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Differential expression of gap junction mRNAs and proteins in the developing murine kidney and in experimentally induced nephric mesenchymes

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Summary

The expression of three gap junction (GJ) proteins, α_1 (Cx43), β_1 (Cx32), and β_2 (Cx26), and their transcripts were examined during the ontogeny of the mouse and rat kidney. These proteins were expressed in two non-overlapping patterns. The α_1 GJ protein was first observed in mesenchymal cells in the 12-day mouse kidney. By day 14 and thereafter, the α_1 protein was detected in the transient S-shaped bodies, but not in the podocytes of the maturing glomeruli. After birth the antigen was retained in a small subset of secretory tubules.

The β_1 and β_2 GJ proteins were similar in their developmental patterns. They were first detected in a small subset of secretory tubules in the subcortical zone of day 17 embryos. These tubules were identified by immunohistochemical markers to be proximal. At birth, practically all proximal tubules expressed the two antigens.

This analysis of GJ proteins was consistent with the results of S1 nuclease protection assays showing that, while the α_1 mRNA appeared early during kidney development and declined around birth, the two β mRNAs appeared later and became intensified during the last days of intrauterine development.

In experimentally induced metanephric mesenchymes, a transient expression of the α_1 GJ protein was seen during the segregation of the tubular anlagen. β_1 and β_2 GJ proteins were not detected in such induced mesenchymes cultivated up to 7 days.

These observations provide evidence for the cell-specific utilization of different GJ genes during different stages of kidney organogenesis. The α_1 gene is activated during the early segregation of the secretory tubule and might contribute to its compartmentalization, while the β_1 and β_2 gene products are not detected until advanced stages of development. The latter gene products might be correlated with the physiological activity of the proximal tubules in vivo, as they are not expressed in experimentally induced tubules detectable with markers for proximal tubules.

Key words: murine embryo, gap junctions, mRNA, protein, kidney differentiation, metanephric mesenchyme, transfilter induction.

Introduction

Gap junctions contain transmembrane channels that directly link adjacent cells and provide pathways for the transfer of low molecular weight molecules and ions from one cell to another (Gilula et al., 1972; Bennett and Goodenough, 1978; Loewenstein, 1981). The junctional channels are bipartite structures formed by the association of two oligomeric structures or connexons, each connexon representing an oligomeric arrangement of six polypeptides. The gap junction (GJ) proteins have been derived from a multigene family, based on a conserved region of about 200 amino-terminal residues that includes four transmembrane and two extracellular domains (Zimmer et al., 1987; Beyer et al., 1987; Milks et al., 1988; Goodenough et al., 1988; Nicholson and Zhang, 1988; Hertzberg et al., 1988; Yancey et al., 1989). Full-length sequences for several of these proteins have been deduced from cDNA analysis: a 32×10^3 M_r protein from mammalian liver (Paul, 1986; Kumar and Gilula, 1986), a 43×10^3 M_r protein from mammalian heart (Beyer et al., 1987), a 26×10^3 M_r protein from mammalian liver (Nicholson and Zhang, 1988), and a 31×10^3 M_r protein from several mammalian tissues, including placenta and skin (Hoh et al., 1991). A 46 ×10^3 M_r protein found in the lens also appears to be a member of this family because of its predicted structural motif (Kistler et al., 1988; Beyer et al., 1989). On the basis of predicted structural similarities, the multigene family has been divided into two classes, α and β (Risek et al., 1990). The α class contains α_1 (43 ×10^3 M_r), α_2 (38 ×10^3 M_r from amphibians) and α_3 (46 ×10^3 M_r); while the β class contains β_1 (32 ×10^3 M_r), β_2 (26 ×10^3 M_r) and β_3 (31 ×10^3 M_r).

