

## ***c-ros*: the vertebrate homolog of the *sevenless* tyrosine kinase receptor is tightly regulated during organogenesis in mouse embryonic development\***

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### **Summary**

The *c-ros* proto-oncogene is the vertebrate homologue of the *Drosophila sevenless* tyrosine kinase receptor. Examination of *c-ros* mRNA transcripts in the mouse embryo reveals a stringent pattern of expression. Only kidney, intestine and lung exhibit *ros*-specific RNA using sensitive techniques such as RNase protection and *in situ* hybridization. The temporal and spatial arrangement of *c-ros* transcripts is coincident with the phenotypic induction and proliferation of epithelium during organogenesis of the kidney and intestine. The data provide

evidence for a role of *c-ros* in the obligate cell-cell interactions that characterize the morphogenic induction and proliferation of epithelial cells in the kidney, intestine and lung. The *c-ros* tyrosine kinase receptor may provide a signal transduction pathway for epithelial-mesenchymal interactions.

Key words. *c-ros*, proto-oncogene, tyrosine kinase, *in situ*, embryogenesis.

### **Introduction**

The process of induction is observed in many phases of embryonic development. Currently, considerable interest and effort is being applied toward the elucidation of the poorly understood molecular events that specify this process. Such an interest has been underscored by the emerging evidence that many growth factors found to be expressed in the vertebrate embryo may mediate signalling for events other than mitogenesis such as morphogenic induction, differentiation or cell survival. Among these growth factors, several share the common feature of binding to cell surface tyrosine kinase receptors (i.e. *steel* factor; *Trk* genes; fibroblast growth factors).

The *steel* (*Sl*) locus in mouse encodes a factor that is crucial for the homing, proliferation and survival properties of various embryonic migratory cell populations (Geissler et al., 1981; Dolci et al. 1991; Keshet et al. 1991). Similarly, the Nerve Growth Factor gene family numbers at least four members (NGF, BDNF, NT-3, and NT-4) (Kaplan et al., 1991a,b; Klein et al., 1991; Soppett et al., 1991; Squinto et al., 1991; Hallböök et al., 1991) and these polypeptides play important roles in the early stages of neuronal differen-

tiation, in subsequent survival (Barde, 1989), and perhaps in proliferative events as well.

As a means of gaining a molecular access to inductive events during murine embryonic development, we have sought to characterize the expression of TK receptors at various stages of embryogenesis. In particular, we have framed our study by focusing on TK receptors with either highly restricted or uncharacterized patterns of expression in normal adult tissues based on the reasoning that these receptors might function primarily during development.

The *ros* proto-oncogene encodes a member of the protein tyrosine kinase gene family that bears the structural features of receptor TKs. Originally described as the *v-ros* oncogene borne by the avian UR2 retrovirus (Wang et al., 1982), human *ros* was isolated by sequence homology with *v-ros* (Nagarajan et al., 1986) and also as an oncogene in an NIH-3T3 transfection and tumorigenicity protocol (Fasano et al., 1984; Birchmeier et al., 1986). Further characterization of the human *c-ros* proto-oncogene (ROS1) revealed two features of particular interest. This TK receptor is scarcely expressed in normal tissues or in tumorigenic cell lines with the exception of human glioblastomas (Birchmeier et al., 1987). Additionally, nucleic acid

sequence comparison revealed that *c-ros* bears significant homology to the *Drosophila sevenless* TK receptor in both the intra- and extra-cellular domains (Birchmeier et al., 1990). The *sevenless* gene product is required for the induction of a predetermined precursor cell to differentiate into photoreceptor number seven in the ommatidium of the *Drosophila* compound eye (Basler and Hafen, 1988; Rubin, 1989; Banerjee and Zipursky, 1990). Like *sevenless*, *c-ros* encodes an unusually large polypeptide (2348 aa) with an extra-cellular domain of 1862 amino acids in the human (Birchmeier et al., 1990).

The available information on *c-ros* placed this gene within the framework of our criteria for a potentially interesting regulatory TK receptor during embryogenesis. In this study, we describe the expression pattern for *c-ros* during murine embryonic development. The data indicate that this TK receptor is expressed in a tightly regulated spatial and temporal manner during organogenesis. Furthermore, *c-ros* expression is apparently confined to the epithelial layer of kidney, intestine and possibly lung, organs that are characterized by formation of epithelial-mesenchymal boundaries suggesting that *c-ros* may play a role in the interactive process of these two tissue layers.

## Materials and methods

### Isolation of mouse *c-ros* genomic clone

A genomic library constructed from adult DBA/2J mouse liver DNA in the lambda phage replacement vector EMBL 3A was purchased from Clontech Labs (Palo Alto, CA). A single positive clone, designated  $\lambda$ La, was identified upon screening 600,000 recombinants with the plasmid pHROShi6, which corresponds to the *c-ros* kinase domain (Nagarajan et al., 1986). Comparison analysis of  $\lambda$ Lak4 with its human homolog resulted in the identification of the exon encoding the juxtamembrane domain within a 4.3 kbp *Bam*HI-*Sal*I fragment (Narayana and Nagarajan, in preparation).

### RNA preparation and RNase protection analysis

Embryos were dissected under the microscope, frozen in liquid nitrogen, and stored at  $-70^{\circ}\text{C}$ . RNA was extracted using RNazol (Cinna/Biotech) following the manufacturer's recommendations. For RNase protection analysis, a subclone of pmros 4.3 that spans 104 nucleotides of the JM exon and includes 36 upstream intronic nucleotides was generated by PCR (Mullis and Faloona, 1987) and subcloned into the pBluescript KS+ vector (Stratagene, pLT-1). RNase protection analyses were performed using the RPA kit (Ambion) following the manufacturer's recommendations. Briefly, a 220 nucleotide  $^{32}\text{P}$ -labelled T3 RNA Polymerase transcript synthesized from pLT-1 after linearization with *Bam*HI was gel purified. The probe was hybridized in solution to 10 or 50  $\mu\text{g}$  of total RNA at  $43^{\circ}\text{C}$  for 16 hours and then digested with RNases A and T1. The RNA hybrids, protected from digestion, were denatured and analyzed on 6% acrylamide/urea gels.

### Northern blot analysis

5  $\mu\text{g}$  of poly(A)+ RNA isolated by oligo (dT) column chromatography (Invitrogen) were separated on 1.1% for-

maldehyde - 1.2% agarose gels and transferred to nylon membranes (Zetabind; CUNO). Hybridization was performed by using random-primed probes in 250 mM sodium phosphate (pH 7.2)-1 mM EDTA-7% sodium dodecyl sulphate (SDS)-1% bovine serum albumin (BSA) at  $65^{\circ}\text{C}$  (Church and Gilbert, 1984). Filters were washed twice for 30 minutes at  $65^{\circ}\text{C}$  in 40 mM sodium phosphate-5% SDS-0.5% BSA, followed by two washes in 40 mM sodium phosphate-1% SDS.

