and members of the TGFβ family display stronger effects on the development of the anterior axis and head (Rosa, 1989; Ruiz i Altaba and Melton, 1989; Smith *et al.* 1990; Thomsen *et al.* 1990). It has been shown that oocytes and early *Xenopus* embryos contain FGF peptides (Kimmelman *et al.* 1988; Slack and Isaacs, 1989). Hence FGF and FGF-R are thought to be intrinsic to amphibian development. Less is known however, about their contribution to comparable events in mammalian embryogenesis (Wilkinson *et al.* 1988; Joseph-Silverstein *et al.* 1989; Gonzalez *et al.* 1990; Curatola and Basilico, 1990; Wanaka *et al.* 1990; Moore *et al.* 1991) and the information regarding FGF receptors falls short of what is known about their ligands.

The FGF family contains seven polypeptides, which are distinguished from other polypeptide growth factors by their affinity to heparin-like extracellular matrix components (for review, Burgess and Maciag, 1989). High affinity cell surface receptors for the FGFs are membrane-spanning protein tyrosine kinases. Their cDNA sequence places them, as a separate subgroup (Lee *et al.* 1989; Dionne *et al.* 1990; Avivi *et al.* 1991; Raz *et al.* 1991), into the receptor tyrosine kinase gene family (RTK, Yarden and Ullrich, 1988). Recently numerous FGF-Rs have been cloned, including three from chicken (cek1, cek2 and cek3; Lee *et al.* 1989; Pasquale, 1990), three from the mouse (*flg*, Safran *et al.* 1990 and Reid *et al.* 1990; *bek*, Kornbluth *et al.* 1988; Raz *et al.* 1991 and KGF-R, Miki *et al.* 1991) and seven from man (*flg* and *bek*, Dionne *et al.* 1990; Johnson *et al.* 1990; Reid *et al.* 1990; Mansukhani *et al.* 1990; *flg*-2, Avivi *et al.* 1991; k-*sam* Hattori *et al.* 1990; FGFR3 and FGFR4, Partanen *et al.* 1991; as well as TK14, Houssaint *et al.* 1990). The cek-1 chicken cDNA shows highest homology to the human and murine *flg* sequence, cek-3 of the chicken appears to be homologous to the mammalian *bek* and k-*sam* and KGF-R cDNA clones (Pasquale, 1990), whereas cek-2 may be

the cognate of murine *flg*-2 (Avivi *et al.* 1991) and human FGFR3 cDNA-s (Partanen *et al.* 1991).

FGF receptors are expressed with considerable variation. *Flg* transcripts were described both with two and with three immunoglobulin-like domains, which however, had similar ligand-binding specificity. Putative secreted forms have been observed also (Johnson *et al.* 1990; Reid *et al.* 1990; Mansukhani *et al.* 1990) and a recent report by Hou *et al.* (1991) suggests that cultured liver cells may express as many as twelve FGF-R variants, different in specific structural domains, but homologous along most of their sequence. Similar local nucleotide sequence variation separates the otherwise very closely related *bek*, k-*sam* and KGF-R cDNAs (Hattori *et al.* 1990; Miki *et al.* 1991; Raz *et al.* 1991). Therefore, it is not clear whether certain FGF-R cDNA clones represent separate loci or allelic variants.

Several FGF-R bind more than one polypeptide growth factor with high affinity. The human *bek* and *flg* receptors bind both acidic (aFGF) and basic FGF (bFGF) with similar affinities (Dionne *et al.* 1990). Mansukhani *et al.* (1990) demonstrated that a *flg* variant with two immunoglobulin-like loops binds both bFGF and *hst*/K-*fgf*. The KGF (keratinocyte growth factor) receptor, which is closely related to *bek* (Kornbluth *et al.* 1988; Miki *et al.* 1991; Raz *et al.* 1991), recognizes KGF and aFGF preferentially (Rubin *et al.* 1989). Moreover FGFR4 specifically binds aFGF rather than bFGF (Partanen *et al.* 1991). One interpretation for the multiplicity of the FGF – FGF-R system is based on the closely similar binding specificity of some FGF-R, like *bek* and *flg* (Dionne *et al.* 1990) and suggests that redundant sets of FGF-R may exist.

In this study we used *in situ* hybridization to compare the developmental expression of *bek* and *flg*. We aimed at an initial assessment of the role of FGF-R in mammalian embryogenesis. It was also expected that the developmental localization of *bek* and *flg* would reveal whether they are indeed redundant, and if they are functionally distinct despite their similar ligand binding specificity.

Materials and methods

Embryos

C57BL/6J mice were kept under a 14 h light, 10 h dark (from 7.30 p.m. to 5.30 a.m.) regime and the time of pregnancy was established by the presence of vaginal plugs the morning following mating. This time was regarded as day 0.5 *post coitum* (*p.c.*). Early embryos (6.0 to 8.0 days *p.c.*) were prepared for sectioning within the decidua. Later embryos were carefully dissected from their embryonic membranes. All embryos were prefixed in 4 % paraformaldehyde at 4°C.

Probes

The probes used in this study derived from a mouse brain cDNA library and they were cloned into BluescriptII KS+. The *bek* probe was a 281 bp long *Nla*IV–*Sma*I fragment starting 121 bp upstream from the assumed initiator methionine and included the hydrophobic leader sequence and part of the first immunoglobulin-like loop of *bek* (Raz *et al.* 1991). The hydrophobic leader sequence of *bek* and KGF-R (Miki *et al.* 1991) are identical (A. Yayon *et al.* unpublished data), therefore our *bek* probe, recognizes both the *bek* and the KGF-R transcripts. The *flg* probe was a 220 bp long *Sty*I–*Nru*I fragment including the leader sequence and entering 24 nucleotides into the first immunoglobulin-like domain (Safran *et al.* 1990). T3 and T7 polymerase-catalyzed transcripts were synthesized in the presence of ^{35}S-UTP for *in situ* hybridization. In each experiment both sense (control) and antisense transcripts were used for hybridization. No significant signals were obtained with sense (control) transcripts of these probes.

In situ *hybridization*

Embedding, sectioning (cryostat), post-fixation, hybridization and washing was as previously described (Orr-Urtreger *et al.* 1990). Post-hybridization washing was performed at high stringency in 2×SSC, 50 % formamide and 0.1 % dithiothreitol at 65°C, followed by RNAase digestion and by a further high-stringency wash. The slides were inspected and photographed in a Wild/Leitz Macroscope or in a Zeiss Photomicroscope III.

Results

Bek and flg expression during gastrulation

To test whether *bek* and *flg* are active in oogenesis, we analyzed their expression in adult ovaries. Both genes displayed low transcript levels in the ovarian stroma (not shown), whereas the ovarian follicles, the corpora lutea and various oocytes contained no detectable FGF-R transcripts. The apparent absence of *bek* and *flg* expression in the oocyte suggested that they may lack maternal regulation. This was in contrast to other RTK genes, like c-*kit* of the mouse, which is active both in male and female gametogenesis (Orr-Urtreger *et al.* 1990), or *torso* and *faint little ball,* which are maternal mutations of *Drosophila* (Sprenger *et al.* 1989; Schejter and Shilo, 1989).

With the appearance of the proamniotic cavity both *bek* and *flg* were expressed in the primitive ectoderm (Fig. 1A–D). The *flg* probe hybridized to most cells along the proamniotic cavity, suggesting that it is active both in the extraembryonic and in the embryonic ectoderm (Fig. 1A and B). *Bek* displayed higher transcript concentrations than *flg*. In the midsagittal plane *bek* transcripts were confined to the embryonic ectoderm through its whole extent and showed no anterior or posterior preference (Fig. 1C and D). In more parasagittal planes, *bek* transcripts accumulated in the extraembryonic ectoderm (not shown). Thus, it seems that *bek* and *flg* are expressed both in the ICM (inner cell mass)-derived and in the trophectoderm-derived ectoderm of the egg cylinder.