Gap junctions mediate important developmental and physiological activities. In excitable tissues, gap junctions provide low-resistance coupling pathways for nerve conductance (Furshpan and Potter, 1968), myocardial contraction (Dreifuss et al., 1966) and the coordination of smooth muscle movement (for review, see Daniel, 1987). The specific function of each type of GJ protein remains largely unknown, although the dramatic elevation of α_1 mRNA seen in the myometrium the day before parturition is thought to be responsible for producing the junctions that synchronize the uterine contractions (Risek et al., 1990). During development, gap junctions have been shown to play important roles in compartmentalizing cells and in transmitting morphogenetic information (for review, see Guthrie and Gilula, 1989). Gap junctions connect the blastomeres of the 8-cell mouse embryo (Lo and Gilula, 1979) and, if junctional communication between cells of these embryos is blocked by treatment with antibodies that bind to GJ proteins, the communication-deficient cells are not retained by the compacted embryo (Lee et al.,1987). *Drosophila* imaginal discs and the gastrulating mouse embryo are both divided into communication compartments by GJ pathways that exist between certain cells and not others (Weir and Lo, 1982; Kalimi and Lo, 1988, 1989). Gap junctions also form communication compartments in developing frog embryos, which physiologically separate the presumptive neural ectoderm from presumptive epidermis (Warner, 1973). When antibodies to mammalian GJ protein were injected into blastomeres of 8-cell *Xenopus* embryos, electrical and ionic coupling was inhibited, and patterning defects (asymmetries) developed in those regions derived from the injected blastomere (Warner et al., 1984). Information transfer through gap junctions has also been found in developing invertebrates. The gradient of hydra head inhibitor was blocked by treatment with antibodies to mammalian GJ proteins (Fraser et al., 1987), and gap junctions appear at the specific time when the information required to form molluscan mesoderm is activated in the 3D macromere by contact with vegetal micromeres (de Laat et al., 1980).

The mammalian kidney provides an opportunity to analyze whether three of the well-characterized GJ proteins (α_1, β_1 and β_2) are utilized differentially during organ development. The kidney develops as a result of interactions between two tissues, the ureter bud and the metanephrogenic mesenchyme (reviewed in Saxén, 1987). When the bud enters the mesenchymal blastema on day 11 of mouse development, the mesenchymal cells induce it to branch. Conversely, the epithelium induces the mesenchyme to form the secretory nephrons first visualized as condensations around the tips of the ureter. The condensed cells then form a renal vesicle which develops a central cavity, and, subsequently, an S-shaped body is formed. The portion of this tube closest to the ureter becomes the distal tubule cells, while the rest develops into proximal tubules and the epithelium of the glomerulus. The continuous branching of the ureter bud and its induction of nephron formation in the mesenchyme generate the metanephric kidney.

Using antibodies and cDNA probes to three GJ proteins and their messengers, we analyzed the spatial and temporal distribution of these GJ proteins during mouse renal development. Results from this study demonstrate that the expression of these GJ proteins correlates with specific cell types.

Materials and methods

RNA analysis

Embryonic kidneys at different developmental stages were obtained by timed matings of Balb/c × C57BL/6 F_1 mice. The appearance of the vaginal plug was noted as day 0. The embryonic kidneys were removed at appropriate post-implantation stages in order to prepare RNA for analysis. For extraction of total RNA from the kidney samples, an acid phenol-guanidinium thiocyanate procedure was applied with RNAzol (Cinna/Biotex). The extracted RNA from the different stages was analyzed by using a mixture of GJ probes (for α_1, β_1 and β_2) in an S1 nuclease protection assay as described previously (Davis et al., 1986; Nishi et al., 1991).

Histology

The kidneys were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, for 1 hour at room temperature, mounted in Epon, and 1 μm sections were cut for staining with toluidine blue and examined in light microscopy.

Immunohistochemistry

Sections from whole embryonic kidneys, transfilter explants and reaggregated mesenchymal cell cultures were analyzed by immunohistochemistry. Affinity-purified rabbit antibodies were prepared against synthetic peptides corresponding to the S epitopes of GJ proteins α_1 and β_1 and the J epitope of β_2 (Milks et al., 1988; Risek et al., 1990). Mouse embryos from matings of CBA males with NMRI females and Spraque-Dowley rat embryos, used for double-staining immunofluorescence experiments, were obtained from the breeding colony of the Department of Pathology, University of Helsinki. For indirect immunohistochemistry, whole embryonic kidneys were rapidly frozen in OCT and sectioned on a Leitz 1720 cryostat without prior fixation. Sections of 5-6 μm were incubated with 3% BSA, 3% goat serum (Vector Laboratories, Burlingame, CA) in PBS (10 mM sodium phosphate, pH 7.5, 0.9% NaCl) for 1 hour at room temperature to reduce non-specific binding. Incubation with preimmune serum or antibodies was performed overnight at 4°C in the BSA- and goat serum-containing buffer, followed by three washes in PBS. FITC- and TRITC-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch, West Grove, PA) diluted 1:200 in PBS was added to the slides for 1 hour at room temperature. The slides were then washed three times in PBS and mounted in PBS-containing glycerol or in Elvanol (Klein et al., 1988). Podocalyxin, a rat podocyte- and endothelium-specific antibody (Schnabel et al., 1989, Miettinen et al., 1990), dipeptidylpeptidase IV, a rat proximal-tubule-specific antibody (Miettinen et al., 1990), as well as brush border and Tamm-Horsfall glycoprotein antibodies (Miettinen and Linder, 1976; Sikri et al., 1979; Dawnay et al., 1980; Ekblom et al., 1980) were generous gifts of Dr A. Miettinen (Department of Bacteriology and Immunology, University of