### Probes

Total gut RNA extracted from 15.5 day mouse embryos was reverse-transcribed in the presence of oligo (dT) primers (Amersham) for 2 hours at  $42^{\circ}\text{C}$ , and the resulting cDNA was amplified by PCR using oligonucleotides derived from the rat *ros* cDNA sequence (Matsushime and Shibuya, 1990). Two *ros*-specific probes were derived: one spanning 1431 bases of the extracellular domain (nucleotides 4026-5457 of the rat sequence), the other ranging from nucleotide 6940 to nucleotide 7320 of the TK domain.

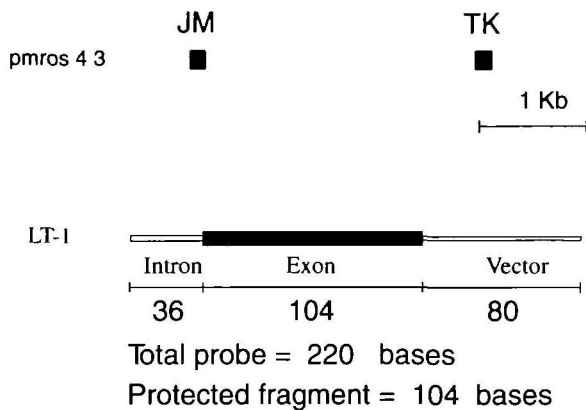
### In situ hybridization

*In situ* hybridization protocols were as described (Martin-Zanca et al., 1990). Briefly, dissected embryos were fixed overnight in 4% paraformaldehyde, dehydrated with alcohols and xylenes, and embedded in paraffin. Embryos were sectioned at 5  $\mu\text{m}$  thickness and mounted on gelatin-coated slides. Slides were deparaffinized, digested with proteinase K (Boehringer Mannheim), acetylated with triethanolamine, dehydrated and hybridized overnight with  $5 \times 10^5$  cts/minute of  $^{35}\text{S}$ -labeled cRNA probe at  $52^{\circ}\text{C}$ . The slides were washed in hybridization buffer, treated with ribonucleases at  $37^{\circ}\text{C}$  for 45 minutes, rinsed in  $2 \times \text{SSC}$ , dehydrated and dipped in Kodak emulsion NTB-2. After drying, the slides were stored for 5-10 days at  $4^{\circ}\text{C}$ , developed in Kodak D-19, and fixed as recommended by the manufacturer. All dark- and bright-field photomicroscopy was done on a Zeiss Axiophot microscope. RNA probes labeled with  $^{35}\text{S}$  were prepared by standard procedures (Krieg and Melton, 1987) by using UTP as the labeled nucleotide.

## Results

To study the expression of *ros* in the mouse, we utilized exonic probes derived from mouse genomic clones as described in Materials and methods and elsewhere (Narayana and L.N., in preparation). Initial efforts to detect the presence of *c-ros* transcripts in embryonic mRNAs by northern analysis or by *in situ* hybridization using genomic probes were unsuccessful. We concluded that if *c-ros* was expressed in the embryo, either the levels of mRNA must be extremely low, or expression must be confined to specific subsets of cells where transcript levels might be high but whose representation would be minimal in the embryo as a whole. In order to determine whether either of the above possibilities was true, we chose to repeat the expression study, by using the RNase protection assay as a means of obtaining the greatest possible sensitivity of detection (Melton et al., 1984).

The mouse *c-ros* genomic clone, pmros 4.3, which contains two *c-ros* exons is depicted in Fig. 1. The 5'-most exon contained in this clone is a 134 nucleotide sequence that encodes the juxtamembrane (JM) intra-



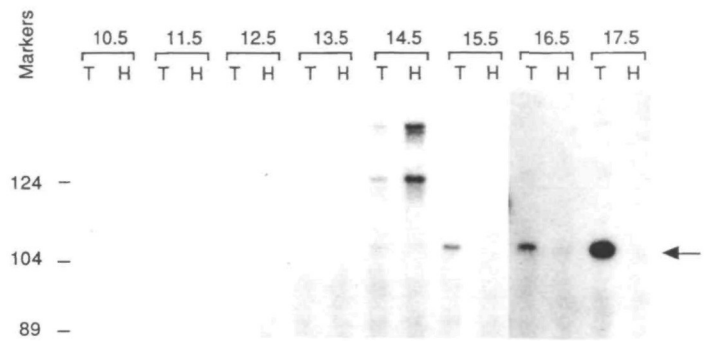
**Fig. 1.** Organization of the *ros* genomic clone, pmros 4.3, used to derive RNase protection assay probes. Thick boxes represent exons encoding the juxtamembrane (JM) and initial (5') tyrosine kinase (TK) domains. The 220 base cRNA used in the RNase protection assay that contains 104 of the total 134 bp present in the JM exon is shown at the bottom (LT-1)

cellular region of the protein. More 3' is a 173 nucleotide exon that encodes TK domain sequences (see Materials and methods for further details). The RNase protection data described here were obtained using the exonic JM probe; however identical results have been obtained with the TK exon probe. Use of sense strand as a cRNA probe in our experiments never resulted in detection of protected fragments (data not shown).

#### *c-ros* expression is temporally regulated during embryogenesis

A subclone of pmros 4.3 that spans 104 nucleotides of the JM exon and includes 36 upstream intronic nucleotides was generated by the polymerase chain reaction (PCR) (Mullis and Faloona, 1987) and subcloned into pBluescript KS+ (pLT1; see Materials and methods). Hybridization of LT-1 probe with *c-ros*-encoding RNA followed by single strand-specific RNase degradation would result in the precise reduction of the 220 base probe to a 104-bp protected fragment (Fig. 1 and Materials and methods).

<sup>32</sup>P-labelled LT-1 cRNA was hybridized to 10 µg of total RNA extracted from either heads (H) or trunks (T) of day 10.5 through 17.5 mouse embryos, submitted to RNase protection analysis as described in Materials and methods, and resolved on acrylamide gels under denaturing conditions (Fig. 2). Protection of the expected 104 nucleotide fragment was first detected in trunk RNA from 14.5 day embryos (Fig. 2). At this stage (day 14.5), the protected fragment was barely detectable although by day 15.5 the protected fragment was clearly evident and its intensity increased through embryonic day 17.5 (see Fig. 2). These results indicate that *c-ros* transcripts are present during embryonic development in a temporally regulated manner, appearing in late gestation when the embryo is undergoing organogenesis. No protection of the *c-ros* exon was