Mouse embryos at the early egg cylinder stage are made of two cell layers, the primitive ectoderm and the visceral endoderm. Around the sixth day of gestation ectodermal cells exfoliate through the primitive streak into the space between these two layers, and the mesoderm forms. Serial sections were used to find nascent mesoderm in 6.5 and 7.5 day-old embryos. At 7.5 days *bek* expression was easily detectable in the primitive ectoderm-neural plate and it culminated in

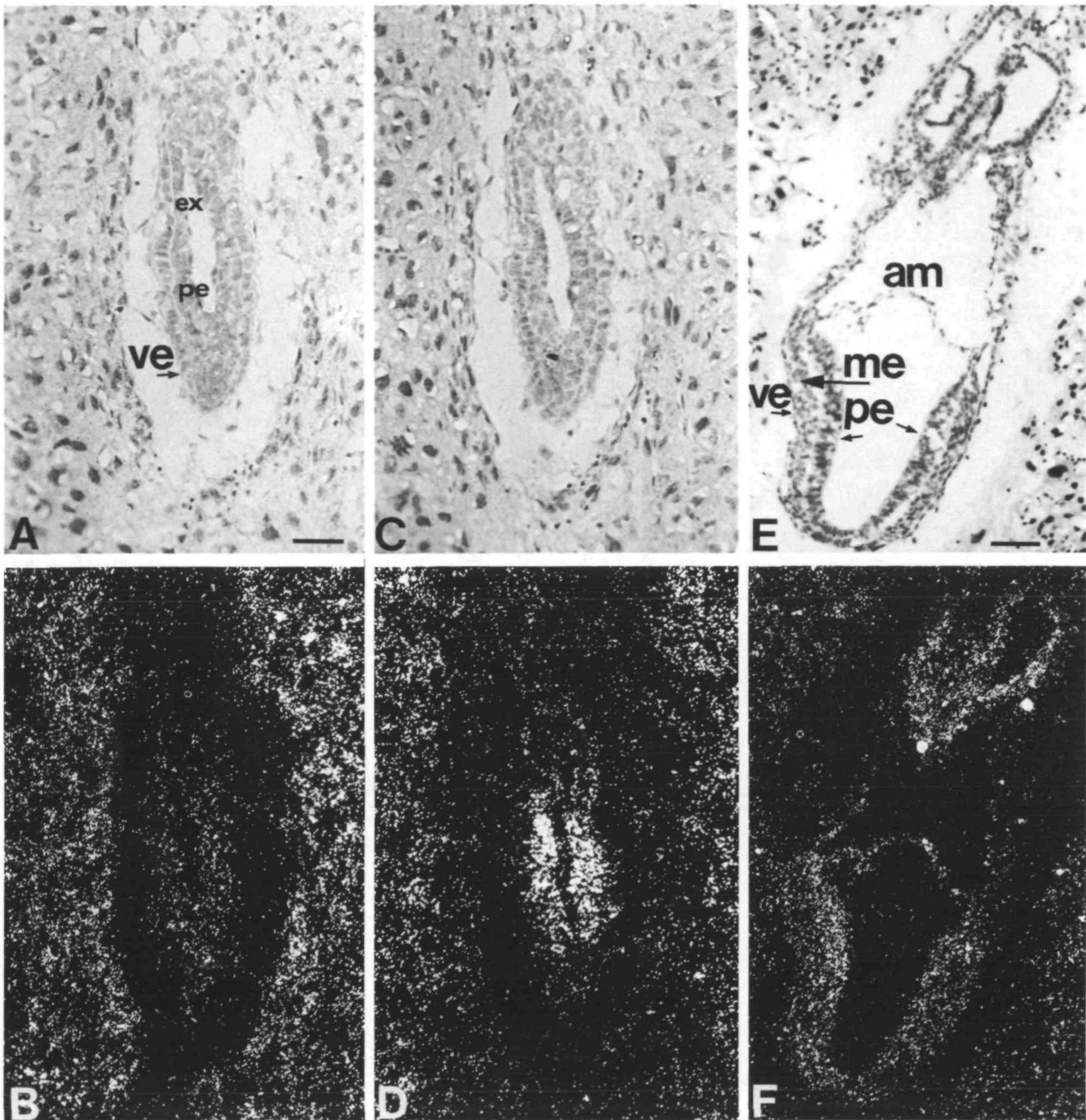

Fig. 1. Expression of *bek* and *flg* during gastrulation. (A,C and E) Bright-field photographs; (B,D and F) dark-field photographs of the same sections respectively of 6.5 day-old (A–D) and 7.5 day-old (E and F) mouse embryos. (B) Hybridization with the *flg* probe; (D and F) hybridization with the *bek* probe. Abbreviations: am, amnion; ex, extraembryonic ectoderm; me, mesoderm; pe, primitive ectoderm; ve, visceral endoderm. Bars: A–D, 50 μm; E and F, 80 μm.

the head fold. In these embryos the non-segmented, presomitic mesoderm was already clearly visible; it however displayed only weak hybridization signals, detectable both in the embryonic and extraembryonic mesoderm (Fig. 1E and F).

In the 8 day-old mouse embryo the mesoderm differentiates into axial, paraxial and lateral mesoderm. The paraxial mesoderm then aggregates into somites and the anterior part of the neural plate develops into the cranial folds. Fig. 2 demonstrates the expression of both FGF-R genes at this stage. Ectodermal expression at this stage exhibited once again the strongest signals in the cranial folds. *Bek* was expressed also in the somites, in the surface ectoderm and in the heart primordium (Fig. 2A–D), whereas *flg* was expressed (at lower levels) in the presomitic mesoderm (Fig. 2E and F). Taken together, at the primitive streak stage the two FGF-R genes were first expressed in the extraembry-

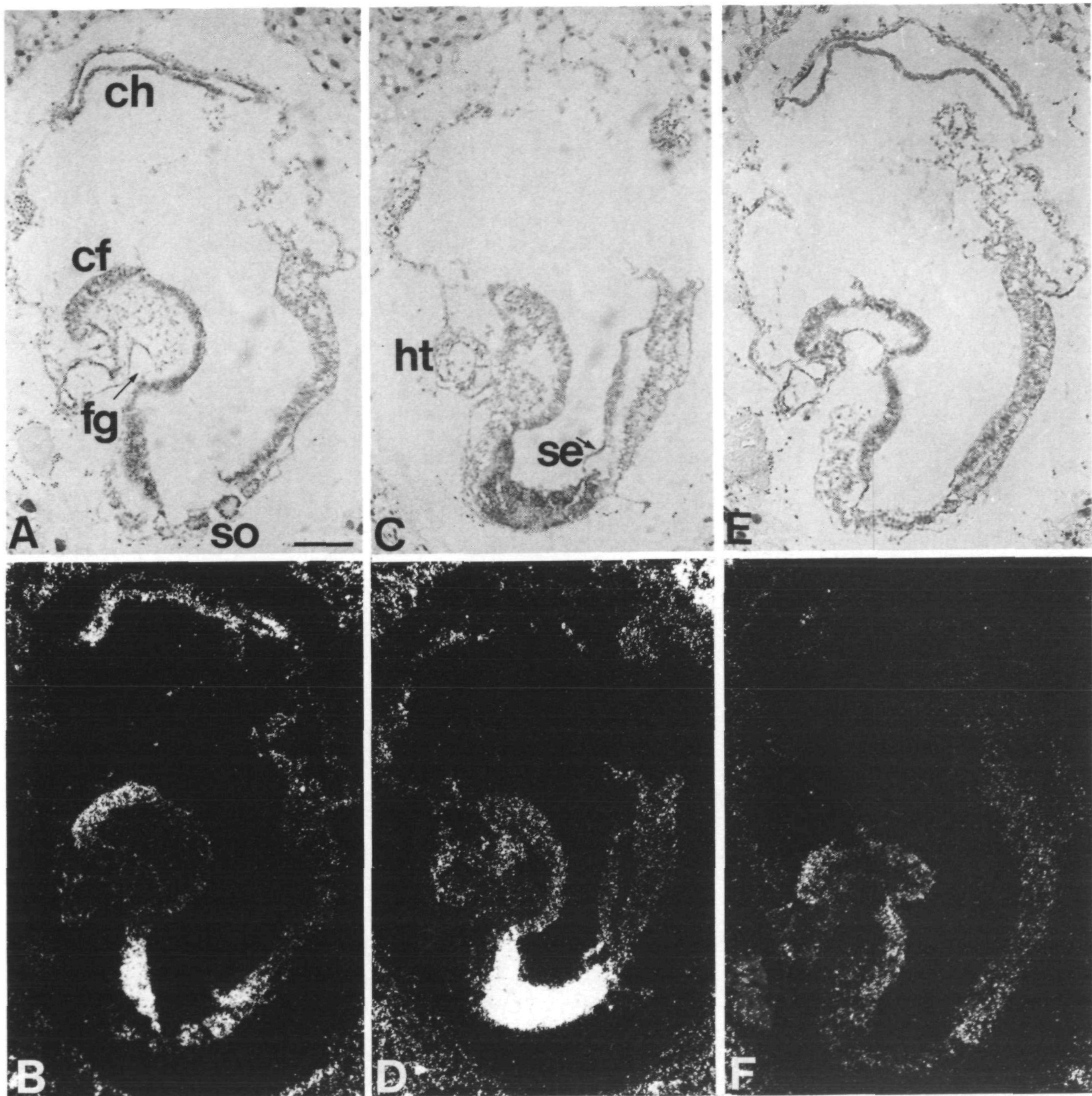

Fig. 2. Expression of *bek* and *flg* in 8 day-old embryos. (A,C and E) Bright-field photographs; (B,D and F) dark-field photographs of the same sections. Near sagittal (A,E) and parasagittal (C) sections. (B, D) hybridization with the *bek* probe; (F) hybridization with the *flg* probe. Abbreviations: cf, cranial folds; ch, chorion; fg, foregut; ht, heart; se, surface ectoderm; so, somites. Bar: 150 μm.

onic and embryonic ectoderm, whereas later in development they appeared also in early derivatives of the mesoderm.