Helsinki). Double-staining immunofluorescence also utilized TRITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch) diluted 1:100 and FITC-conjugated goat anti-rat IgG (Cappel Laboratory, Cochranville, PA) diluted 1:500. Immunofluorescence was analyzed using a Zeiss Axiophot microscope with epifluorescence. Photographs were taken with Fuji Neopan 1600 ASA black-and-white professional print film.

Confocal microscopy was performed with the confocal scanning laser beam fluorescence microscope developed at the European Molecular Biology Laboratories, Heidelberg. The design and operating principles of this microscope have been described previously (Bacallao et al., 1989; Stelzer et al., 1989). FITC was excited at 488 nm by an argon laser (2020-05 SpectraPhysics, Inc., Mountain View, CA), and serial optical sections were made at 0.3-μm or 0.5-μm intervals.

Transfilter induction

Metanephric kidney rudiments were dissected from 11-day mouse and 13-day rat embryos. To separate the mesenchyme from the epithelial ureter bud, the explants were incubated in 0.75% pancreatin-2.25% trypsin for 1.5 minutes at 0°C. The manual separation was performed under a stereomicroscope at room temperature in Eagle's Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS; Myoclone Plus, GIBCO, Paisley, Scotland). Fragments of spinal cord from the same embryos were used as inducers in the transfilter experiments (Saxén and Lehtonen, 1978). In some experiments, the transfilter contact was interrupted after 22 hours and 48 hours to follow the development of the mesenchyme in prolonged cultures after a short induction pulse.

The isolated mesenchymes were transferred onto Nuclepore filters (General Electron Co., Pleasanton, CA) with an average pore size of 1.0 μm. A piece of spinal cord was glued beforehand on the opposite side of the filter, using agarose (Grobstein, 1956; Saxén and Lehtonen, 1987). The transfilter explants were cultured in MEM with 10% FCS and harvested at different intervals for immunohistochemistry.

Results

Temporal analysis of GJ mRNA expression during kidney development

The expression of GJ mRNA from three genes (α_1, β_1, β_2) during kidney development was studied by applying an S1 nuclease protection assay to RNA that was isolated at different developmental stages.

For this analysis, probes for all three transcripts were added together so that all three products could be analyzed simultaneously (Fig. 1). In the 13-day mouse embryonic kidney, the α_1 transcript was seen to be present at high levels. These levels remained high throughout development, but declined around birth. The β_1 transcript was readily detected by day 15, and the expression of this mRNA increased throughout development. On day 17, transcription of the β_2 message was detected, and the accumulation of this transcript increased dramatically after birth. The overall developmental pattern of expression differed for α_1 versus β_1 and β_2. The α_1 transcript was expressed at high abundance during early development, but the expression decreased significantly after birth. Conversely, the expression of the β_1 and β_2 transcripts increased significantly during development and after birth.

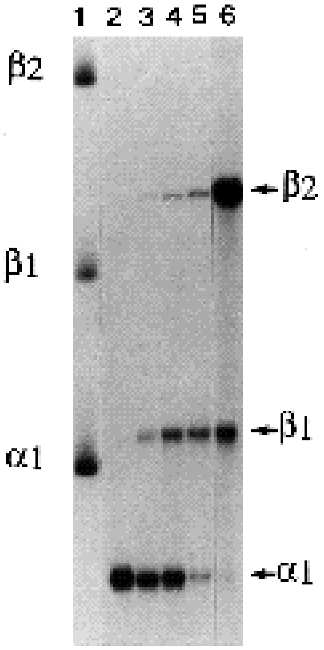


Fig. 1. Analysis of gap junction mRNA by an S1 nuclease protection assay during mouse kidney development. Three antisense single-stranded DNA probes were hybridized as a mixture to the different samples of kidney RNA (5 μg total RNA/sample). The lanes contain the following samples: (1) Undigested probes; (2) 13-day embryonic kidney RNA; (3) 15-day embryonic kidney RNA; (4) 17-day embryonic kidney RNA; (5) newborn kidney RNA; (6) 4-week-old kidney RNA.