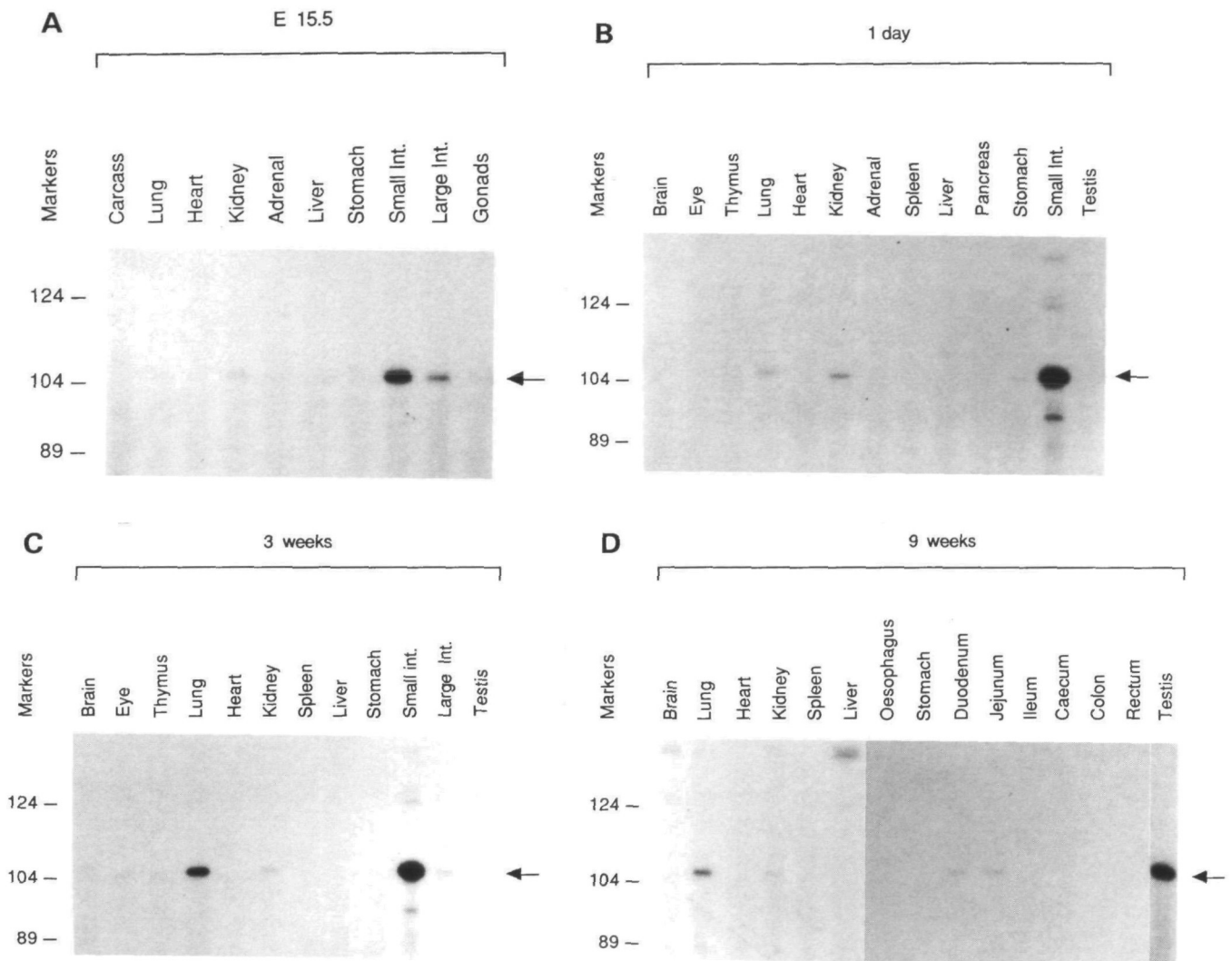
**Fig. 2.** RNase protection analysis of *c-ros* transcripts in embryonic mouse mRNAs from 10.5 to 17.5 days of gestation. Approximately 10 µg of total RNA from embryonic trunks (T) and heads (H) were hybridized to the <sup>32</sup>P-labeled cRNA probe LT-1 (Fig. 1). Co-electrophoresed DNA size markers are indicated at the left. The arrow at the right indicates the 104 base pair *ros* protected fragment. The larger smears present in 14.5 day sample lanes H are due to DNA contamination in the RNA samples.

detected with RNA extracted from heads (H) of the embryonic stages examined (Fig. 2).

#### *c-ros* expression is confined to specific tissues during organogenesis

We next wished to determine whether *c-ros* expression existed in a broad spectrum of tissues or whether it was confined to specific organs or tissues of the embryo. Dissections of various organs from 15.5 day embryos were performed and the organs were collected for preparation of RNA. The remaining carcasses (after organ dissection) were included in the analysis as a source of enriched cartilage, muscle and dermal tissues and to determine whether expression was distributed throughout embryonic tissues. Analysis of head RNA was excluded since no *c-ros* transcripts had been detected in earlier studies (see Fig. 2). 10 µg of total RNA from the dissected embryonic organs were hybridized to LT-1 probe as previously described in Fig. 2. Protected fragments indicative of *c-ros* transcripts were observed in two developing organs at this stage in embryogenesis: the intestine (small and large) and embryonic kidney (Fig. 3A). The above data provide the first evidence that, in addition to temporal regulation, the *c-ros* TK receptor is also spatially regulated during embryonic development.

We next determined whether *c-ros* expression was confined to embryonic development or whether transcription was maintained following birth and in the adult. Analysis of RNA from day one neonatal animals revealed *c-ros* expression to be maintained in the intestine and kidney (Fig. 3B). Moreover, two additional sites of *c-ros* expression were observed in these animals: stomach (at threshold levels of detection that may represent contamination of the RNA preparation with intestinal tissue) and lung. *c-ros* mRNA levels are maintained in lung and intestine three weeks after birth while kidney transcript levels are reduced (Fig. 3C). In



**Fig. 3.** *c-ros* expression in various organs of embryonic, neonatal and adult mice. RNA was isolated from the following age organs as described in Materials and methods: (A) embryonic day 15.5 [E 15.5]; (B) 1 day post-partum, (C) 3 weeks and (D) 9 weeks. 10  $\mu$ g (panels A, B) or 50  $\mu$ g (panels C, D and carcass panel A) of total RNA were used in RNase protection assays employing probe LT-1 (Fig. 1). Organs from which RNA was isolated and migration of MW standards (bases) are indicated.

the adult (Fig. 3D), *c-ros* transcripts remain present in kidney and intestine albeit at the threshold of detectability. In contrast, *c-ros* expression remains moderately abundant in adult lung and high levels of mRNA appear in mature testes.