Activity of the two FGF receptors in lateral mesoderm and limb development

Transversal sections of 9.5 day-old embryos were hybridized with *bek* and *flg* probes. *Bek* was expressed in the lateral and splanchnic mesoderm. Its transcripts were apparent also in the cranial and dorsal neural tube and in the surface ectoderm of the embryo (Fig. 3B,E–G). *Bek* hybridization signals in the embryonic integument originated in the most superficial cell layer (the surface ectoderm) and not in the section–slide interface (edge effect), as it can be judged by comparing these signals with those in consecutive sections hybridized with *flg* (Fig. 3C and H). Transcripts of both *bek* and *flg* were apparent along the anterior part of the body wall and a pair of sites with

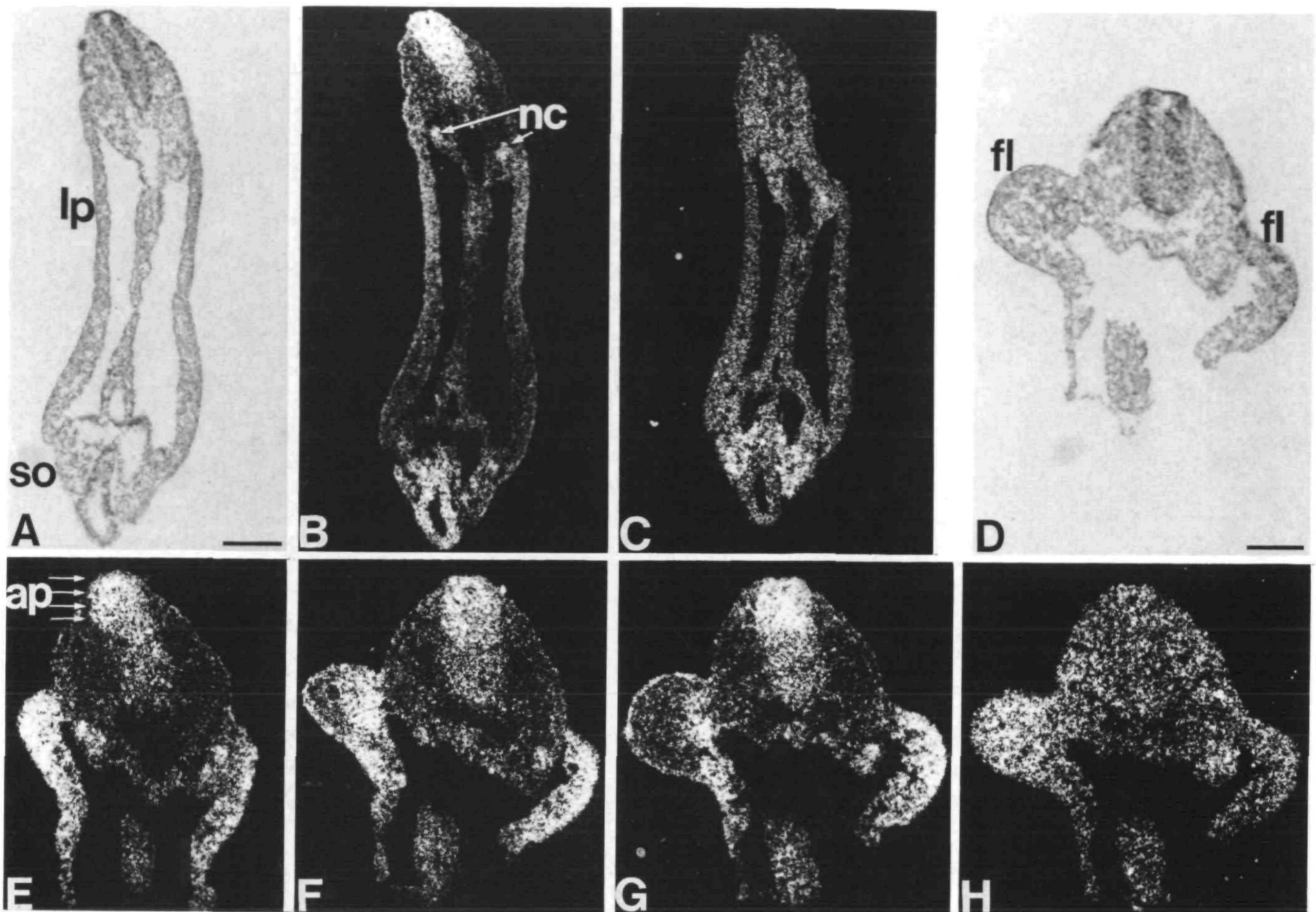

Fig. 3. Expression of *bek* and *flg* in the lateral mesoderm and limb bud (9.5 days *p.c.*). (A–C) Transverse sections hybridized with *bek* (B) or *flg* (C) probes. (D,G and H) Transverse sections through the level of the forelimb bud, hybridized with *bek* (G) and *flg* (H) probes. (E–G) Serial sections hybridized with *bek*. G is the most anterior section of the series. Abbreviations: ap, alar plate (arrows indicate its approximate extent); fl, forelimb bud; lp, lateral plate; nc, nephrogenic cords; so, somite. Bars: A–C, 200 μm; D–H, 140 μm.

relatively high concentrations of transcripts could be seen in the dorsal roof of the embryonic coelom, which corresponded to the nephrogenic cords.

Bek transcripts were unevenly distributed (Fig. 3D–G) in the early (9.5 days *p.c.*) forelimb bud. Their concentration increased in posterior and proximal direction towards a position close to the body wall. Similar observations were also made one day later, when the first signs of mesenchymal condensations became visible (Fig. 4B). The expression of *flg* was more homogeneous than that of *bek* (Fig. 3H), but later, at day 10.5, this gene also was expressed in a nonhomogeneous manner along the limb bud (Fig. 4C). Next, at 11.5 days *p.c.*, *bek* expression localized to mesenchymal aggregates corresponding to the future bones of the limb. This process was less evident in consecutive sections hybridized with the *flg* probe (Fig. 4D–F). Thus the expression of *bek* followed more closely the osteogenic mesenchymal condensation than *flg*. Fig. 4 demonstrates another characteristic difference between the localization of *bek* and *flg*, already observed in younger embryos. It shows *bek* transcripts in the surface ectoderm of the limb, as a continuous contour, which seems to be strongest in the interdigital web (at 14.5 days *p.c.*, see Fig. 5E). In contrast *flg* transcripts are distributed diffusely in the mesenchyme.

To continue studying the role of FGF-R in limb development, 12.5 and 14.5 day-old embryos were investigated (Fig. 5). Ossification in the limb starts in the long bones (humerus, radius and ulna) and continues radially towards the metacarpal bones and finally reaches the phalanges and the carpal bones. *Bek* and *flg* expression followed this order. Our data demonstrate that *bek* transcripts occupy the chondrification centers at 12.5 days (Fig. 5B) and the body of the distal bones (phalanges and carpals) at 14.5 days *p.c.* Later they appear in the periphery of the developing radius, in which ossification (calcification) has already commenced (Fig. 5E; see also Rugh, 1968). This temporal order of expression suggested that the FGF system may have a genuine role in limb and bone development. The presence of FGF in the regenerating limb blastema of urodele amphibians also supports this notion (Boilly *et al.* 1991).