In summary, the data from this analysis provides evidence that all three GJ genes are expressed during the development of the kidney, that these genes are developmentally regulated, and that there is a change in the relative abundance of these transcripts during kidney organogenesis.

Spatial and temporal immunolocalization of GJ proteins in the developing kidney

Expression pattern of the α_1 GJ protein

Stages of the early development of the metanephric kidney are illustrated in Fig. 2.

By immunohistochemistry, the α_1 GJ protein was detectable in the 12-day embryo. At this early stage of kidney development, the ureteric bud has branched only two or three times and patches of immunofluorescence were observed in the mesenchyme near these branches. This immunofluorescence in the mesenchyme was not seen in samples stained with preimmune sera or with the antibodies for other GJ proteins. By day 14, the expression of α_1 was localized to a particular subset of kidney mesenchymal cells: it was detected only on the cells that had formed the S-shaped bodies. The cells expressing α_1 in the 14-day mouse kidney were predominantly found in a subcortical zone containing early S-shaped bodies (Fig. 3A). Higher magnification of one of these regions (Fig. 3B) shows the relationship between the ureter bud and the S-shaped bodies on either side of it. The highest staining intensity of the α_1 antigen was in the crevice furthest from the ureter-derived collecting duct. The epithelial cells of this region are the presumptive glomerular podocytes, but when they mature, the α_1 antigen is concomitantly downregulated; double immunostaining with α_1 antibody and antipodocalyxin reveals no overlapping (Fig. 3C-F).

Expression pattern of the β_2 GJ protein

The β_2 GJ protein could not be detected until around day 17.