#### *Differing kinetics of expression*

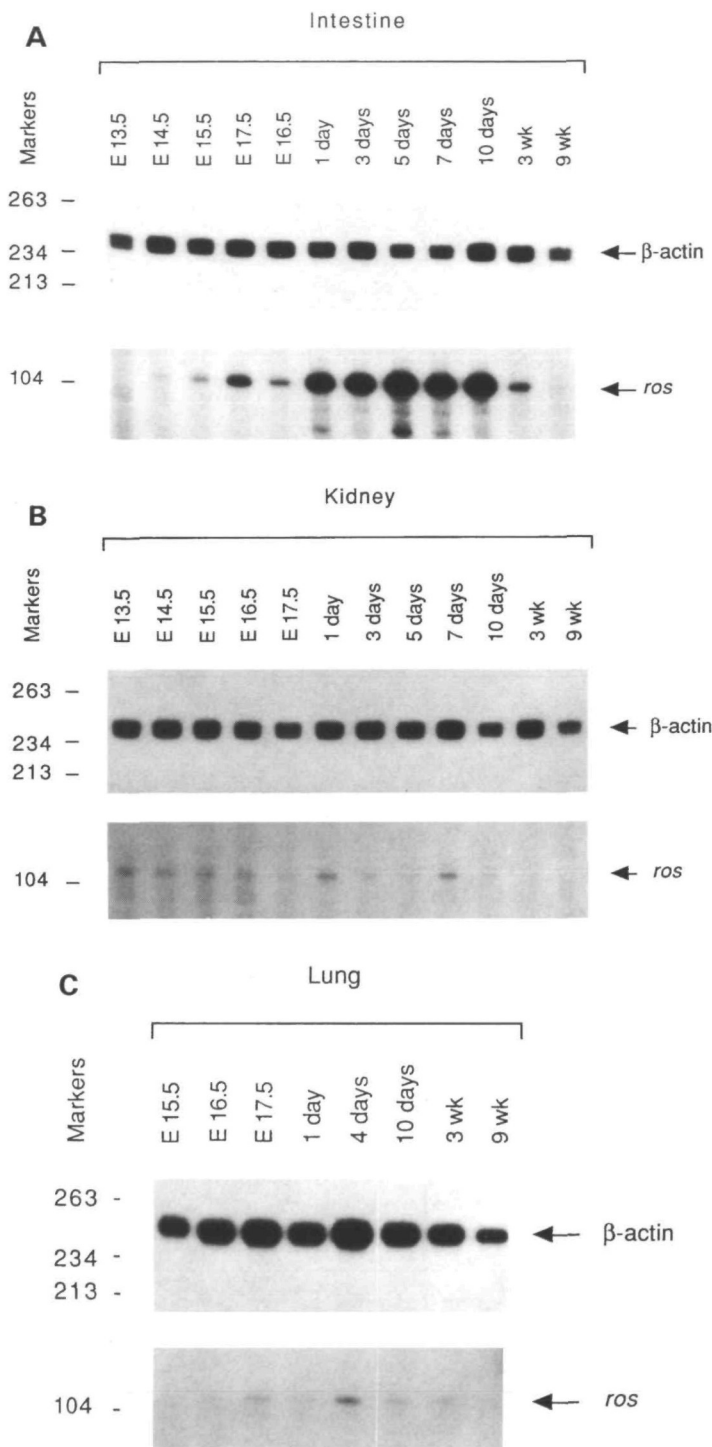
Having determined a subset of tissues that express *c-ros* transcripts, we next performed a time course experiment in order to measure more directly relative levels of *c-ros* mRNA within each tissue. The LT-1 *ros* probe was hybridized to 10  $\mu$ g of total RNA from intestine, kidney and lung from various stages of embryonic and postnatal development (Fig. 4). A 250 base  $\beta$ -actin cRNA probe was included (Alonso et al., 1986) in the same reaction as a means of assessing relative levels of RNA present in each hybridization (Fig. 4A-C).

As previously observed, we first detect *c-ros* ex-

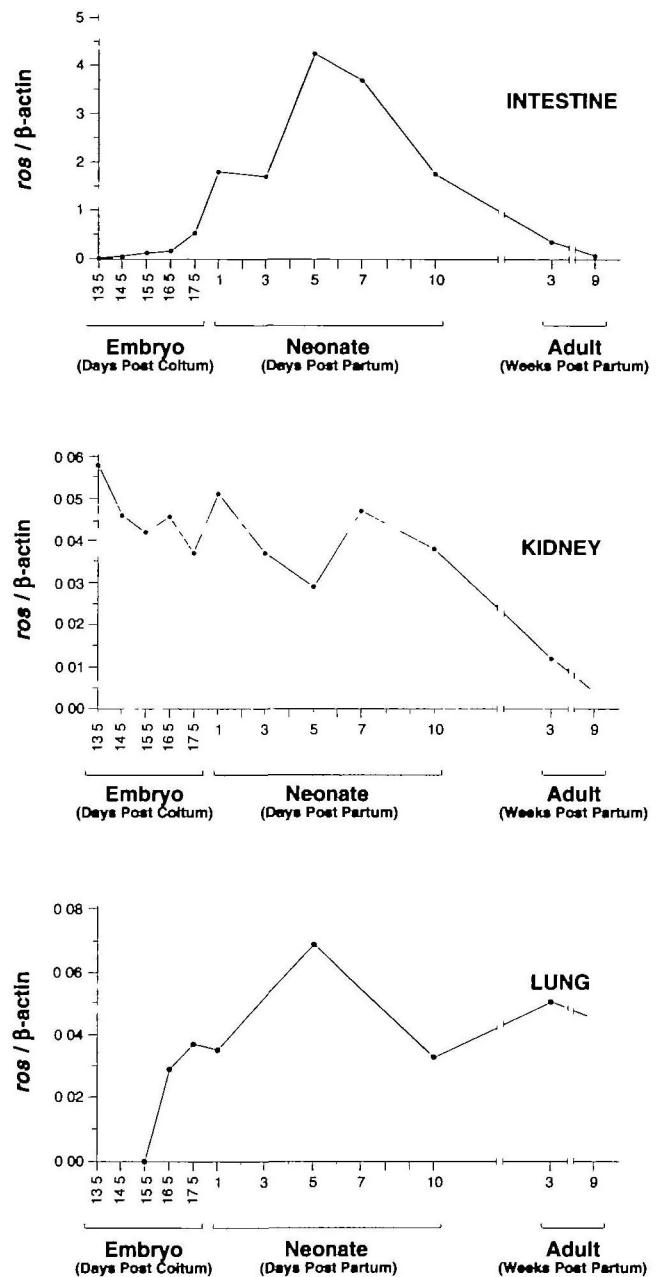
pression in the intestine on embryonic day 14.5 (Fig. 4A). An incremental gradient of *c-ros* transcripts is observed during the remainder of fetal development which reaches a maximum peak at birth and is sustained for the ensuing 10 days. Our next time point (3 weeks of age) shows that *c-ros* mRNA levels have dropped to embryonic levels and in the adult mouse (9 weeks of age), detection of transcripts is extremely limited due to the low expression levels (Fig. 4A).

Expression of *c-ros* mRNA in the kidney is highest during embryogenesis (Fig. 4B). Transcripts are already present at maximum levels in kidney by embryonic day 13.5 and are detectable through postnatal day 10 although, by this stage, expression has decreased to very low levels that approach our threshold of detection (Fig. 4B).

In the lung, *c-ros* transcripts were first observed at embryonic day 16.5 as indicated in Fig. 4C. In this organ, mRNA levels do not appear to fluctuate



**Fig. 4.** Developmental expression analysis of *ros* in intestine (A), kidney (B) and lung (C). Tissues of embryonic, neonatal and adult animal were analysed by RNase protection using probe LT-1 (Fig. 1). The ages of the donor animals are indicated along with migration of MW standards (bases). "E" indicates the embryonic age. A  $\beta$ -actin probe was used in the same hybridization mixture with *ros* as an internal control for RNA quantitation (see Materials and methods). Note that in Fig. 4A lanes 16.5E and 17.5E are reversed.



