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Original Article

Neuronal characteristics in embryonic renal stroma

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ABSTRACT The metanephric mesenchyme is considered a homogeneous population of predetermined, but pluripotent cells with a nephrogenic bias. By an inductive stimulus, the mesenchyme is programmed to differentiate into the various epithelial phenotypes of the secretory nephron. A fraction of the mesenchymal cells, however, remains in the interstitium between the nephrons and differentiates into spindle-shaped, clear-cytoplasmic renal stroma. We have analyzed the molecular nature of these cells in order to discover the specific cell types that could be involved in the morphogenetic processes during kidney differentiation. *In situ* hybridization reveals neurofilament light protein mRNA, and immunohistology shows neurofilament light and medium proteins in the stromal cells around kidney tubules. By immunohistochemistry these peritubular stromal cells can be distinguished from the neuronal cells of the renal microganglion: the peritubular stromal cells are neurofilament-positive but L1 neural cell adhesion protein-negative, whereas the neuronal cells with axonal extension are both neurofilament-positive and L1 neural cell adhesion protein-positive. Proliferation index of the stromal cells was low as compared to tubular cells, as shown by bromodeoxyuridine incorporation.

KEY WORDS: *embryonic kidney, neurofilament, stroma*

Introduction

The stroma of the embryonic metanephric kidney consists of clear-cytoplasmic, spindle-shaped cells. In the adult mammalian kidney this cell type is sparce (Wolgast, 1985), but it is abundant in fetal kidneys (Kissane, 1983). The molecular characteristics and developmental role of the renal stromal cells have remained unresolved. In organ culture the stromal cells affect the branching morphogenesis of the ureter as well as the tubule-formation of the mesenchymal cells (Unsworth and Grobstein, 1970; Sariola *et al.*, 1988a). Epidermal growth factor promotes cell proliferation of embryonic renal stromal cells (Weller *et al.*, 1991) and prevents their programmed cell death or apoptosis (Koseki *et al.*, 1992; Coles *et al.*, 1993).

The results of viral transfection experiments with cultured kidney rudiments have suggested that the nephrogenic mesenchymal cells are pluripotent with differentiation options toward both epithelial and stromal cell lineages (Herzlinger *et al.*, 1992). In contrast to this are the results from interspecies chick-quail chimeras and neural crest labeling studies showing that neural crest cells are migrating into metanephric mesenchyme (LeDouarin and Teillet, 1974; Bronner-Fraser and Fraser, 1988). Moreover, early avian metanephric kidney contains adrenomedulla-like cells with catecholamine granules (LeDouarin and Teillet, 1974). A neural crest marker molecule, disialated ganglioside GD_3, is expressed in the stromal cells of the mouse embryonic kidney (Sariola *et al.*, 1988a), and cultures of mouse metanephric kidney rudiment contained neuronal cells (Sariola *et al.*, 1988b). The data suggests that neural-crest-derived cells are present in the embryonic kidney and may have their own developmental bias different from that of other cells of the metanephric mesenchyme. We have analyzed neurofilament proteins and mRNA, as well as the expression of neural cell adhesion molecule L1 of the embryonic metanephric kidneys. Incorporation of bromodeoxyuridine (BrdU) in different cell types was compared to analyze the cell proliferation index of the stromal and epithelial cells.

Neurofilaments are cytoskeletal intermediate filaments that are 10 nm in diameter and several microns long. Three polypeptides of apparent molecular weights of 200 kDa, 160 kDa, and 68 kDa constitute neurofilament heavy (NF-H), medium (NF-M) and light (NF-L) proteins, respectively (reviewed by Liem, 1993). Neurofilaments are found mainly in axons, and during neurogenesis the three subunits are differentially expressed. The NF-M and NF-L proteins are expressed simultaneously during early embryonic development, but the NF-H expression begins at later stages or postnatally (Shaw and Weber, 1982; Julien *et al.*, 1986; Carden *et*

Abbreviations used in this paper: BrdU, bromodeoxyuridine; NF-L, neurofilament light; NF-M, neurofilament medium; NF-H, neurofilament heavy.