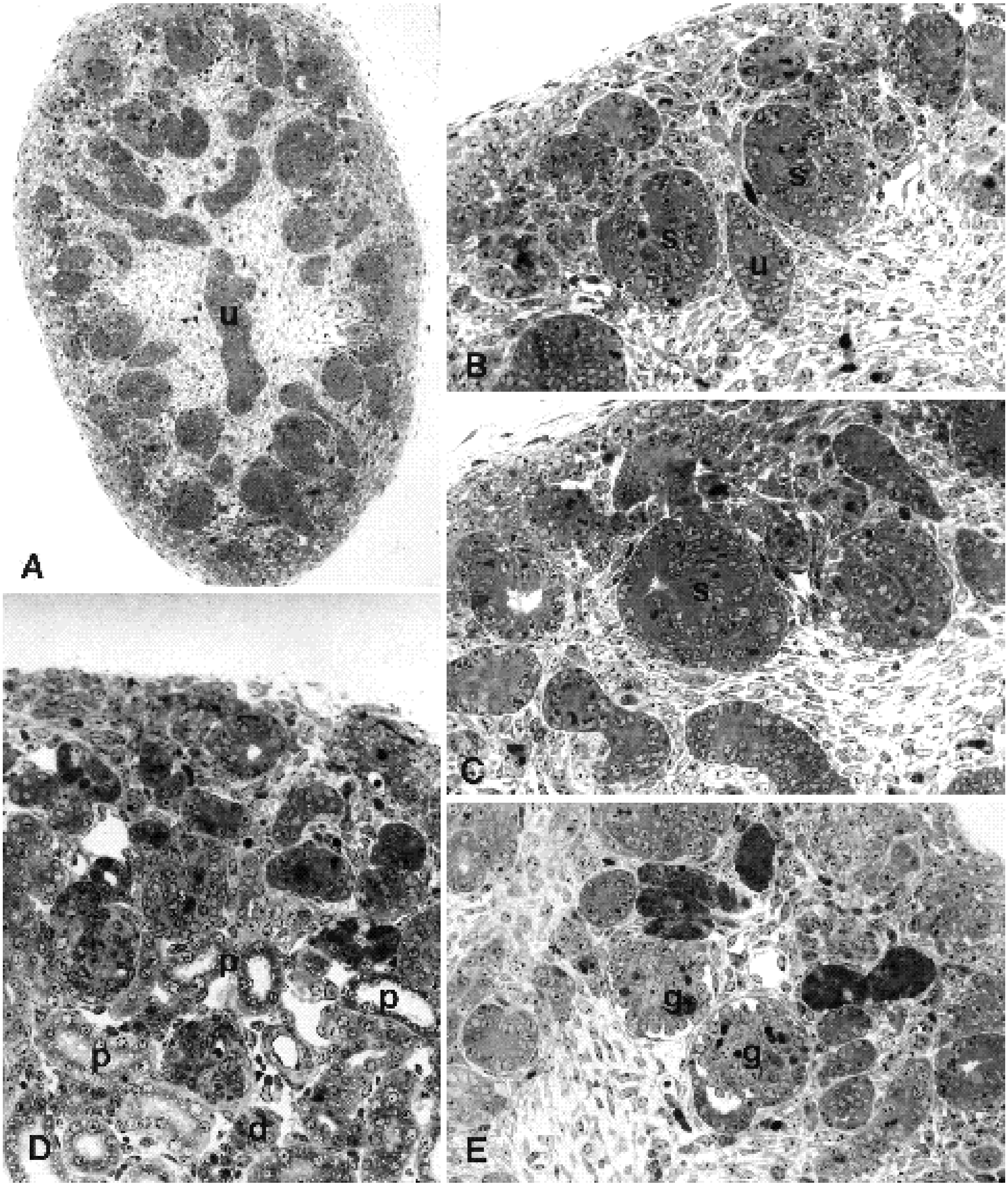
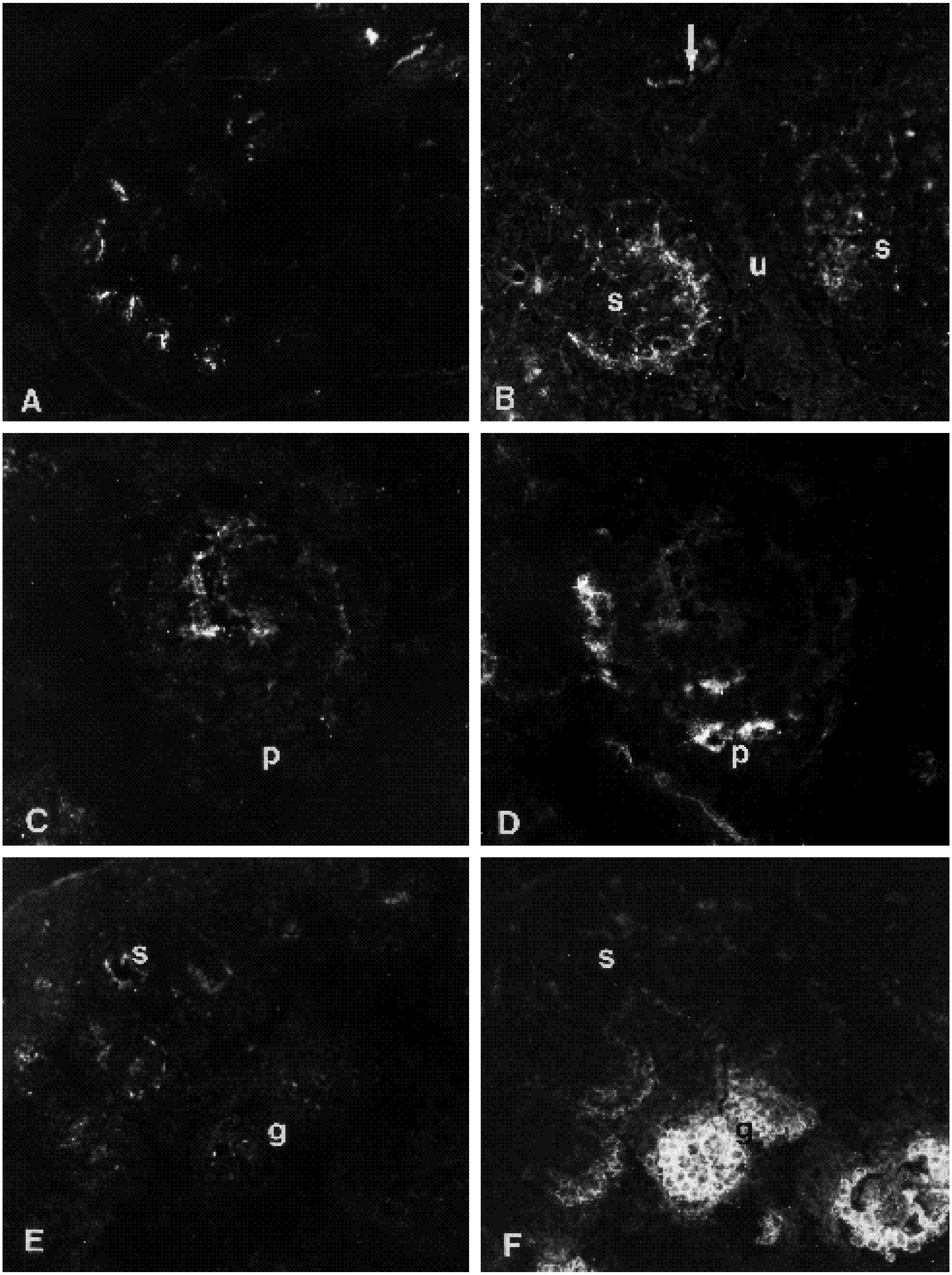