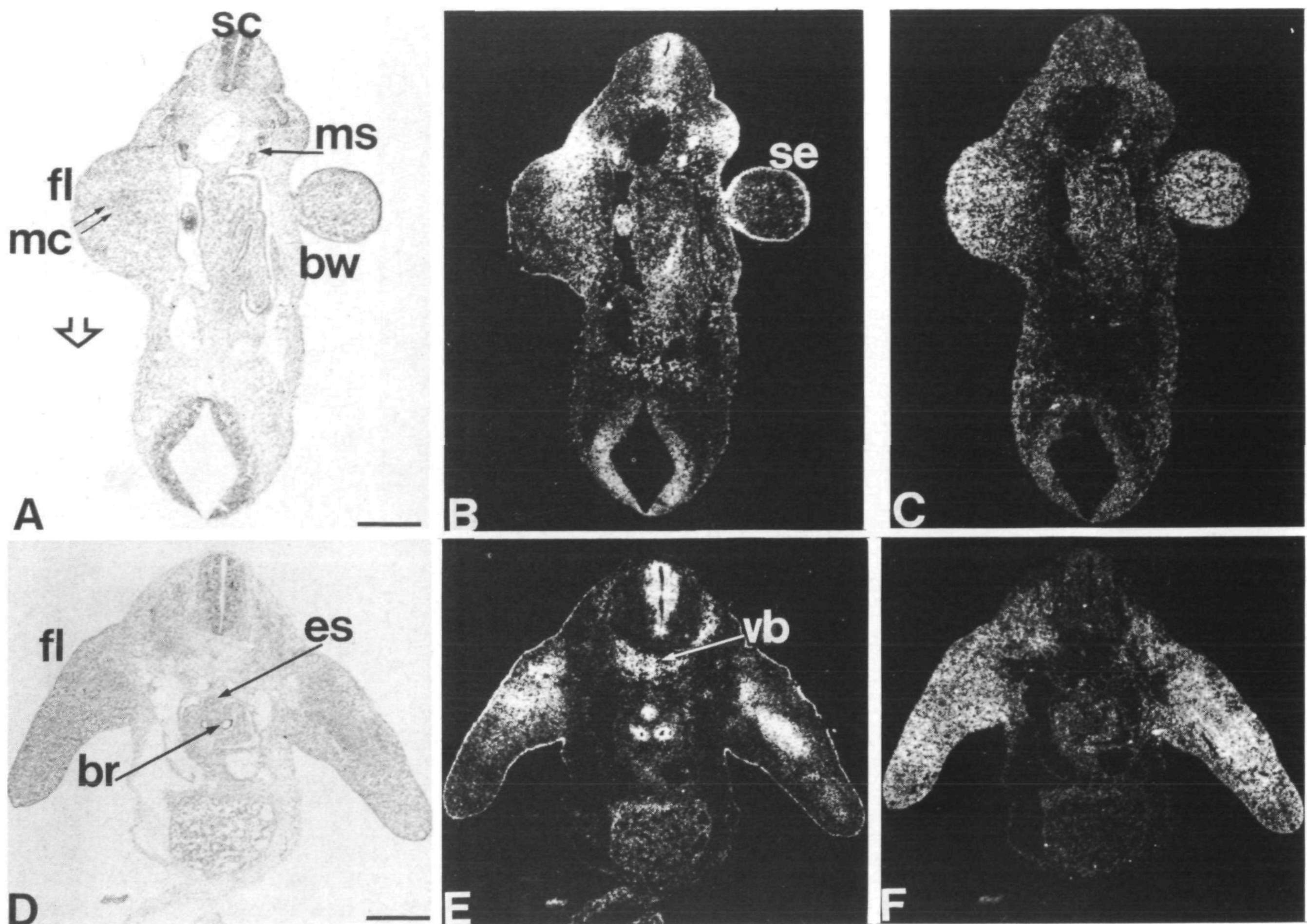

Fig. 4. Expression of *bek* and *flg* in the forelimbs of 10.5 (A–C) and 11.5 (D–F) day-old embryos (transverse sections). (B, E) Hybridization with the *bek* probe; (C,F) hybridization with the *flg* probe. Abbreviations: br, bronchus; bw, body wall; es, esophagus; fl, forelimb; mc, mesenchymal condensation; ms, mesonephros; sc, spinal cord; se, surface ectoderm; vb, vertebral body. Outlined arrow points to anterior part of the embryo. Bars: A–C, 360 μm; D–F, 500 μm.

Both FGF receptors are active in numerous aspects of organogenesis

Sagittal, parasagittal and transversal sections of whole embryos (Figs 6, 7F–K and 8) demonstrate that the two FGF receptors are active in most developing organs. Sagittal sections show that both are transcribed in distinct areas of the *nervous system*. They are present in the cerebral ganglia, in the ependymal layer and in elements of the developing cortex and spinal cord. *Bek* is present also in the choroid plexus (Fig. 6B,E). In the spinal cord it is initially expressed in the alar plate (9.5–10.5 days, Figs 3, 4) and later (12.5 and 14.5 days *p.c.*) it concentrates, together with *flg*, to the ependymal layer (Fig. 8).

Both FGF-R are active in the development of the *sense organs*. Fig. 7B and C show the expression of *bek* and *flg* in the otic vesicle at 10.5 days *p.c.* At 14.5 days of gestation only *bek* expression could be detected in derivatives of the otic vesicle, when it appeared in the semicircular and cochlear ducts (Fig. 7J). *Bek* and *flg* apparently also contribute to eye development. This is suggested by their presence in the lens, by *bek* expression in the corneal epithelium and by the expression of *flg* in the cornea and sclera (not shown).

Bek expression in the *digestive and excretory system* could be observed at the earliest in the foregut pocket of 8 day-old embryos (Fig. 2A and B). *Bek* transcripts were detected in the epithelium of the pharynx (Fig. 6A,B,D and E; Fig. 7A,B,D and E), the oesophagus (Fig. 4D and E and Fig. 8A,B,D and E) and the cloaca (Fig. 8G and H). The highest concentration of *bek* transcripts was observed in the stomach epithelium (Fig. 8G and H). This expression pattern is specially noteworthy because amplification of the human analogue of the murine *bek* transcript, k-*sam*, is associated with dedifferentiated stomach cancer (Hattori *et al.* 1990). *Bek* is also expressed in the mesodermal wall of the intestine, which is derived from the splanchnic mesoderm (Fig. 6B,E and Fig. 8B,H). Diverse derivatives of the embryonic foregut, the liver, the pancreas, the epithelium of the bronchial tree and the alveoli of the lung also express *bek*. No *flg* transcripts were, however, apparent in these structures (Figs 6–8). In contrast the genital eminence expressed both FGF-R at

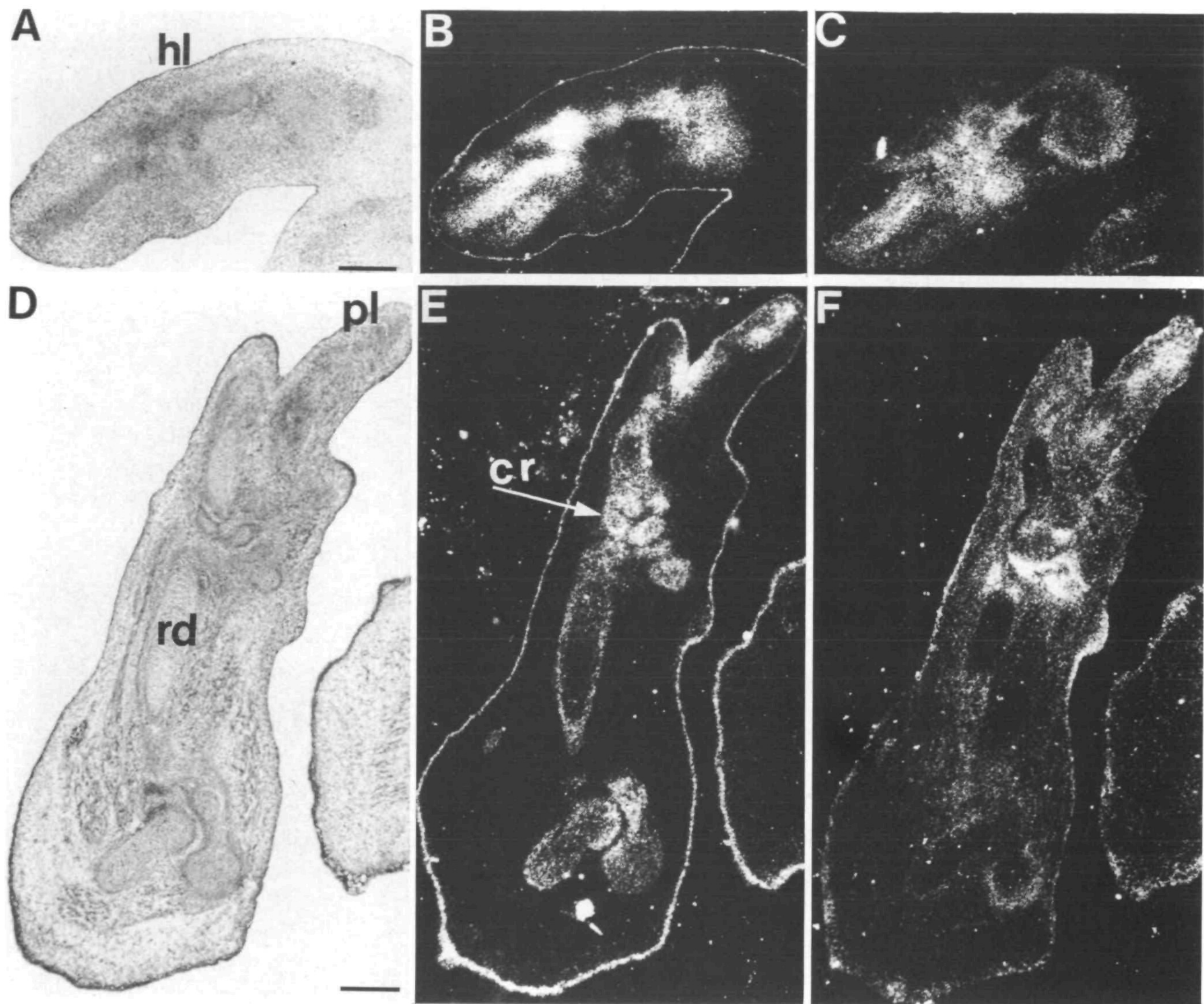

Fig. 5. Expression of *bek* and *flg* in the limbs of 12.5 (A–C) and 14.5 (D–F) day-old embryos. (B,E) Hybridization with the *bek* probe; (C,F) hybridization with the *flg* probe. Abbreviations: cr, carpals; hl, hind limb; pl, phalanx (distal); rd, radius. Bars: A–C, 400 μm; D–F, 300 μm.

high level. *Bek* transcripts were more prominent in the surface ectoderm and in the invaginating epithelium at the entrance of the urethra, which derives from the ectoderm, whereas *flg* was more prominent in the mesenchyme (Figs 6 and 8).