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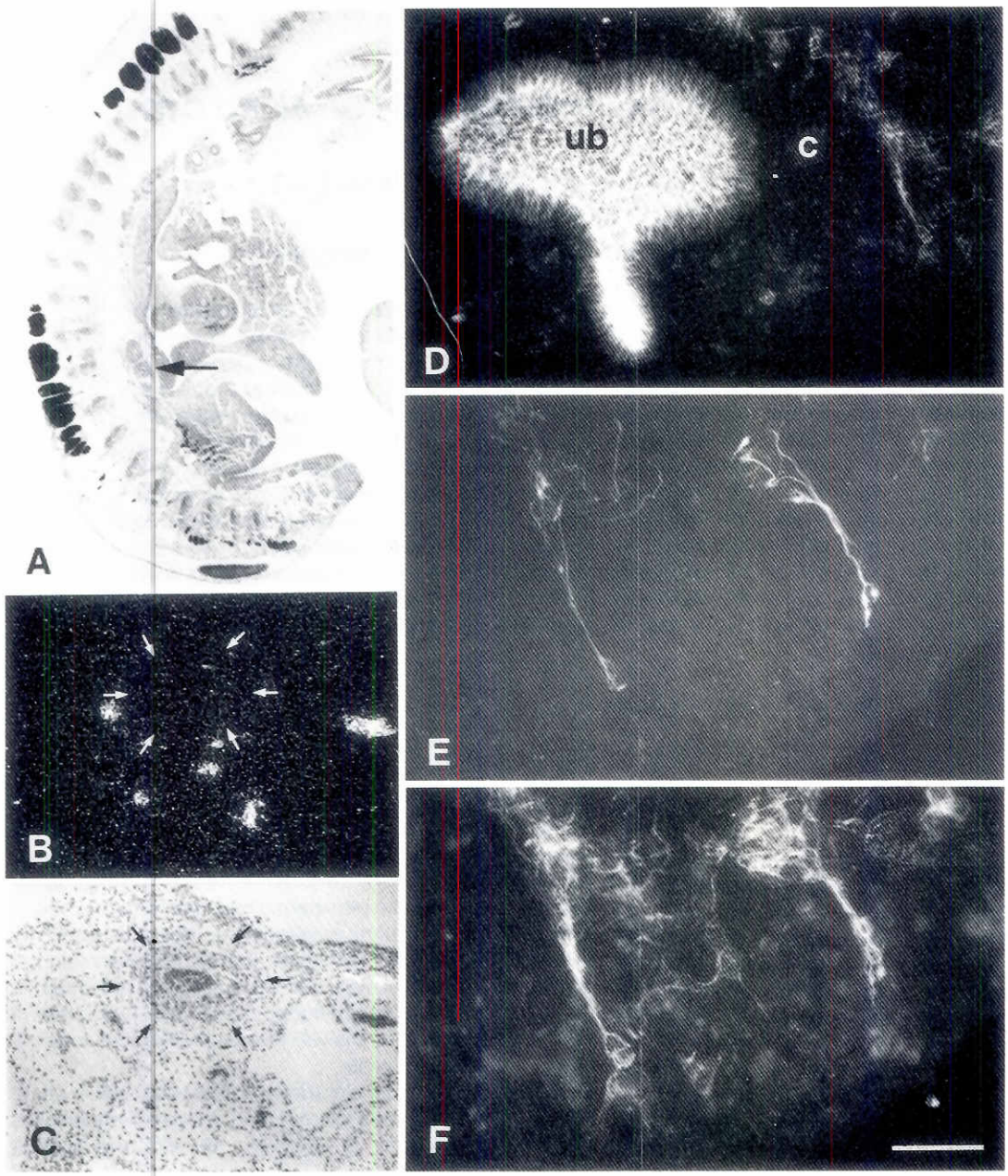


Fig. 1. Neurofilament expression in early rat kidneys. (A) *In situ hybridization of E13.5 whole embryo with neurofilament light chain (NF-L) cRNA probe. Spinal ganglions are massively labeled. Metanephric kidney is marked by an arrow.* **(B,C)** *Dark and bright field illustrations of the urothelium area of E13.5 embryo hybridized with NF-L cRNA. NF-L transcripts are seen in small clusters in medial, cranial and caudal sides of the first condensate in metanephric kidney (small arrows).* **(D)** *Whole-mount immunohistochemistry of E13.5 metanephric kidney labeled with L1 neural cell adhesion molecule. The ureter bud epithelium (ub) is labeled, and a few cells around the condensed mesenchyme (c).* **(E,F)** *A high magnification of the same explant double labeled with the monoclonal antibody 13AA8 against neurofilaments* **(E)** *and the polyclonal antibodies against L1* **(F).** *Bars, A, 1000 μm; B,C, 180 μm; D, 100 μm; E,F: 50 μm.*

al., 1987). Neurofilaments form the normal radial structure of axons (Friede and Samorajski, 1970; Hoffman *et al.*, 1987; Cleveland *et al.*, 1991; Yamasaki *et al.*, 1992) with NF-L subunit core and NF-M and NF-H coassembly (Delacourte *et al.*, 1980; Geisler and Weber, 1981; Liem and Hutchison, 1982; Hisanaga and Hirokawa, 1988; Troncoso *et al.*, 1990). NF-L is able to self-assemble and form a long intermediate network in axons, but NF-M and NF-H produce only very short filamentous structures (Delacourte *et al.*,1980; Geisler and Weber, 1981; Liem and Hutchison, 1982; Aebi *et al.*, 1988; Hisanaga and Hirokawa, 1988, 1989; Troncoso *et al.*, 1990; Heins *et al.*,1993). Although the neurofilaments are characteristic of neuronal cells, they have also been found in certain neural crest-derived and Schwann cells.

We show that the renal stroma includes a spindle-shaped cell lineage that expresses both NF-L and NF-M from the early stage of development on to neonates. Thus, the mesenchyme of the kidney rudiment contains cells with either a tubulogenic or stromal differentiation option, and the stromal cells are characterized by their neural-type intermediate filament structure. The proliferation index of the spindle shaped stromal cells remained very low, showing that during kidney morphogenesis the difference in cell proliferation contributes to the diminishing proportion of the stroma. The histogenesis of the spindle-shaped stromal cells remains open, but the expression of neural characteristics in renal stroma raises the possibility that these cells are of neural crest rather than mesodermal origin.