Fig. 2. Stages of development of the metanephric kidney. (A) A kidney of a 14-day-old mouse embryo. Low magnification showing several pretubular aggregates in the cortical zone and the ureter (u) in the centre. (×120). (B and C) Higher magnifications of the cortical zone of a kidney of a 16-day-old mouse embryo showing early and later stages of S-shaped bodies (s). (×300). (D and E) Two views of a kidney of a 18-day-old mouse embryo showing maturing proximal (p) and distal (d) tubules and early glomeruli (g). (E, ×300; D, ×200).

Fig. 3. Immunohistochemical localization of α_1 gap junction protein (GJ) in the developing mouse and rat kidneys. (A) A section through a 14-day mouse embryonic kidney showing a subcortical ring of cells expressing the α_1 GJ protein. (×75). (B) Higher magnification of the subcortical zone illustrating a terminal branch of the ureter (u, arrow) and two mesenchyme-derived early S-shaped bodies (s) expressing the α_1 GJ antigen predominantly in their lower crevice. (×300). (C, D) Double immunofluorescence view of a late S-shaped body of a 17-day rat embryonic kidney stained with antibodies against α_1 GJ antigen (C) and against podocalyxin visualizing the maturing podocytes (p, D). The GJ-protein becomes downregulated when the podocytes mature. (×360). (E, F) Double immunofluorescence view of the cortex of a kidney from 20-day rat embryo stained as above. The α_1 GJ protein is still expressed in the S-shaped bodies (s) and in the immature glomeruli (g, E), but not in the maturing podocytes expressing podocalyxin (F). (×200).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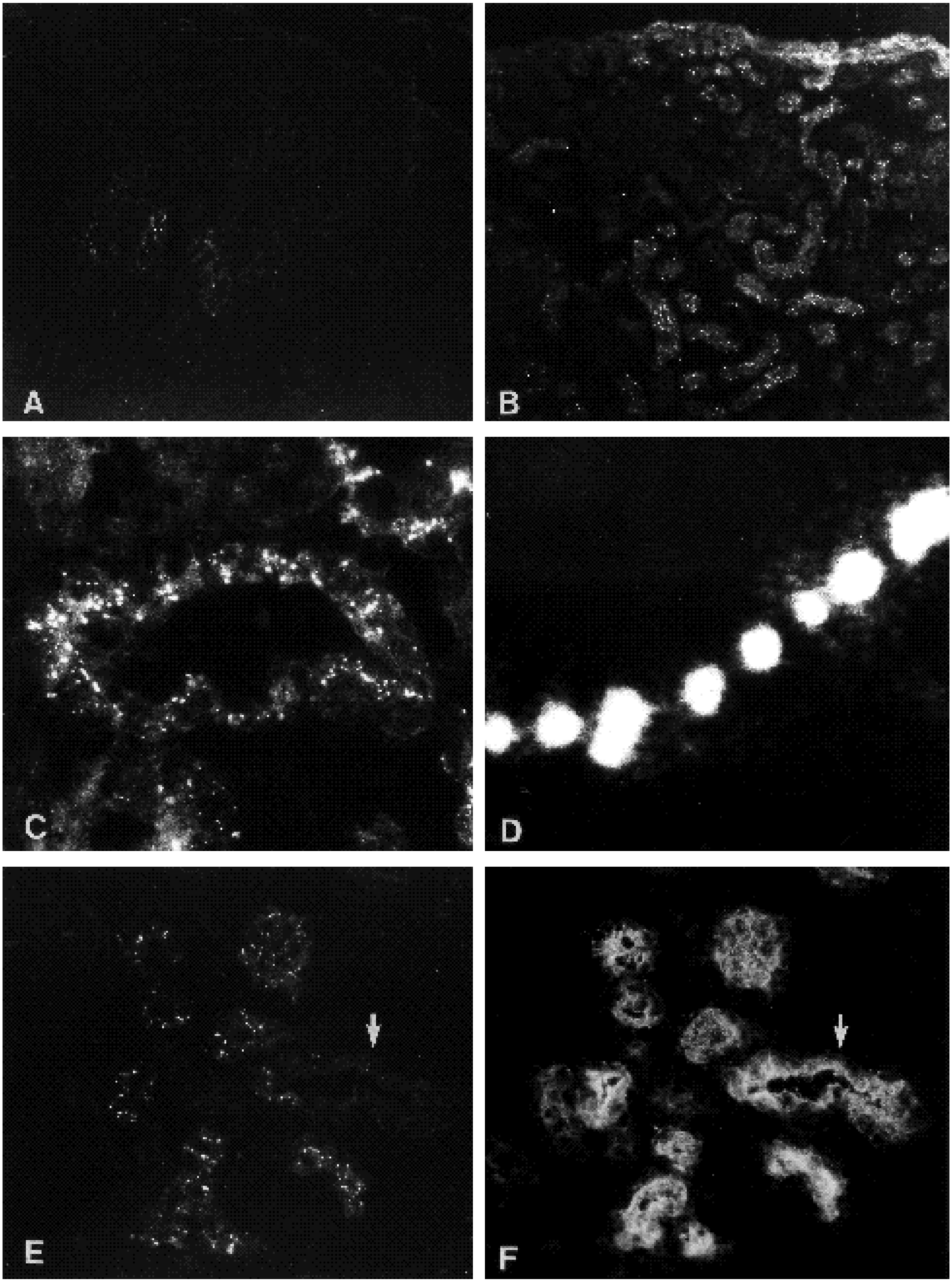
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Fig. 4. Immunohistochemical localization of the β_2 GJ protein in the tubules of developing mouse and rat kidneys. (A) Small subcortical groups expressing the GJ antigen in the kidney of a 17-day mouse embryo. (×120). (B) A similarly treated cortical region of kidney of a newborn mouse where a majority of the proximal tubules express β_2 GJ protein. (×100). (C) A proximal tubule of a 17-day mouse embryonic kidney composed of β_2 GJ-positive cells. (×500). (D) Confocal microscopy section through a β_2 GJ-positive tubule in a 17-day mouse embryonic kidney. (×12 000). (E, F). Double immunofluorescent staining of β_2 GJ protein (E) and dipeptidylpeptidase IV (DPY) (F) of a section through a 20-day rat kidney. Note the tubular portions already expressing DPY, but not yet the gap junction protein (arrows). (×350).