Figs 6, 7 and 8 demonstrate the two FGF receptors' activity in *osteogenesis* in addition to what was also seen during limb development. The most notable observation was that both receptors were apparently expressed in all prospective bones; they were present in the basicranium, scapula, the ribs and in the sternum, as well as in the long and short bones of the extremities (Figs 5–8) and in the vertebrae (Figs 6, 7). *Bek* transcripts are present in the body of the vertebrae at day 12.5 and by day 14.5 of embryonic development, when calcification starts in the vertebral body, they appear in the periphery of the prevertebrae. As in limb development, also in the developing vertebral column *bek* is expressed earlier than *flg* (Fig. 4).

Both *bek* and *flg* are expressed in the craniofacial area. *Flg* transcripts are characteristically present in the diffuse mesenchyme of the trunk (Fig. 6F; Fig. 8C,F and I), face, tongue and mandibula (Fig. 6C and F; Fig. 7H and K). These areas however, are mostly free from *bek* transcripts (Fig. 6B and E; Fig. 7G and J). At 10.5 and 11.5 days of gestation both FGF-R are expressed in the maxillary process and in the mandibular and hyoid arches (Fig. 7A–E). *Bek* expression is not uniform along the branchial arch, and as shown in Fig. 7B, its transcripts are distributed in a gradient increasing from the base of the arch towards its distal tip. Parasagittal sections taken at 12.5 and 14.5 days of development show that the *flg* message is more abundant in the facial mesenchyme, whereas *bek*

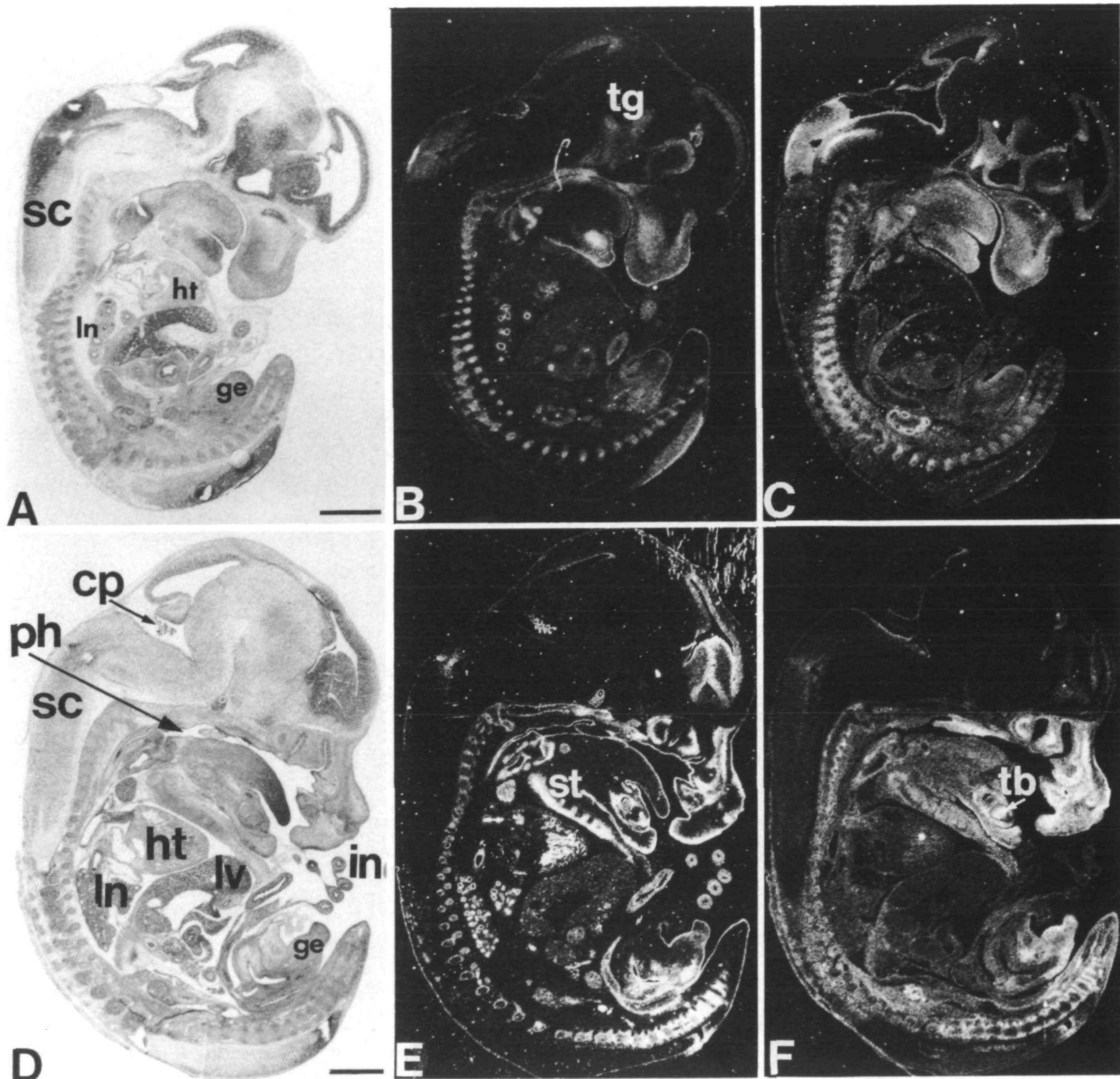

Fig. 6. Expression of *bek* and *flg* in 12.5 (A–C) and 14.5 (D–F) day-old mouse embryos (sagittal sections). (B,E) Hybridization with the *bek* probe; (C,F) hybridization with the *flg* probe. Abbreviations: cp, choroid plexus; ge, genital eminence; ht, heart; in, intestine; ln, lung; lv, liver; ph, pharynx; sc, spinal cord; st, sternum; tb, tooth bud; tg, trigeminal ganglion. Bars: 1 mm.

transcripts are concentrated more to the frontal bone, the maxilla, the mandibula, and certain areas of the fore and hindbrain (Fig. 7F–K). *Bek* was also detected in the ossicles of the middle ear, which derive from the first and second branchial arches (Fig. 7J).

A common feature is that *bek* is frequently expressed in the epithelium, whereas *flg* is expressed mainly in the mesenchyme, in the majority of the developing organs surveyed by us. Two examples, kidney and skin development, illustrate this issue further.

Bek and flg expression in the developing kidney

Both *bek* and *flg* are expressed already in the nephrogenic cords of 9.5 day-old embryos (Fig. 3). They are also present in the mesonephros (Fig. 4 and unpublished data) and their relative expression can be compared well in the developing permanent mammalian kidney, the metanephros (Fig. 9). The kidney develops through interactions between the epithelium of the metanephric diverticulum (ureteric bud) and the metanephric (intermediate) mesoderm. Fig. 9A–C demonstrates the expression of *bek* and *flg* in the embryonic kidney at 12.5 days of development. *Bek* is mostly expressed in the epithelium of the ureteric bud and its branches (Fig. 9B), whereas abundant *flg* transcription can be seen in the surrounding mesenchymal condensations and in their derivatives, the meta-