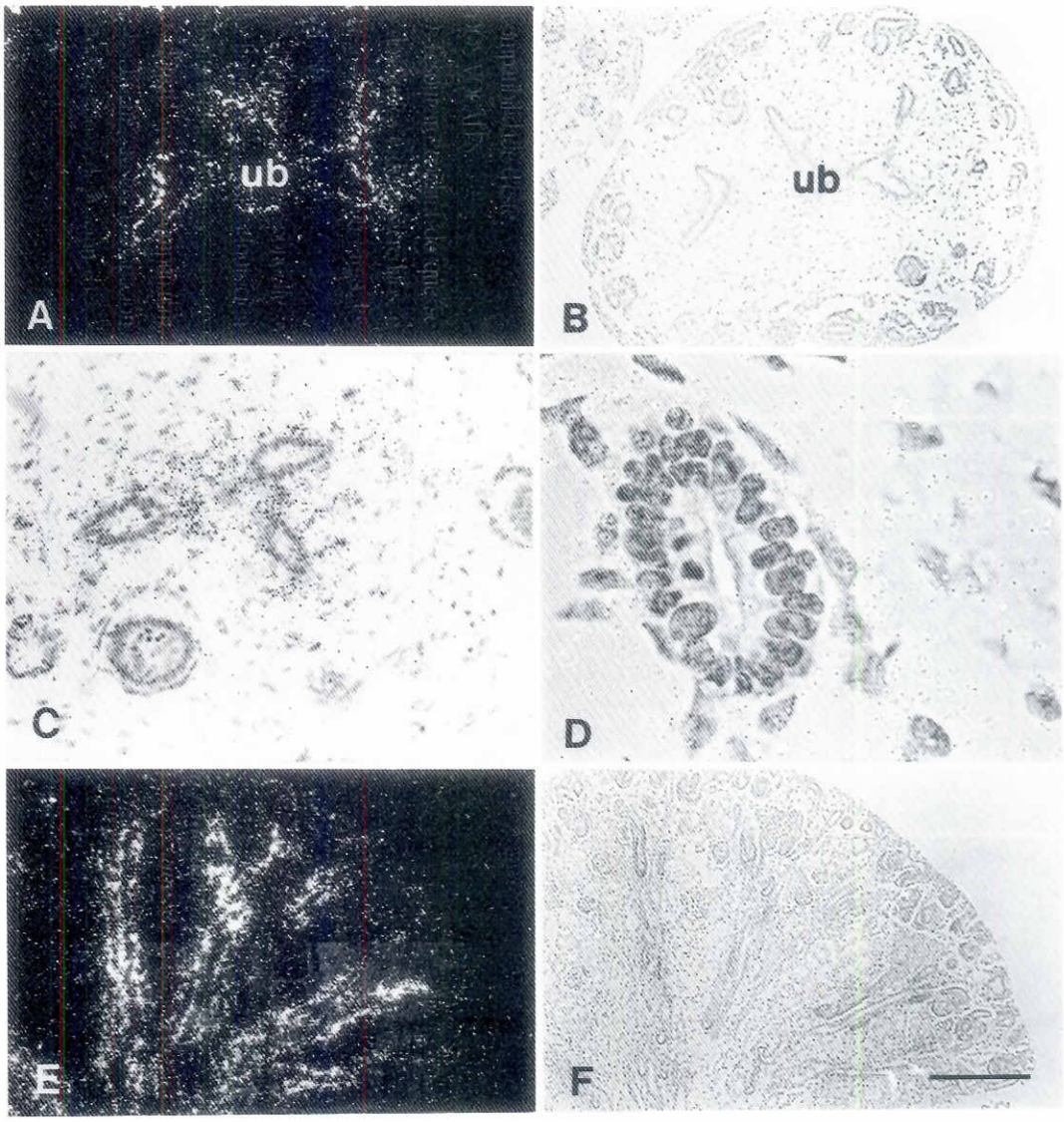


Fig. 2. Peritubular expression of NF-L mRNA in embryonic rat kidneys. (A,B) *Dark and bright field illustrations of in situ hybridization of NF-L mRNA in E16 metanephric kidney. The cells surrounding the medullary branches of the ureter express NF-L transcripts.* **(C,D)** *High magnification of the medulla shows that the silver grains are over the spindle-shaped peritubular cells trapped in a loose extracellular matrix.* **(E,F)** *At E20 NF-L transcripts are still seen in the peritubular region around the collecting tubules. In adult kidney we did not see NF-L expression with in situ hybridization (data not shown). Bars, A,B: 120 μm; C: 70 μm; D: 30 μm; E,F: 250 μm.*

Results

Both immunohistochemical staining and *in situ* hybridization revealed an identical pattern of expression for neurofilament proteins and NF-L transcripts. At day 13.5 the embryonic rat kidney rudiment consists of the branching ureter bud and the nephrogenic mesenchyme. NF-L mRNA was seen in a few cells adjacent to the first group of condensing pretubular cells, but not within the condensate (Fig. 1A-C). The transcripts were seen at the medial, caudal and cranial sides of the pretubular condensate, but not lateral to it (Fig. 1B and C). L1 neural cell adhesion protein (L1) is widely expressed in different epithelial and neural cell types (Probstmeier *et al.*, 1990; Nolte and Martini, 1992). Double labeling of the E13 kidney rudiment with an antibody against L1 and neurofilaments (Fig. 1D-F) showed that L1 was expressed not only in the neurofilament-positive microganglion derived neuronal cells, but also in the ureter bud (Fig. 1D). The antibodies against L1 could therefore, conveniently, be used to define the spatial relationship of neurofilament-positive cells with ureter bud-derived epithelial cells. As with *in situ* hybridization, immunocytochemistry detected neurofilament-positive cells adjacent to the first pretubular condensate (Fig. 1D-F).

At later stages of renal development *in situ* hybridization revealed expression of NF-L mRNA in cells around the branches of the ureter bud (Fig. 2A-F). These NF-L mRNA-positive peritubular cells did not resemble L1-positive neuronal cells in their morphol-