No β_2 antigen was detected in day 12 and day 15 kidneys. At day 17, β_2 GJ protein was detected in only a few tubular cells in each section of kidney where dots of fluorescence were observed (Fig. 4). After day 17, more cells expressed this antigen, and it was abundant in the tubules of newborn and adult kidneys.

The cells expressing the β_2 GJ protein were seen only in those areas of the kidney that contained proximal tubules. The identification of the β_2-positive cells with proximal tubules was made by immunofluorescence. First, adjacent sections of 20-day embryonic mouse kidney were stained with antibodies to the β_2 GJ protein and to proximal tubule brush border antigen and antibodies to distal tubule Tamm-Horsfall glycoprotein. These studies demonstrated that the β_2 GJ antigen was located specifically on the brush-border-positive proximal tubule cells (data not shown). Second, double immunofluorescent staining of sections of 20-day rat embryonic kidney (Fig. 4E, F) localized the β_2 antigen only to those tubules that expressed dipeptidylpeptidase IV, a marker for proximal tubule cells. As the kidney matured, more proximal tubules in each section were positive for the β_2 antigen and, by two weeks after birth, all brush border antigen-positive tubules were also positive for the β_2 antigen. This suggests that, in the kidney, the β_2 GJ protein is a specialized product of the proximal tubule cells.