**Fig. 5.** Quantitative analysis of *ros* expression in intestine, kidney and lung during mouse development by laser scanning densitometry. Films shown in Fig. 4 were analysed using an Ultrosan XL densitometer (LKB). The values [*ros*]/[ $\beta$ -actin] were then plotted with respect to the age of the animal.

appreciably through adulthood (see also Fig. 3D and note that less RNA was present in Fig. 4A, lane labelled 9 weeks).

The data in Fig. 4 were quantitated by laser scanning densitometry, normalized to the levels of  $\beta$ -actin from the same gel track, and plotted to allow better visual evaluation (Fig. 5). This direct comparison shows that the three major organs possessing detectable levels of *c-ros* transcripts in our assay exhibit distinctive kinetics of mRNA expression through embryogenesis and after

birth (Fig. 5). These data further suggest that appreciable levels of *c-ros* transcripts exist in kidney prior to embryonic day 13.5 (see below).

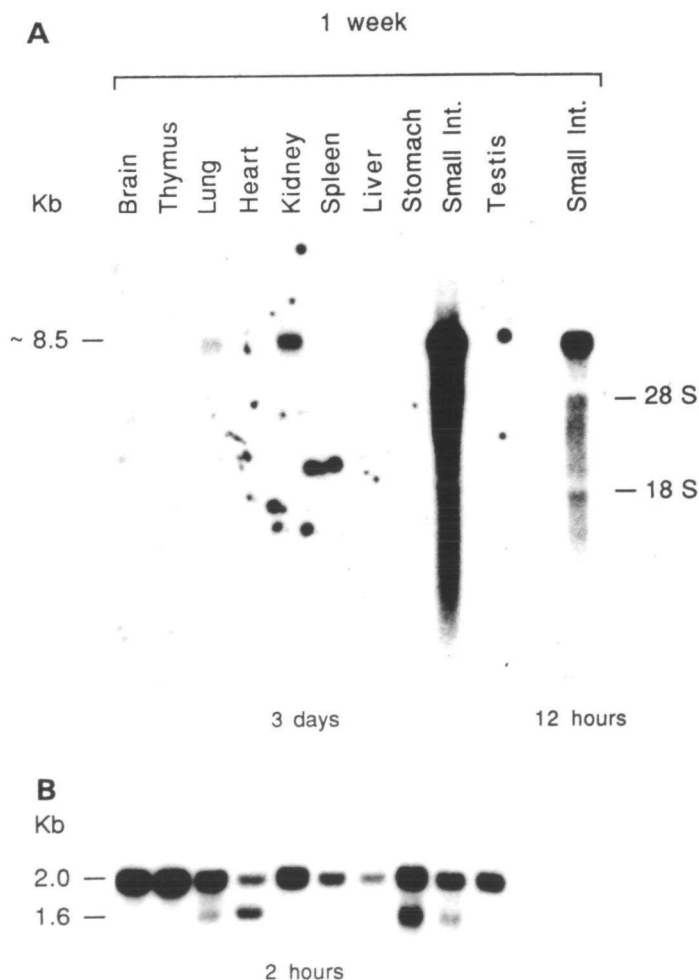
#### Northern analysis

The identification of tissues that express abundant levels of *c-ros* transcripts provided a source of cDNA for PCR amplification and cloning of larger, more complex probes that would allow greater sensitivity for the detection of *ros* transcripts using northern analysis and RNA *in situ* hybridization (for details, see Materials and methods). 5  $\mu$ g of oligo (dT)-cellulose-enriched poly(A)<sup>+</sup> mRNA from the indicated sources were analyzed in the northern transfer analyses for one-week (Fig. 6) and nine-week-old mice, respectively (not shown). As illustrated in Fig. 6, a transcript of approximately 8.5 kb hybridizes to the cDNA *ros* probe in lung (faintly), kidney and small intestine. A similar

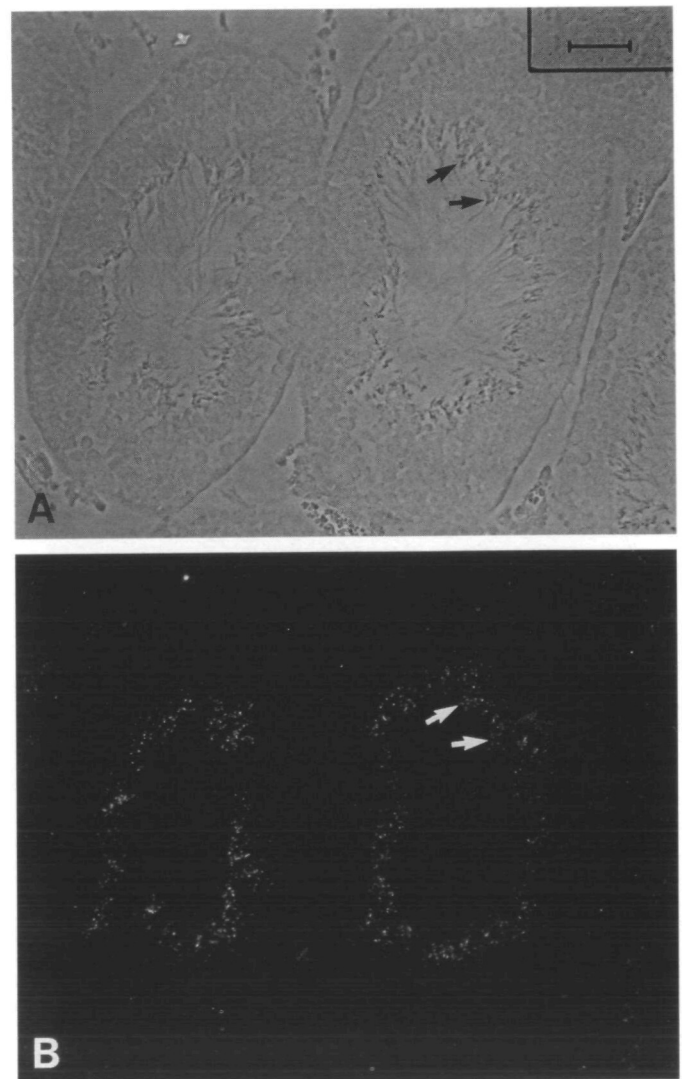
experiment with RNAs isolated from nine-week-old mice further confirms the previous RNase protection data. In adult mice, *c-ros* mRNA levels remain constant, albeit low in lung and transcript levels are greatly reduced in kidney and small intestine (not shown).

A novel abundant transcript of approximately 4.5 kb was seen in testes of adult mice. These data are consistent with previous experiments (Fig. 3D) in which the appearance of an RNase-protected *c-ros* fragment was observed in testes. However, it must be noted that a 4.5 kb transcript is insufficient to encode the predicted full-length *c-ros* polypeptide of approximately 2350 amino acid (Birchmeier et al., 1990).