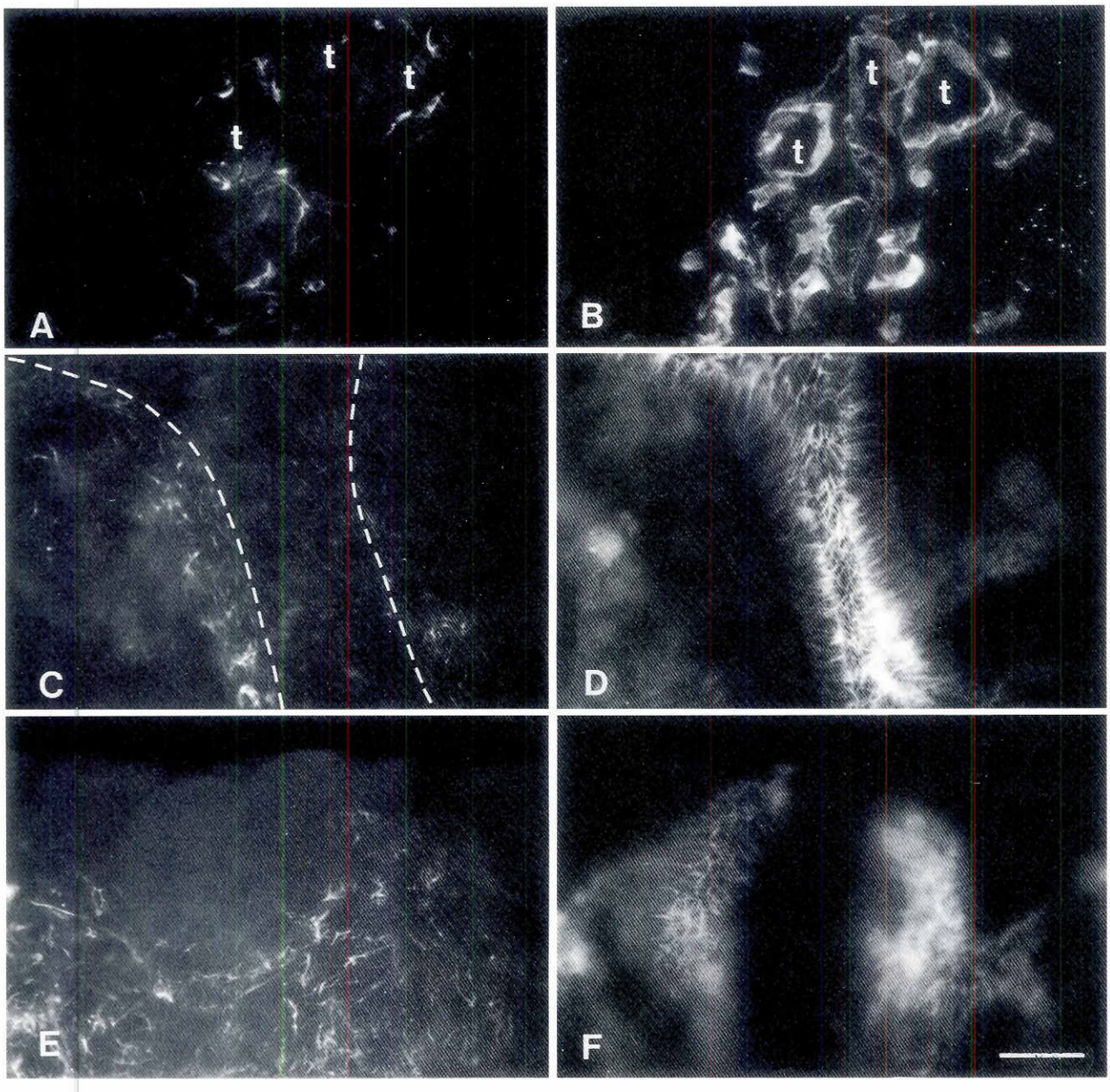


Fig. 3. Expression of neurofilament proteins around kidney tubules in E18 kidneys. *A frozen section double labeled with anti-neurofilament* **(A)** *and anti-laminin* **(B)**, *t, kidney tubules. Whole-mount preparations of E16 kidneys reveal neurofilament expression* **(C and E)** *around the L1 neural cell adhesion molecule-positive collecting tubules* **(D and F). (C and D)** *Medullary region;* **(E and F)** *the cortical region. Note that neurofilaments are not seen in the uppermost region of the kidney cortex in* **E**. *Bars, A,B: 30 μm; C-F: 50 μm.*

ogy. The peritubular NF-L transcripts were seen in the spindle-shaped and clear-cytoplasmic stromal cells (Fig. 2C and D). Other stromal cells further away from the branches of the ureter bud were negative for NF-L transcripts, but by morphological criteria they resembled the NF-L mRNA-positive peritubular cells (Fig. 2A-C).

The expression of neurofilaments in the peritubular stromal cells in the E16 metanephric kidneys is shown in Fig. 3. Whole-mount immunolabeling of embryonic kidneys provides a good "three-dimensional" view of different structures. In addition to the stromal expression of neurofilaments (Fig. 3C-F), such explants revealed typical neuronal cells with neurites in a microganglia-like structure that was located adjacent to the stalk of the ureter (Fig. 4). This group of cells could also be seen by *in situ* hybridization in the hilus of the kidney (Fig. 4A).

A clear molecular difference between the neurofilament-positive stromal cells and the microganglion neuronal cells could be demonstrated by double-immunolabeling the whole-mount explants with the polyclonal antibody against L1 neural cell adhesion protein