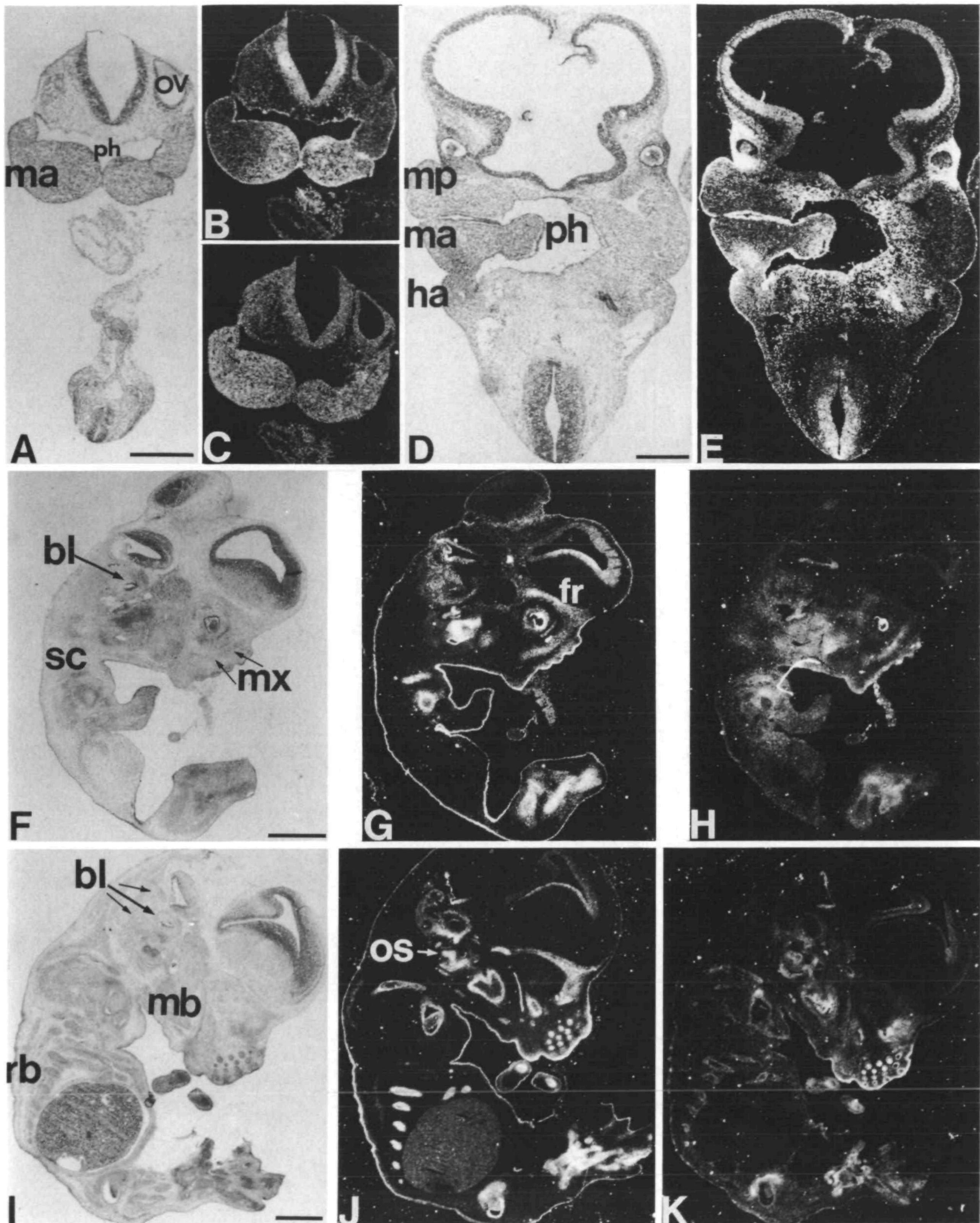

Fig. 7. Expression of *bek* and *flg* in the craniofacial area. Transverse sections through the mandibular arches of 10.5 (A–C) and 11.5 (D and E) day-old mouse embryos, and parasagittal sections of 12.5 (F–H) and 14.5 (I–K) day-old mouse embryos. Sections B,E,G and J were hybridized with the *bek* probe and sections C,H and K with the *flg* probe. Abbreviations: bl, bony labyrinth and semicircular ducts; fr, frontal bone; ha, hyoid arch; ma, mandibular arch; mb, mandibula; mp, maxillary process; mx, maxilla; os, middle ear ossicles; ov, otic vesicle; ph, pharynx; rb, ribs; sc, scapula. Bars: A–E, 0.5 mm; F–K, 1 mm.

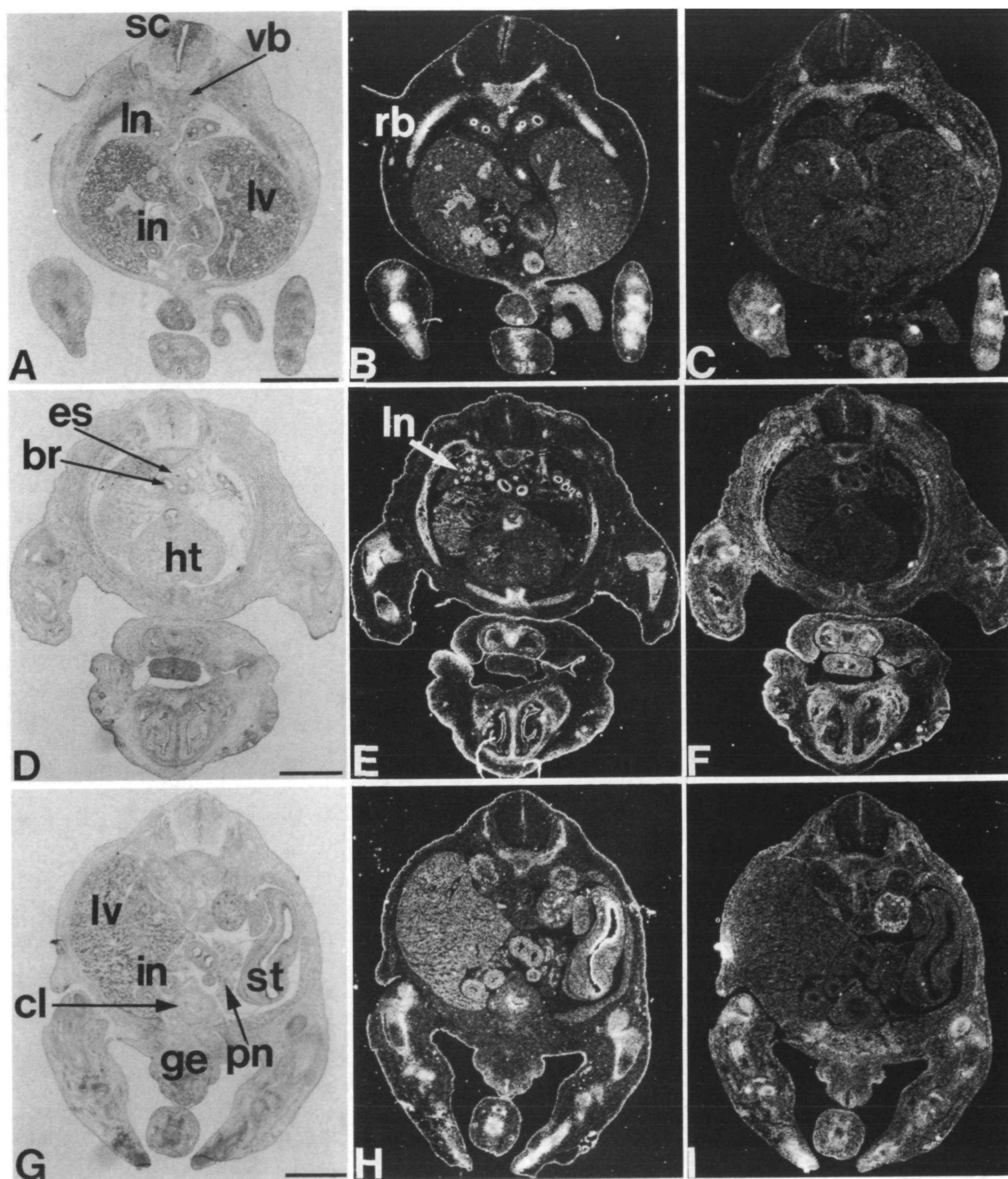

Fig. 8. Expression of *bek* and *flg* in 12.5 (A–C) and 14.5 (D–I) day-old mouse embryos (transverse sections). (B,E and H) Hybridization with *bek* probe, (C,F and I) hybridization with *flg* probe. (A–C) Sections at the level of the upper abdomen; (D–F) sections at the level of chest and through the mouth (coronal view); (G–I) sections at the level of the mid-abdomen. Abbreviations: br, bronchi; cl, cloaca; es, esophagus; ge, genital eminence; ht, heart; in, intestine; ln, lung; lv, liver; pn, pancreas; rb, rib; sc, spinal cord; st, stomach; vb, vertebral body. Bars: 1 mm.

nephric vesicles (Fig. 9E). In the 14.5 day-old embryonic kidney *bek* is present in the epithelium of the collecting tubules (Fig. 9F), which is derived from the metanephric diverticulum, while *flg* transcripts occupy mesenchymal elements in the cortical areas of the embryonic kidney (Fig. 9F).