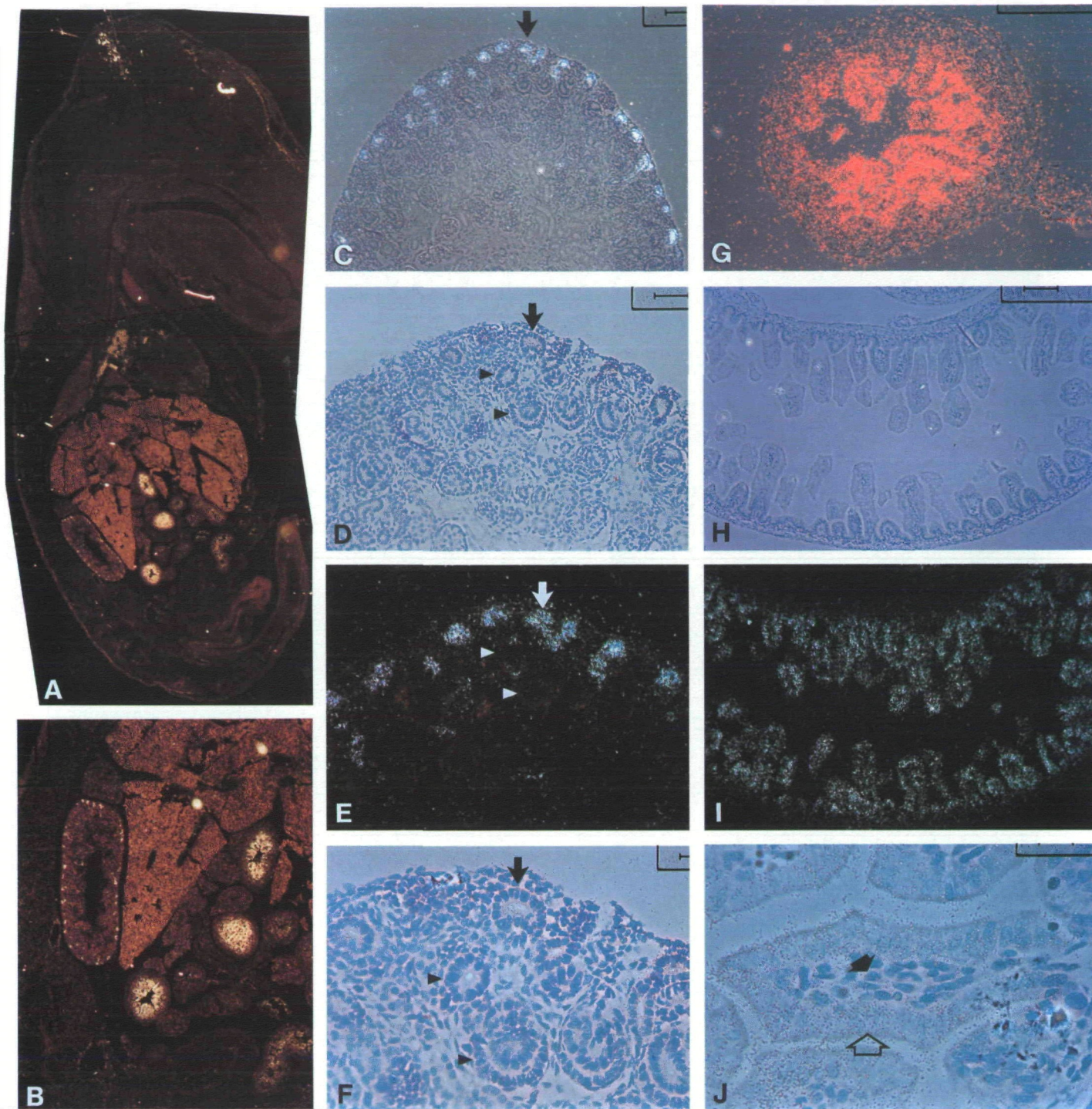
We next determined the precise location in testes of the novel 4.5 kb transcript by RNA *in situ* hybridization. The results demonstrate that the novel transcript is present only in mature stages of germ cell development (spermatids and spermatozoa; see arrows in Fig.



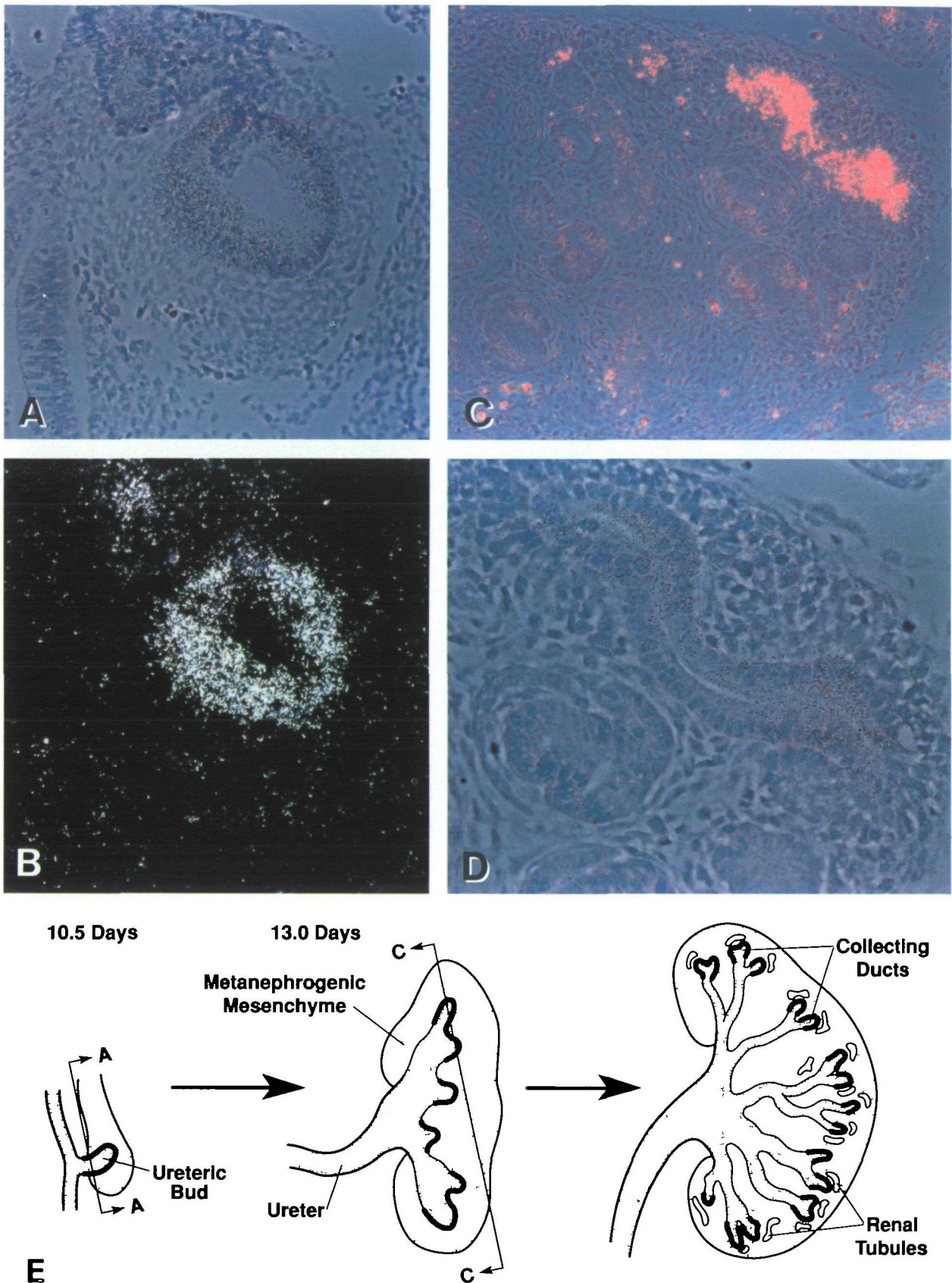
**Fig. 6.** Northern blot analysis of *c-ros* transcripts in 1-week-old mouse tissues. Poly (A)<sup>+</sup>-selected RNAs (~5  $\mu$ g) from 1-week-old mouse tissues were analyzed as described in Materials and methods using a 1.5 kb mouse-specific *c-ros* probe derived from the extracellular domain (panel A). The same filter was hybridized to a  $\beta$ -actin probe to control for the amount of RNA loaded in each lane (panel B).