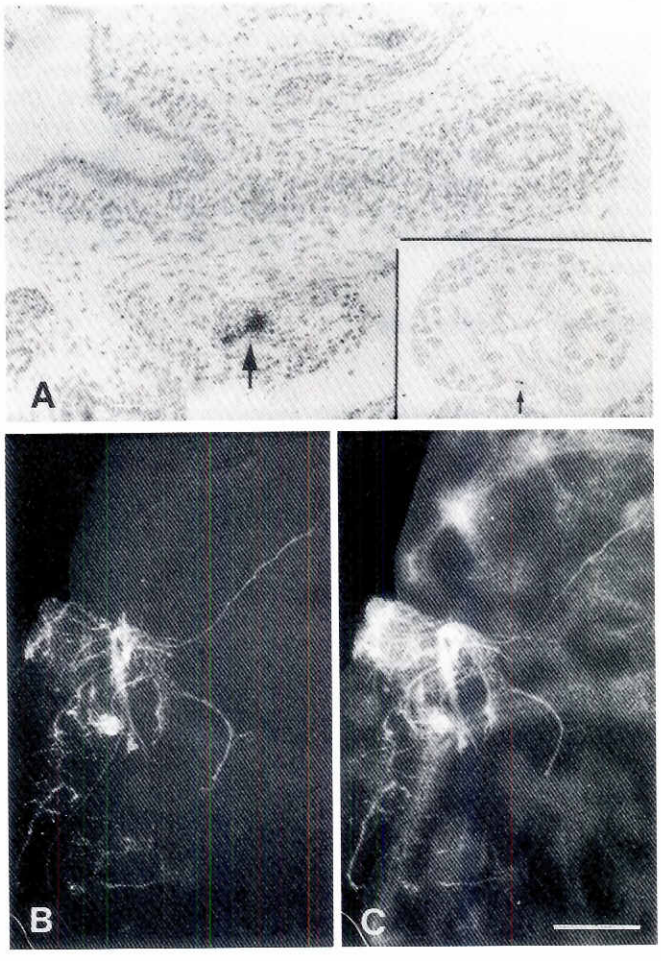


Fig. 4. Neuronal microganglion in the hilus of the kidney. (A) *In situ hybridization of E17 metanephric kidney with NF-L cRNA shows the microganglion adjacent to the ureter (localization of the area is shown in the insert).* **(B and C)** *show the same cells in E16 kidney whole-mount with anti-neurofilament* **(B)** *and anti-L1 neural cell adhesion molecule* **(C)** *antibodies. Bars, A: 70 μm, insert: 650 μm; B,C: 100 μm.*

and with the 13AA8 monoclonal antibody against neurofilaments. L1 was not expressed in the neurofilament-positive peritubular stromal cells (see Fig. 3C-F). Coexpression of L1 and neurofilament proteins was detected only in the microganglion-like group of cells and in neurites derived from it (see Fig. 4B-C). Thus, the peritubular stromal cells have not only a typical morphology, but they can also be characterized by their immunocytochemical pattern.

To analyze which of the three neurofilament proteins are expressed in the embryonic kidney, whole-mount preparations of E15 to E17 kidneys were stained by monoclonal antibodies that specifically recognize either NF-L, NF-M, or NF-H proteins. The peritubular stromal cells expressed only NF-L and NF-M, but no NF-H, whereas the cells of the renal microganglion expressed all neurofilament proteins (data not shown).

Bromodeoxyuridine incorporation in the embryonic kidneys

The proliferation index of different cell types of the mouse embryonic kidney at day 16 is shown in Table 1. BrdU incorporation assay indicates that the cell proliferation is high both in the condencing pretubular mesenchyme in the S-shaped epithelia, and in the cortical branches of the ureter bud, but low in the renal stroma.

Discussion

The present study was undertaken to analyze molecular features and proliferation characteristics of the spindle-shaped, clear cytoplasmic stromal cells of the embryonic kidney. Unexpectedly, NF-L and NF-M proteins, and NF-L transcripts were found not only in the cells of the renal microganglion, but also in the cells of the peritubular stroma. As compared to the epithelial cells, these cells showed a low rate of BrdU incorporation, which suggests that low cell proliferation may contribute to the diminishing proportion of the stroma during late kidney morphogenesis.

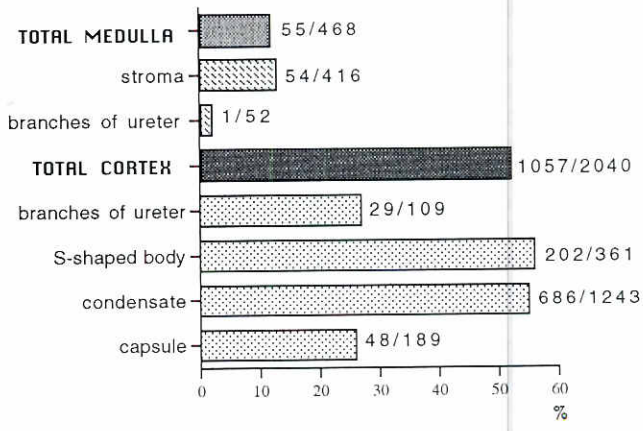
Two-step induction of kidney morphogenesis

Kidney development is an example of organogenesis guided by heterotypic interactions between several cell lineages which differentiate and assemble in a synchronized manner. The interaction between the ureter bud and the mesenchymal blastema leads to a series of sequential metabolic and morphological changes (Saxén *et al.*, 1968; Ekblom *et al.*, 1981; Saxén, 1987). A variety of heterotypic tissues can trigger the tubulogenesis and lead to formation of typical kidney tubules (Grobstein, 1955; Lombard and Grobstein, 1969; Unsworth and Grobstein, 1970). Before the ureter bud enters the blastema, the nephrogenic mesenchyme has developed a "tubulogenic bias" as no other embryonic mesenchyme can be converted into tubules of the secretory nephron (Saxén, 1970). After invasion of the ureter bud, the tubulogenic cells are initially rescued from apoptosis (Koseki *et al.*, 1992). The surviving, "induced" cells are then stimulated to proliferate, they become aggregated and ultimately convert into epithelial structures.

Increasing intercellular adhesion leading to aggregation of the induced cells is a prerequisite for their further differentiation and polarization (Saxén *et al.*, 1988). It has been suggested that this stage is not affected primarily by the ureter bud, but that a two-step

TABLE 1

PROFLIFERATION INDEXES IN VARIOUS RENAL CELL TYPES AT DAY E16



mechanism operates during tubulogenesis (Gossens and Unsworth, 1972). Initially the contact with the ureter bud might lead to induction of certain key molecules, such as WT-1 and pax-2, that selectively activate the genes required for further nephrogenesis and repress the others (Dressler and Douglass, 1992; van Heyningen and Hastie, 1992; Kreidberg *et al.*, 1993). The subsequent second step of induction would be regulated by an interaction between the induced pretubular cells and the surrounding stroma. In accordance with this proposal, an antibody against the cell surface ganglioside GD_3, an antigen expressed in the stroma of the embryonic mouse metanephros, perturbs nephrogenesis (Sariola *et al.*, 1988a). The two-step induction hypothesis provides a possible explanation for coordinated and controlled complex morphogenesis of the cell lineages taking part in the various functions of a mature nephron. Only the nephrons in a proper local environment (i.e. neurons, blood vessels, stroma etc.) continue their differentiation, while the others cease to differentiate and undergo apoptosis.