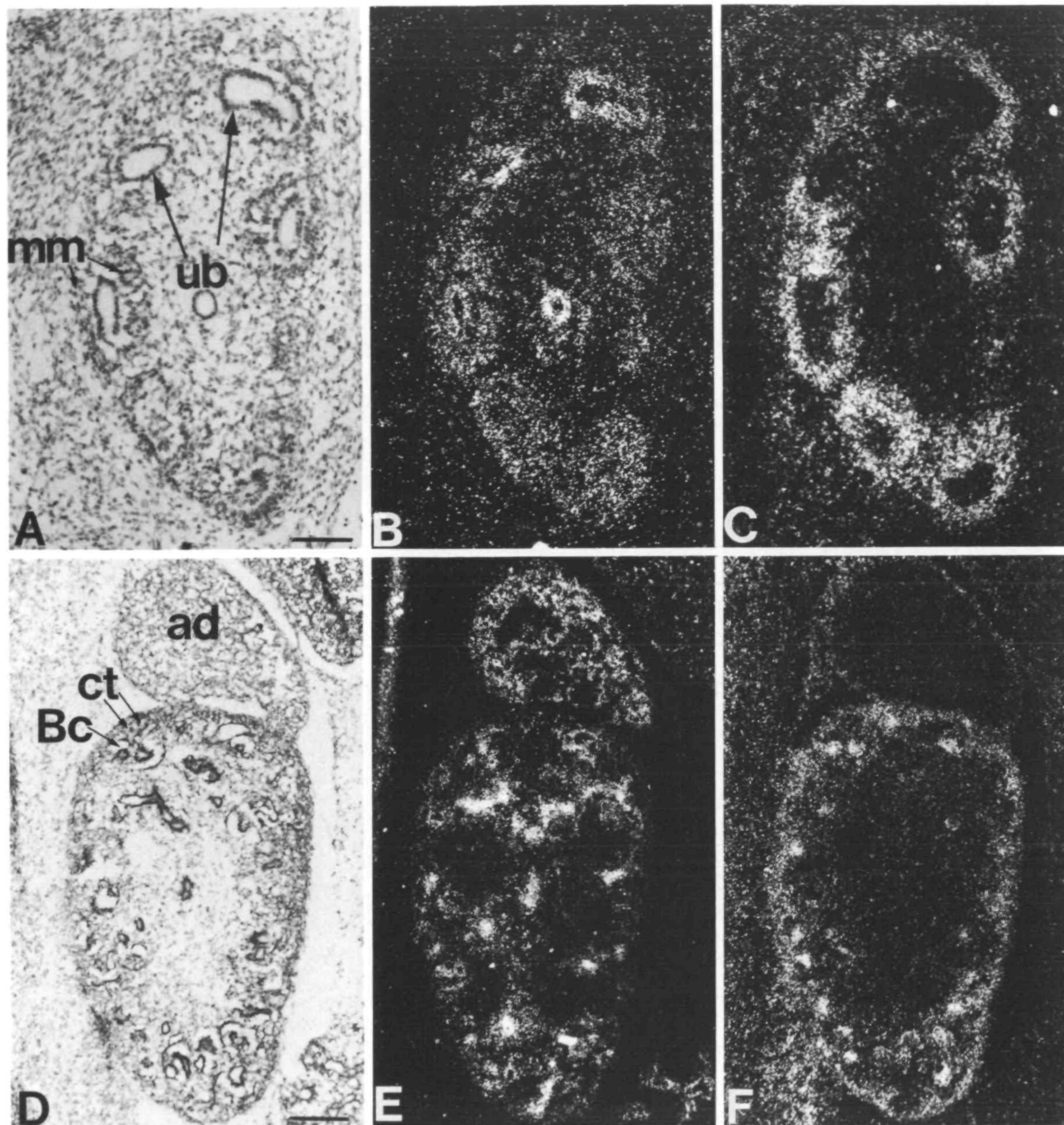

Fig. 9. Expression of *bek* and *flg* in the kidney of 12.5 (A–C) and 14.5 (D–F) day-old mouse embryos (sagittal sections). (B,E) Hybridization with the *bek* probe; (C,F) hybridization with the *flg* probe. Abbreviations: ad, adrenal gland; Bc, Bowman's capsule; ct, collecting tubules; mm, metanephric (condensed) mesenchyme and vesicles; ub, ureteric bud and its branches (epithelium). Bars: A–C, 100 μm; D–F, 160 μm.

FGF receptor expression in the developing skin

Skin and its appendages (hair, teeth and certain membrane bones) develop from interactions between ectodermal and mesodermal elements. The integument of the early embryo is covered by a single ectodermal cell layer, the surface ectoderm. This cell layer displayed one of the most characteristic developmental expression patterns of the *bek* gene. It can be seen, in most of the figures presented in this report, that the hybridization signals of *bek* trace the contour of the embryo (Figs 3–8). Higher magnification revealed that this signal originates in the surface ectoderm (Fig. 10B). The latter develops into the actively dividing layer of the adult epidermis, the stratum germinativum, and gives rise to keratinocytes, which produce the keratinized protecting layer of the adult skin. In 14.5 day-old embryos *bek* is present in the epidermis (Fig. 10A,B) and *flg* transcripts accumulate immediately beneath it, in the mesenchyme of the prospective dermis (Fig. 10C,E). This relationship between the two receptors' expression pattern demonstrates that *bek* and *flg* occupy adjacent positions at the epithelial–mesenchymal interface of the developing skin.

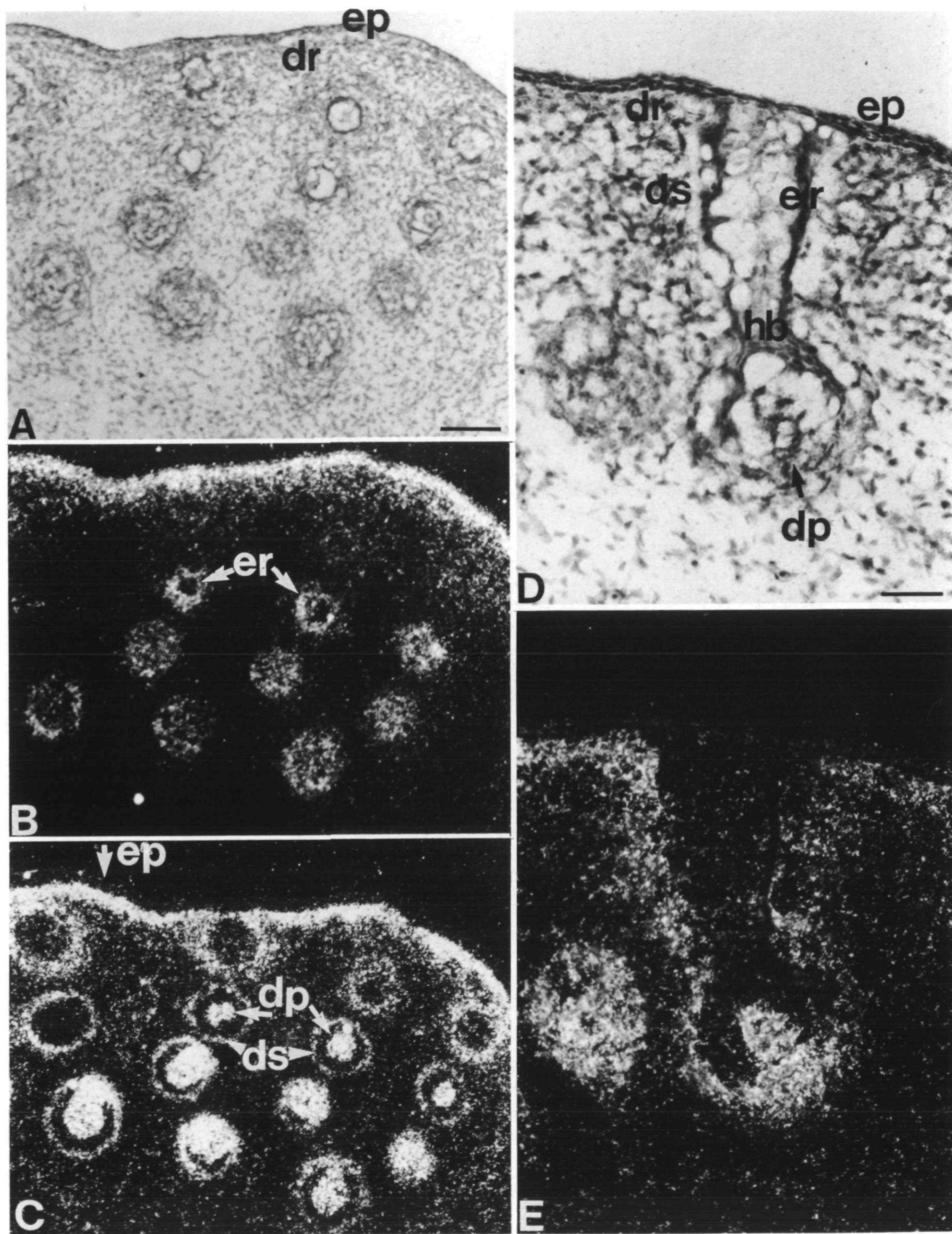

Fig. 10. Expression of *bek* and *flg* in the skin and vibrissal hair roots of 14.5 day-old embryos. (A–C) Cross sections through the hair root at various levels hybridized with *bek* (B) and *flg* (C). (D,E) Longitudinal section through the hair root hybridized with *flg*. Abbreviations: dp, dermal papilla; dr, dermis; ds, dermal sheath; ep, epidermis; er, epithelial root sheath; hb, hair bulb. Bars: A–C, 125 μm; D and E, 50 μm.

Hair follicles develop from invaginations of the epidermis. Mesodermal cells then penetrate into the base of these invaginations and form the dermal papilla. Longitudinal and transversal sections through the hair root, shown in Fig. 10, demonstrate that mesodermal derivatives, the dermal papilla and sheath, express the

flg receptor, whereas the ectodermal components, the epithelial root sheath and bulb, contained *bek* transcripts. In hair and skin development, as in the development of most organs, *bek* occupies epithelial positions, whereas *flg* occupies mesenchymal positions. Additional skin derivatives, teeth, and mammary glands, like hair, also develop by epithelial–mesenchymal interactions and *bek* and *flg* here too occupy epithelial or mesenchymal components respectively (Fig. 6E and F and unpublished).

These patterns of expression demonstrate that *bek* and *flg* occupy distinct locations within different organs. Unique expression patterns were seen in the diffuse mesenchyme of the face, in the extremities and in the trunk. This domain of *flg* expression, which precedes myogenesis, may be connected with the expression of *flg* in myoblasts (Moore *et al.* 1991). No *bek* transcripts were found in this domain, which emphasizes that the two receptors and their possible transcriptional variants, are functionally distinct throughout development.