**Fig. 7.** *c-ros* expression in adult testis. Bright-field (A) and dark-field (B) section through seminiferous tubules of mature adult mouse testis. Spermatozoa heads are indicated by arrows.



**Fig. 8.** *c-ros* expression in embryonic kidney and gut from 17.5 day mouse embryos. Dark-field (A, B) of a sagittal section. Bright-field (C, D, F) and dark-field (E) sagittal section of a kidney. Vertical arrows point to cross sections of the branching ureteric tips (collecting ducts) at their distal ends. Horizontal arrows indicate renal tubules cells undergoing differentiation towards formation of glomeruli (see Fig. 9 panel E for illustration). Panels G-J represent bright-field (G, H, J) and dark-field (I) sections through embryonic intestine. The cells of the lamina propria (black arrow) and the epithelium (hollow arrow) are indicated in panel J. In panel A the apparent brightness in brain region, and in liver is non-specific



**Fig. 9.** *c-ros* expression in early kidney development. (A) Bright-field and (B) dark-field view of section through the laterally branching ureteric bud of a 10.5 day mouse embryo (see panel E for illustration and plane of section) (C and D) Bright-field view of a section through a 13.5 day mouse embryo kidney emphasizing expression along the tips of branching tubules (see panel E for illustration and plane of section). (E) Graphic illustration of kidney development (adapted from Wessells, 1977). 10.5 indicates approximate stage of early ureteric bud migration into the metanephrogenic mesenchyme. Line and arrows indicate plane of section in A and B. 13.5 indicates more advanced branching of the ureteric epithelium with arrows indicating plane of section in panels C and D. The last illustration in panel E shows later stages of development in which renal tubules appear and fuse with collecting ducts to form glomeruli (see Fig. 8 panels C-F). Approximate extent of *c-ros* expression is indicated in bold outline of the branching ureteric bud tips

7). This localization is consistent with the appearance of transcripts in testes with the onset of male sexual maturity. Assuming that this novel transcript is translated, either a greatly modified and reduced *c-ros* peptide is produced in mature spermatozoa or expression in this site may be spurious and of no biological significance.

#### *ros* expression is confined to epithelial compartments

The availability of cDNA clones allowed us to reinvestigate the expression of *c-ros* in the embryo by RNA *in situ* hybridization. The *in situ* hybridizations in Fig. 8 show various magnifications of representative sections from 17.5 day embryos. Low power magnification (Fig. 8A and B) shows that the *c-ros* expression pattern adheres to our previous RNase protection data. *c-ros* probe hybridization is evident exclusively in the intestine and kidney in this section. High power magnification of the gut and kidney provide further resolution to the expression profile. These results (Fig. 8C-J) indicate that *c-ros* transcripts are confined to the epithelial compartment of these organs. In the intestine (Fig. 8G-J), expression is found throughout the luminal epithelium with no apparent differences in grain density in the regions destined to form crypts.

In the embryonic kidney, *c-ros* transcripts localize to pockets of the cortical layer (Fig. 8A-C). At higher magnification, these pockets of *c-ros* expression can be identified as the terminal endings of the branching ureteric tubules that induce mesenchyme to form epithelial precursors of renal tubules (see vertical arrows Fig. 8C-F; Mugrauer et al., 1988; Saxen and Sariola, 1987). *c-ros* expression disappears rapidly as the primary induced epithelium (renal tubules) undergoes differentiation into the comma-shaped precursors of glomeruli (see horizontal arrows Fig. 8D-F; Mugrauer et al., 1988). These data, taken together with the kinetics of *c-ros* expression described for lung and intestine, suggest that the initial, and highest, *c-ros* transcript levels in these organs correlate with interactive epithelial-mesenchymal events.

The RNase protection data obtained from isolated embryonic kidneys indicated that *c-ros* transcripts have reached essentially maximal levels by embryonic day 13.5 (Figs 4B and 5). Examination of *c-ros* expression in kidney by *in situ* hybridization from earlier stages in development exhibits a pattern consistent with that seen at late fetal stages (Figs 9 and 8 C-F). *c-ros* transcripts are present in abundance in the early ureteric bud epithelium as it first branches into the surrounding metanephrogenic mesenchyme (Fig. 9A,B,E). At embryonic day 13.5, the ureteric bud has elaborated into secondary branches. *c-ros* transcripts are present along the branching tips of the epithelial collecting ducts (Fig. 9C,D,E). This pattern is maintained in late kidney development with expression at the tips of the collecting ducts but not in the renal tubules that are induced from mesenchyme later to form glomeruli.

The present data indicate that the *c-ros* TK receptor is both temporally and spatially regulated in its

expression. The observed pattern during embryonic development persists until maturity and appears to be confined to the epithelial compartments of organs (intestine and kidney) that interact in the transmission of epithelial-mesenchymal inductive signals (Dauca et al., 1990; Saxen and Sariola, 1987).

#### Discussion

Tyrosine kinase (TK) receptors have recently assumed prominence in the study of vertebrate development. Experiments from many laboratories indicate that TK receptors such as *c-kit* (*Sl* receptor), platelet-derived growth factor receptor (PDGF-R), *trk* family members (NGF family receptors) and FGF receptors, among others, have important functions during embryogenesis.

These observations fit well with preceding data obtained in studies with the fruitfly *Drosophila melanogaster*. The combined powers of genetics and molecular biology available with this organism have allowed the identification and characterization of numerous genes with key functions during embryogenesis. *Sevenless* (Hafen et al., 1987; Basler and Hafen, 1988; Bowtell et al., 1988; Simon et al., 1989) and *torso* (Sprenger et al., 1989) were the first examples of TK receptors that participate in the signal transduction of morphologic events.