The developmental fate of the embryonic renal stroma

The developmental fate of the embryonic renal stroma is unknown, but it represents only a minor population in adult kidneys. Either the peritubular cells have a low proliferation rate or they undergo apoptosis. Koseki *et al.* (1992) described apoptosis in peritubular stromal cells. These apoptotic cells apparently represent the stromal cells with neuronal characteristics. Even though precise calculations are not available, most of the apoptosis in the embryonic kidney seems to occur in the stroma (Koseki *et al.*, 1992). Coles *et al.* (1993) recently estimated that 50% of the metanephric cells die through apoptosis during kidney differentiation. We now show by the BrdU incorporation assay that the proliferation index in the stromal cells is much lower than in epithelial cells or in the induced condensates. This changes the ratio of stromal versus epithelial cells during morphogenesis and will ultimately result in epithelial predominance in neonatal kidney, even without apoptosis of the stromal cells. Thus, both a low proliferation index and apoptosis of stromal cells lead to the paucity of stroma in adult metanephros.

L1 neural cell adhesion molecule in epithelial collecting ducts and neurons

L1 neural cell adhesion molecule (Rathjen and Schachner, 1984) has been detected in several neural and non-neural cell types (Probstmeier *et al.*, 1990; Nolte and Martini, 1992). It affects neuronal outgrowth through both homophilic and heterophilic association of cell adhesion molecules (Sonderegger and Rathjen, 1992). We now show that L1 is expressed on embryonic collecting tubules and renal neurons. Of the two different renal cell types with neurofilaments, L1 is expressed only on the typical neuronal cells, but not on the stromal cells. The practical consequence of this finding is that immunocytochemistry can be used in defining these two cell lineages. It also indicates that, if L1 is involved in the pattern formation of the stromal cells or in the epithelial-mesenchymal interaction, it acts through the heterophilic rather than homophilic binding principle.

The developmental origin of the renal stroma

The transplantation studies with chick-quail chimeras (LeDouarin and Teillet, 1974) and neural crest labeling with a vital dye (Bronner-Fraser and Fraser, 1988) have traced trunk neural crest cells to mesonephric and metanephric kidney. The neural crest gives rise to numerous neuronal and non-neuronal phenotypes, such as peripheral neurons, Schwann and glial cells, pigment cells, head mesenchyme, and cartilage (reviewed by LeDouarin, 1982). Our present observations show that the spindle-shaped stroma of the embryonic kidney includes cells that possess neuronal characteristics. The unexpected neuronal feature of the stromal cells raise the possibility that they may be the neural crest derivatives in the embryonic metanephric kidney, a hypothesis that deserves further experimental studies.

Materials and Methods

Animals

Sprague-Dawley rat and CBAxNMRI mouse embryos were used throughout the study. Animals were mated overnight (E0). In mice, the appearance of the vaginal plug was considered as day 0. Embryonic rat kidneys were microsurgically dissected at different stages beginning from E13, and the stage of the embryos was further verified by the size of the ureter bud.

Antibodies

The mouse monoclonal antibody 13AA8 (diluted 1:2 in phosphate buffered saline: PBS) against purified bovine neurofilament polypeptides (Virtanen *et al.*, 1985) reacts with all three neurofilament proteins of mouse cerebellum (Tienari *et al.*, 1987). This antibody was used in double labeling experiments with different polyclonal antibodies. The different subunits of the neurofilament triplet were analyzed in the whole-mount preparations with monoclonal antibodies detecting specifically either NF-L, NF-M, or NF-H (Boehringer Mannheim, Germany). These three antibodies were used at 1:10 dilution. The rabbit polyclonal affinity purified antibody against mouse brain neural cell adhesion molecule LI (Rathjen and Schachner, 1984) was diluted 1:100. Rabbit polyclonal antibody (diluted 1:400 in PBS) against mouse EHS-laminin was from Gibco-BRL (Paisley, Scotland). The secondary rhodamine-conjugated goat anti-mouse IgG (dilution 1:100) and the fluorescein-conjugated donkey anti-rabbit IgG (dilution 1:200) antibodies were from Jackson Immunoresearch (West Grove, PA, USA).

Immunohistochemistry

The dissected kidneys were fixed with -20°C methanol for 5 min at room temperature and rinsed with PBS containing 11.6% sucrose and 1% BSA, and stained by whole-mount immunofluoresence as previously described (Sariola *et al.*, 1988b). Kidneys of different developmental stages were also dissected for frozen section immunohistochemistry, mounted in Tissue Tek O.C.T. compound (Elkhard, IN, USA), frozen in dry ice and sectioned at 6 μm. Sections were fixed with -20°C methanol for 5 min or acetone for 10 min, rinsed with PBS, and double-stained with different antibodies against tubular and neuronal antigens for 1 h or over night. Sections were stained with secondary antibodies for 1 h and mounted with Elvanol.

Construction of the probes

The cDNA probe encoding for the neurofilament light (NF-L) protein was subcloned into pGEM™-4Z vector from the full-length rat NF-L cDNA (J. Palgi, unpublished results) as a *XbaI*/*KpnI* 120 bp insert corresponding to nucleotides 396-516 of the partial NF-L sequence (Julien *et al.*, 1985). This probe was used for *in situ* hybridization as described (Wilkinson and Green, 1990).