Discussion

Developmental localization suggests distinct roles for bek *and* flg

The overall nucleotide sequence homology of the *bek* and *flg* cDNA-s is 71 %. Their kinase domains are 90 % homologous and the second and third immunoglobulin-like domains are close to 80 % similar. In contrast, their first immunoglobulin-like loops and the respective interkinase segments are more divergent (Raz *et al.* 1991). In agreement with this structural homology, the two receptors display almost identical binding specificity. Because of this similarity it was argued that *bek* and *flg* may be functionally redundant (Dionne *et al.* 1990). Our localization studies, however, reveal that *bek* and *flg* have clearly distinguishable spatial specificity during development. Not only the characteristic epithelial–ectodermal preference of *bek* distinguishes it from *flg,* but also their differential expression during osteogenesis, kidney and hair follicle development, all point to the functional distinction of these two receptors.

The *bek* probe, which was used in the present study, includes the hydrophobic leader sequence and part of the first immunoglobulin-like loop of the *bek* cDNA (Raz *et al.* 1991). This area is identical in *bek* and KGF-R. Therefore, our probe does not discriminate between the probably allelic *bek* and KGF-R transcripts (Miki *et al.* 1991; Raz *et al.* 1991; Dionne *et al.* 1990; A. Yayon *et al.* unpublished). The present results however, are consistent with the suggestion that *bek* and *flg* have independent patterns of expression. The strongest argument pointing to the independent expression of *bek* and *flg* is the absence of *bek* transcripts in the undifferentiated mesenchyme, which appears to be a specific domain for *flg* expression.

It is unusual that two receptors with similar structures and binding specificities should be conserved through evolution in man, mouse and chicken (Dionne *et al.* 1990; Pasquale, 1990; Raz *et al.* 1991). Our data suggest that these genes may acquire functional specificity through their developmental localization. An additional influence on the specificity of this system could be the localization of the heparin-binding sites for FGF in the extracellular matrix (Vainio *et al.* 1989; Trautman *et al.* 1991; Yayon *et al.* 1991). Hence, the evolution of the FGF system, because its diversity, which seems to derive from the spatial restriction of its receptors and ligands, may not have required the complex binding site polymorphism of other receptor–ligand systems. A system of specifically localized receptors, with redundant binding specificity, however, could be highly sensitive to quantitative and spatial changes. The transforming activity of *hst* /K-*fgf,* isolated from Kaposi sarcoma and from stomach cancer (Delli Bovi *et al.* 1987; Taira *et al.* 1987), as well as the association of the k-*sam* protooncogene with stomach cancer (Hattori *et al.* 1990), may be related to such mechanisms.

FGF-R expression during gastrulation

Our data suggest that *bek* and *flg* are probably devoid of maternal expression. They were however, readily detectable in the egg cylinder (6–6.5 days *p.c.*). Transcripts of the two FGF-R were concentrated in the ectoderm along the proamniotic cavity. We assume that FGF-R expression in the primitive ectoderm may be connected to the role of FGF in mesoderm induction. In *Xenopus* FGF affects posterior and lateral rather than anterior areas of the rostrocaudal axis (for reviews, Ruiz i Altaba and Melton, 1989; Smith, 1989), whereas *bek* and *flg* are expressed along the whole anterioposterior extent of the primitive ectoderm. Whether in mammals, as in amphibians, FGF affects the posterior part of the axis, remains to be determined.

Little is known about the expression of the various members of the FGF family in the early mouse embryo. *Int-2,* an FGF-like protooncogene, somewhat similarly to our FGF receptors, is expressed in the early migrating mesoderm and neuroectoderm (Wilkinson *et al.* 1988). Another protooncogene of the FGF family, *hst*/K-*fgf,* is selectively transcribed in embryonic carcinoma cells (Brookes *et al.* 1989; Curatola and Basilico, 1990). Quite recently Haub and Goldfarb (1991) as well as Hebert *et al.* (1991) reported that a member of the FGF family, FGF-5, is first expressed, after implantation, in the primitive ectoderm of the egg cylinder and later in the lateral mesoderm including the limb bud. Although FGF-5 is only one of seven members of the FGF family and its specific receptor is not known, there appears to be a basic similarity between its expression pattern and that of the two FGF receptors described here.

Other polypeptide growth factors, like members of the TGFβ gene family, which are also potent mesoderm inducers (Whitman and Melton, 1989), are expressed in the oocyte (Rappolee *et al.* 1988; Lyons *et al.* 1989). In the early embryo TGFβ1 was already present in the heart anlage at 8 days of gestation (Akhurst *et al.* 1990), whereas BMP-4 transcripts first became distinctive in

the posterior mesoderm of the 8.5 day neurula (Jones *et al.* 1991).

Bek and *flg* expression in late presomitic–early somitic stage embryos coincides with the expression of the TGFβ family. This temporal and spatial coincidence supports the notion that a multiplicity of polypeptide growth factors and receptors, are localized together at the time of gastrulation both in amphibia and in mammals. Further relevant connections can be made between the pattern-forming activity of homeoboxes and polypeptide growth factors in the amphibian embryo (Ruiz i Altaba and Melton, 1989) and between the localization of retinoic acid receptors and retinoid-binding proteins (Ruberte *et al.* 1991) with our two FGF receptors in late presomitic and early somitic mouse embryos.

FGF receptor expression during organogenesis

Bek and *flg* are expressed in ectoderm and mesoderm derivatives during the development of many organs. A general characteristic of their developmental regulation appeared to be the constancy of their localization. *Bek* and *flg* transcripts usually appeared very early within the first anlage of an organ and accompanied it, in progressively differentiated form, throughout embryogenesis, as in limb, kidney, heart, sense organ, or skin development. Similar constancy of developmental expression was observed also with two other receptor tyrosine kinases, c-*kit* (Orr-Urtreger *et al.* 1990) and the α receptor of PDGF (Orr-Urtreger *et al.* unpublished data) and may be characteristic for the PDGF-R and FGF-R subfamilies of receptor tyrosine kinases.

There is little information regarding the developmental expression of the ligands of *bek* and *flg*. Basic FGF, a ligand shared by both, appears at sites similar to the localization of the two receptors during late embryogenesis, as shown by Gonzalez *et al.* (1990) for rat embryos and by Joseph-Silverstein *et al.* (1989) for chicken embryos. They report that these polypeptides are present in skeletal and heart muscle precursors, in bone and cartilage differentiation and in the developing hair root. A recent report describes the expression of FGF-5 in the splanchic mesoderm and in muscle precursors (Haub and Goldfarb, 1991). Much insight can be expected into the FGF system's role in organogenesis from comprehensive localization studies, which will compare the expression of different FGF-R with that of their various putative ligands.

During gastrulation and also during organogenesis *bek* and *flg* appeared in domains shared with other polypeptide growth factors and transcriptional regulators. In the heart TGFβ1 and 2 displayed expression patterns related to those of *bek* (Potts and Runyan, 1989; Choy *et al.* 1990; Millan *et al.* 1991). Moreover Vgr-1 expression was predominantly detected in squamous epithelia of the developing skin, oesophagus and stomach, areas of expression shared with the *bek* receptor. Similarly to *flg* TGF-β2 and BMP-2 are also expressed in the dermis (Pelton *et al.* 1989; Lyons *et al.* 1989; Lyons *et al.* 1990; Jones *et al.* 1991). Like the two FGF-R, members of the TGFβ family are also expressed during cartilage and bone differentiation (Pelton *et al.* 1990; Schmid *et al.* 1991; Millan *et al.* 1991). A notable example suggesting complex gene interactions involving FGF receptors may be found in early limb development. Concentration gradients along the limb bud, which are formed both by *bek* and by TGFβ (BMP-2A, Lyons *et al.* 1990) transcripts, invite comparison with the gradients of homeobox gene transcripts in the limb bud (Oliver *et al.* 1989; Dolle *et al.* 1989*a*). It is possible that cellular interactions mediated by the TGFβ and FGF systems in limb development are connected with the pattern-forming activity of homeoboxes, retinoic acid derivatives and their receptors (Dolle *et al.* 1989*b*; reviewed by Eichele, 1989; Brockes, 1990).

Colocalisation of *bek* and *flg* with other putative transcriptional regulators indicate the FGF system's possible involvement in additional interactions. *Bek* expression in the alar plate of the spinal cord is coincident with that of a transcriptional regulator, Pax-3 (Goulding *et al.* 1991), whereas the expression of *flg* in the intervertebral disc is coincident with the expression of Pax-1 in the same structure. A mutant allele of Pax-1, *undulate (un)*, displays abnormal vertebral column development (Balling *et al.* 1988). To test the interaction between Pax-1 and the two FGF-R we investigated their expression in homozygous *un* embryos, but no change could be detected. This suggests that *bek* and *flg* may occupy a position upstream to Pax-1 in a developmental hierarchy.

Analysis of spontaneous mutants at the relevant loci, gene targeting or other gene transfer experiments are elements of a comprehensive approach to explore the place of the FGF-R in the molecular interactions of early mammalian development. Their role in later development, especially in the epithelial–mesenchymal interactions of organogenesis, could be amenable to a molecular approach using various organ culture systems.

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