The present study uncovers the *c-ros* proto-oncogene as another example of a TK receptor-encoding gene with expression confined to a limited number of developing cell populations during embryogenesis. Where *in situ* analysis was informative, *c-ros* expression is localized to epithelial compartments of organs, which are characterized by reciprocal epithelial-mesenchymal interactions during their ontogeny. We have studied expression primarily in kidney and intestine and less exhaustively in lung since the comparatively lower levels of transcripts found in this organ rendered *in situ* analysis less interpretable. Several other structures including teeth and salivary gland undergo epithelial-mesenchymal inductive events. *c-ros* expression in those tissues was not detected.

#### *Expression in the intestine*

We first detect *c-ros* expression in the intestine in the embryonic period after which the primary induction of the epithelium has occurred as a consequence of reciprocal epithelial-mesenchymal interaction. Grafting and tissue studies have demonstrated that the intestinal mesenchyme exerts a strong inductive influence on the endoderm to become epithelium, whereas the epithelium is quite refractile to inductive influence from heterologous mesenchyme (Gumpel-Pinot, 1978; Dauca et al., 1990). In the rodent, the entire epithelium of the intestine undergoes transformation from endoderm and proliferates during fetal development (Hermos et al., 1971). The mature, functional intestine is not fully formed until the third postnatal week (Henning, 1979, 1981).

*c-ros* is transcribed only in the epithelial compartment of the forming intestine. At our sensitivity of detection, *c-ros* kinetics of expression coincide with the acquisition and concomitant proliferation of epithelial phenotype. Transcript levels decrease three weeks after birth coinciding with overt differentiation of the epithelium into the various specialized cells found at the mature brush borders. In the adult intestine only the cells in the crypts retain stem cell characteristics. It is possible that these cells are responsible for the faint *c-ros* expression that remains detectable in adult intestine.

#### *Expression in the kidney*

The kidney is an organ of mesodermal origin. The ureteric bud invades the presumptive (metanephrogenic) kidney mesenchyme, at which time reciprocal inductive events take place (Fig. 9A,E; for review see Gilbert, 1988 and references therein). First, the mesenchyme induces branching of the ureter. Next, the tips of the branched ureteric epithelium induce the cortical metanephrogenic mesenchyme to differentiate progressively into renal tubules and glomeruli. This process occurs in a spatially distributed manner such that inspection of late gestation kidney from the cortex toward the interior allows sequential examination of the various stages of renal tubule differentiation (Figs 8 and 9).

The inductive events characterizing the development of the kidney are particularly well documented (Grobstein, 1955, 1956, 1967; Wessels, 1977; Saxen et al., 1986; Saxen, 1987). Culturing of metanephrogenic mesenchyme in the absence or presence of the ureteric bud have demonstrated the causal interaction between the ureteric epithelium and the induction of mesenchymal cells to transform into tubule epithelium. Studies utilizing filters of varying pore size interposed between the inducing tissue and the metanephrogenic mesenchyme further indicate that cell-cell interaction between the two tissues is required for induction of tubules to take place (Grobstein, 1955, 1956; Saxen, 1987) and have been widely interpreted to exclude diffusible factors as the inducing agents.

In kidney, *c-ros* expression is detectable at the tips of the branching ureteric epithelium from the earliest stages of induction of the ureter to branch into the metanephrogenic mesenchyme. As the epithelial tubules fuse with the forming renal tubules *c-ros* transcripts disappear. These data are most consistent with a role for the *c-ros* receptor in the processes that lead to establishment, identity or branching of epithelium rather than its actual specialized functions. We base this interpretation on the following facts. First, *c-ros* expression is highest in the embryonic organs and expression greatly diminishes upon differentiation. Second, in addition to their differing embryonic parentage (mesoderm versus endoderm respectively), the epithelia of the kidney and intestine are highly specialized tissues containing differentiated cell types that have unrelated functions, yet both express *c-ros*.

The *c-kit* TK receptor is also expressed in specific

subsets of embryonic cells that arise in unrelated embryonic compartments (primordial germ cells, melanoblasts and hematopoietic stem cells; Orr-Urtreger et al., 1990; Keshet et al., 1991; Manova and Bacharova, 1991). These differing cell types share the property of originating in embryonic locations that are distant from their ultimate site of differentiation and function. The present study suggests that the *c-ros* TK receptor may also play a role in a generic function (epithelial induction, maintenance and/or proliferation) that is required in the ontogeny of kidney and intestine, organs with differing germ layer origins.

#### *c-ros and sevenless are related*

We have noted the evolutionary relationship between vertebrate *c-ros* and the *Drosophila sevenless* receptor (Birchmeier et al., 1990; Chen et al., 1991; Matsushime and Shibuya, 1990). As with other TK receptors, the greatest homology is found in the catalytic TK domain. However, across considerable evolutionary distance, *ros* and *sevenless* have retained several additional parallels that merit consideration. (1) Both proteins share a conserved 5-6 amino acid insertion in the TK domain (Chen et al., 1991; Hanks, 1991). (2) The extracellular domain for both proteins is unusually large for a TK receptor (approximately 1900 amino acids) (Birchmeier et al., 1990; Matsushime and Shibuya, 1990). (3) Sequence conservation extends into the extracellular, putative ligand binding domain (Birchmeier et al., 1990).

The ligand for the *sevenless* TK receptor, *bride of sevenless* (*boss*), has been identified (Hart et al., 1990). The direct interaction of the *sevenless* and *boss* proteins has been recently demonstrated in an elegant set of experiments by Zipursky and co-workers (Kramer et al., 1991). This membrane-bound interaction of receptor and ligand fits well with the biology of the forming ommatidium and the close juxtaposition of the inducing photoreceptor eight (expressing *boss*) with the R7 precursor cell that expresses *sevenless*.

In light of the extracellular domain conservation between *sevenless* and *ros*, and the existence of close cell-cell interaction in epithelial-mesenchymal interactions of kidney and intestine, we entertain the speculation that a putative ligand to *ros* may bear structural homology to the *boss* protein. Such a ligand would foster strong cell-cell interaction. Proteins like *boss* that contain seven transmembrane domains are known to function as receptors that often couple to G-proteins as a basis for signal transduction (Libert et al., 1989; Houamed et al., 1991; Masayuki et al., 1991). A molecule of this type would permit reciprocal signalling between two interacting cell types.

#### *Concluding remarks*

The data presented in this study add to the recently expanding identification of TK receptors that have tightly regulated expression patterns during vertebrate development. The *c-ros* proto-oncogene exhibits spatial and temporal kinetics of RNA expression that strongly suggest: (1) a role for this TK receptor in the dynamic

interaction of epithelia and mesenchyme witnessed in the organogenesis of kidney, intestine and lung; and (2) insights into the location and possible structure of its cognate ligand. The above information provides a cornerstone that, with continued characterization of the protein, *in vitro* embryonic organ culture studies and homologous recombination experiments in ES cells should lead to the identification of the *ros* ligand and an understanding of the precise function of this receptor/ligand complex during organogenesis in vertebrates.

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