In situ hybridization

E13-15 whole embryos and kidneys from E13-21 embryos were fixed in phosphate-buffered 4% paraformaldehyde overnight at 4°C or 1-2 h at room temperature, respectively. Adult rat kidneys were perfusion fixed with the same fixative. The tissues were embedded in paraffin, and serial sections were cut at 5 μm on triethoxysilane- and acetone-treated slides. The ^{35}S-UTP-labeled sense and antisense cRNA probes were prepared and the *in situ* hybridization was performed according to Wilkinson and Green (1990). The probes were diluted to the final concentration of $20x10^6$

cpm/ml, and 10-40 μl of the hybridization buffer with the label was added to each slide. Hybridization was carried out for 15-20 h at 52°C and the high-stringency washes with 50% formamide (Merck, Darmstadt, Germany) and 30 mM of dithiothreitol (Sigma, St. Loius, MO, USA) at 65°C for 1 h. The slides were RNase A (Boehringer Mannheim) treated (20 μg/ml) for 30 min at 37°C, washed and dehydrated, and coated for autoradiography with NTB-2 emulsion (Eastman Kodak, New Haven, CT, USA). After 14 days exposure at 4°C, the sections were developed in Kodak D-19, fixed with Kodak Unifix (Kodak-Pathé, Chalon-Sur-Saone, France), counterstained with hematoxylin (Shandon, Pittsburgh, PA, USA) and mounted with Permount (Fisher Scientific, NJ, USA).

Bromodeoxyuridine incorporation

BrdU was injected intraperitoneally to mice at day 16 of pregnancy. After 2 h the mice were sacrified and the embryos were fixed in Bouins fixative, embedded in paraffin, and serially sectioned at the level of the kidneys. The incorporated BrdU was analyzed by indirect immunoperoxidase staining with anti-BrdU antibody and Vectastain ABC-kit (Vector Laboratories, Burlingame, CA, USA), and the proliferation index was counted as the ratio of labeled and unlabeled nuclei. BrdU label and the mouse anti-5-bromo-2'-deoxyuridine antibody were from Amersham (Buckinghamshire, UK).

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References

AEBI, U., HÄNER, M., TRONCOSO, J., EICHNER, R. and ENGEL, A. (1988). Unifying principles in intermediate filament (IF) structure and assembly. *Protoplasma 145:* 73-81.

BRONNER-FRASER, M. and FRASER, S. (1988). Cell lineage analysis reveals multipotency of some avian neural crest cells. *Nature 335:* 161-164.

CARDEN, M., TROJANOWSKI, J., SCHLAAEPFER, W. and LEE, V. (1987). Two-stage expression of neurofilament polypeptides during rat neurogenesis with early establishment of adult phosphorylation patterns. *J. Neurosci. 7:* 3489-3504.

CLEVELAND, D., MONTEIRO, M., WONG, P., GILL, S., GEARHART, J. and HOFFMAN, P. (1991). Involvement of neurofilaments in the radial growth of axons. *J. Cell Sci. 15:* 85-95.

COLES, H., BURNE, J. and RAFF, M. (1993). Large-scale normal cell death in the developing rat kidney and its reduction by epidermal growth factor. *Development 118:* 777-784.

DELACOURTE, A., FILLIATREAU, G., BOTTEAU, F., BISERTE, G. and SCHREVEL, J. (1980). Study of the 10-nm filament fraction during the standard microtubule preparation. *Biochem. J. 191:* 543-546.

DRESSLER, G. and DOUGLASS, E. (1992). Pax-2 is a DNA-binding protein expressed in embryonic kidney and Wilms tumor. *Proc. Natl. Acad. Sci. USA 89:* 1179-1183.

EKBLOM, P., LEHTONEN, E., SAXÉN, L. and TIMPL, R. (1981). Shift of collagen type as early response to induction of the metanephric mesenchyme. *J. Cell. Biol. 89:* 276-283.

FRIEDE, R. and SAMORAJSKI, T. (1970). Axon caliber related to neurofilaments and microtubules in sciatic nerve fibers of rats and mice. *Anat. Rec. 167:* 379-388.

GEISLER, N. and WEBER, K. (1981). Self-assembly *in vitro* of the 68,000 mw component of the mammalian neurofilament triplet protein into intermediate-sized filaments. *J. Mol. Biol. 151:* 565-571.

GOSSENS, C. and UNSWORTH, B. (1972). Evidence for two-step mechanism operating during *in vitro* mouse kidney tubulogenesis. *J. Embryol. Exp. Med. 28:* 615-631.

GROBSTEIN, C. (1955). Inductive interaction in the development of hte mouse metanephros. *J. Exp. Zool. 130:* 319-340.

HEINS, S., WONG, P., MÜLLER, S., GOLDIE, K., CLEVELAND, D. and AEBI, U. (1993). The rod domain of NF-L determines neurofilament architecture, whereas the end domains specify filament assembly and network formation. *J. Cell Biol. 123:* 1517-1533.

HERZLINGER, D., KOSEKI, C., MIKAWA, T. and AL-AWQATI, Q. (1992). Metanephric mesenchyme contains multipotent stem cells whose fate is restricted after induction. *Development 114:* 565-572.

HISANAGA, S. and HIROKAWA, N. (1988). Structure of the peripheral domains of neurofilaments revealed by low angle rotary shadowing. *J. Mol. Biol. 202:* 297-305.

HISANAGA, S. and HIROKAWA, N. (1989). The effects of dephosphorylation on the structure of the projections of neurofilaments. *J. Neurosci. 9:* 959-966.

HOFFMAN, P., CLEVELAND, D., GRIFFIN, J., LANDES P., COWAN, N. and PRICE, D. (1987). Neurofilament gene expression: a major determinant of axonal caliber. *Proc. Natl. Acad. Sci. USA 84:* 3472 - 3476.

JULIEN, J-P., MEIJER, D., HURSTI, J. and GROSVELD, F. (1986). Cloning and developmental expression of the murine neurofilament gene family. *Mol. Brain Res. 1:* 243-250.

JULIEN, J-P., RAMACHANDRAN, K. and GROSVELD, F. (1985). Cloning of a cDNA encoding the smallest neurofilament protein from rat. *Biochim. Biophys. Acta 825:* 398-404.

KISSANE, J. (1983). Development of the kidney. In *Pathology of the Kidney.* (Ed. R. Heptinstall). Little, Brown and Co., Boston, pp. 61-81.

KOSEKI, C., HERZLINGER, D. and AL-AWQATI, Q. (1992). Apoptosis in metanephric development. *J. Cell Biol. 119:* 1327-1333.

KREIDBERG, J., SARIOLA, H., LORING, J., MAEDA, M., PELLETIER, J., HOUSMAN, D. and JAENICSH, R. (1993). WT-1 is required for early kidney development. *Cell 74:* 679-69.

LEDOUARIN, N. (1982). *The Neural Crest.* Cambridge University Press, Cambridge.

LEDOUARIN, N. and TEILLET, M-A. (1974). Experimental analysis of the migration and differentiation of neuroblasts of the autonomic nervous system and of neuroectodermal derivatives, using a biological cell marking technique. *Dev. Biol. 41:* 162-184.

LIEM, R. (1993). Molecular biology of neuronal intermediate filaments. *Curr. Opin. Cell Biol. 5:* 12-16.

LIEM, R. and HUTCHISON, S. (1982). Purification of individual components of the neurofilament triplet, filament assembly from the 70,000-dalton subunit. *Biochemistry 21:* 3221-3226.

LOMBARD, M-N. and GROBSTEIN, C. (1969). Activity of various embryonic and postembryonic sources for induction of kidney tubules. *Dev. Biol. 19:* 41-45.

NOLTE, C. and MARTINI, R. (1992). Immunocytochemical localization of the L1 and N-CAM cell adhesion molecules and their shared carbohydrate epitope L2/HNK-1 in the developing and differentiated gustatory papillae of the mouse tongue. *J. Neurocytol. 21:* 19-33.

PROBSTMEIER, R., MARTINI, R., TACKE R. and SCHACHNER, M. (1990). Expression of the adhesion molecules L1, N-CAM, and J1/tenascin during development of the murine small intestine. *Differentiation 44:* 42-55

RATHJEN, F. and SCHACHNER, M. (1984). Immunocytological and biochemical characterization of a new neuronal cell surface component (L1 antigen) which is involved in cell adhesion. *EMBO J. 3:* 1-10.

SARIOLA, H., AUFDERHEIDE, E., BERNHARD, H., HENKE-FAHLE, S., DIPPOLD, W. and EKBLOM, P. (1988a). Antibodies to cell surface ganglioside GD_3 perturb inductive epithelial-mesenchymal interactions. *Cell 54:* 235-245.

SARIOLA, H., HOLM, K. and HENKE-FAHLE, S. (1988b). Early innervation of the metanephric kidney. *Development 104:* 589-599.

SAXÉN, L. (1970). Failure to demonstrate tubule induction in a heterologous mesenchyme. *Dev. Biol. 23:* 511-523.

SAXÉN, L. (1987). *Organogenesis of the Kidney.* Cambridge University Press, Cambridge.

SAXÉN, L., KOSKIMIES, O., LAHTI, A., MIETTINEN, H., RAPOLA, J. and WARTIOVAARA, J. (1968). Differentiation of kidney mesenchyme in an experimental model system. In *Advances in Morphogenesis, 7* (Eds. M. Abercombie, J. Brachet and T. J. King). Academic Press, London, pp. 251-293.

SAXÉN, L., VAINIO, S., JALKANEN, M. and LEHTONEN, E. (1988). Intercellular adhesion and induction of epithelialization in the metanephric mesenchyme. In *Regulatory Mechanisms in Developmental Processes* (Eds. G. Eguchi, T. Okada and L. Saxén). *Cell Differ. Dev. (Suppl.) 25:* 111-118.

SHAW, G. and WEBER, K. (1982). Differential expression of neurofilament triplet proteins in brain development. *Nature 298:* 277-279.

SONDEREGGER, P. and RATHJEN, F. (1992). Regulation of axonal growth in the vertebrate nervous system by interactions between glycoproteins belonging to two subgroups of the immunoglobulin superfamily. *J. Cell Biol. 119:* 1387-1394.

TIENARI, J., VIRTANEN, I., SOINILA, S. and LEHTONEN, E. (1987). Neuron-like derivaties of cultured F9 embryonal carcinoma cells express characteristics of parietal endoderm cells. *Dev. Biol. 123:* 566-573.

TRONCOSO, J., MARCH, J., HÄNER, M. and AEBI, U. (1990). Effect of aluminium and other multivalent cations on neurofilaments *in vitro:* an electron microscopic study. *J. Struct. Biol. 103:* 2-12.

UNSWORTH, B. and GROBSTEIN, C. (1970). Induction of kidney tubules in mouse metanephrogenic mesenchyme by various embryonic mesenchymal tissues. *Dev. Biol. 21:* 547-556.

VAN HEYNINGEN, V. and HASTIE, N. (1992). Wilm's tumor: reconciling genetics and biology. *Trends Genet. 8:* 16-21.

VIRTANEN, I., MIETTINEN, M., LEHTO, V-P., KARINIEMI, A-L. and PAASIVUO, R. (1985). Diagnostic applications of monoclonal antibodies to intermediate filaments. *Ann. NY Acad. Sci. 455:* 635-648.

WELLER, A., SOROKIN L., ILLGEN E-M. and EKBLOM, P. (1991). Development and growth of mouse embryonic kidney in organ culture and modulation of development by soluble growth factor. *Dev. Biol. 144:* 248-261.

WILKINSON, D. and GREEN, P. (1990). *In situ* hybridization and the three-dimensional reconstruction of serial sections. In *Postimplantation Mammalian Embryos: A Practical Approach* (Eds. A. Copp and D. Cockroft). Oxford University Press, London, pp. 155-171.

WOLGAST, M. (1985). Renal interstitium and lymphatics. In *The Kidney: Physiology and Pathophysiology* (Eds. D. Seldin and G. Giebisch). Raven Press, New York, pp. 497-518

YAMASAKI, H., BENNETT, G., ITAKURA, C. and MIZUTANI, M. (1992).Defective expression of neurofilament protein subunits in hereditary hypotrophic axonopathy of quail. *Lab. Invest. 66:* 734-743.

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