Expression pattern of the β_1 GJ protein

The β_1 antigen was expressed in the embryonic kidney in the same way as β_2: it appeared late during organogenesis, around day 17 in the mouse, and became localized first in a subset of proximal tubules (Fig. 5A). Towards the end of kidney development, new β_1-positive tubules were detectable until most (if not all) proximal tubules expressed the antigen in the newborn kidney (Fig. 5B).

Expression of the GJ proteins in experimentally induced nephric mesenchymes

To explore further the appearance of the GJ antigens and their localization, we used the transfilter technique by which isolated kidney mesenchymes can be experimentally triggered to develop into advanced tubular structures (see Methods). In the rat, the uncommitted mesenchyme is dissected from 13-day embryos and brought into transfilter contact with a fragment of spinal cord. On day 4 after setting up the culture, the α_1 GJ protein could be detected in many tubular structures (Fig. 6A-C). Immunostaining with the β_1 and β_2 GJ antigens yielded invariably negative results up to 7 days in vitro.

In the mouse, the uncommitted nephric mesenchyme is dissected from 11-day embryos and brought into contact with the inducer. We have previously shown that a short transfilter induction “pulse” of 24 to 28 hours is sufficient to program the mesenchyme into epithelial transformation and tubule formation (Wartiovaara et al., 1974; Saxén and Lehtonen, 1978). Using markers for the various segments of the secretory nephron, we have shown that all three main segments, the distal and proximal tubules and the glomerular podocytes, will differentiate after this pulse (Ekblom et al., 1980, 1981; Lehtonen et al., 1983). Here, however, a 22-hour induction was not long enough to trigger the α_1-GJ antigen in the observed, cytokeratin-positive tubules, but an additional 26 hours of transfilter contact yielded positive tubules in mesenchymes subcultivated for 3 days (Fig. 6D). Interest-

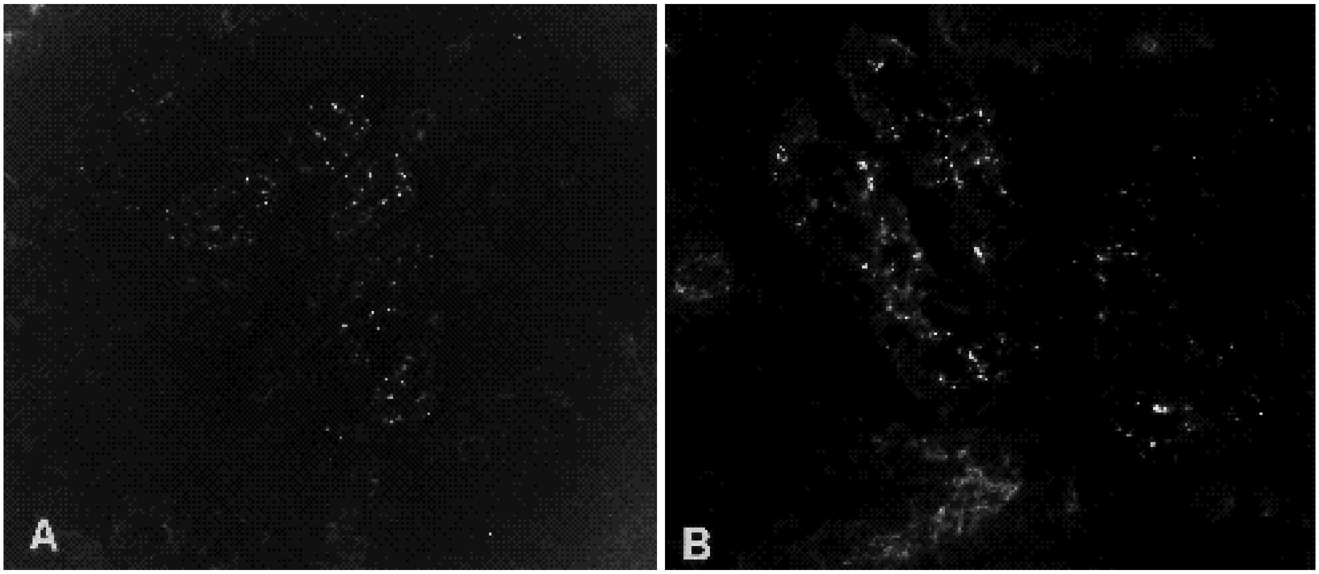

Fig. 5. Immunofluorescence localization of the β_1 GJ protein first expressed in some secretory tubules of a 17-day embryonic kidney (A) (×260) and detected in the newborn kidney of the mouse (B). (×300).

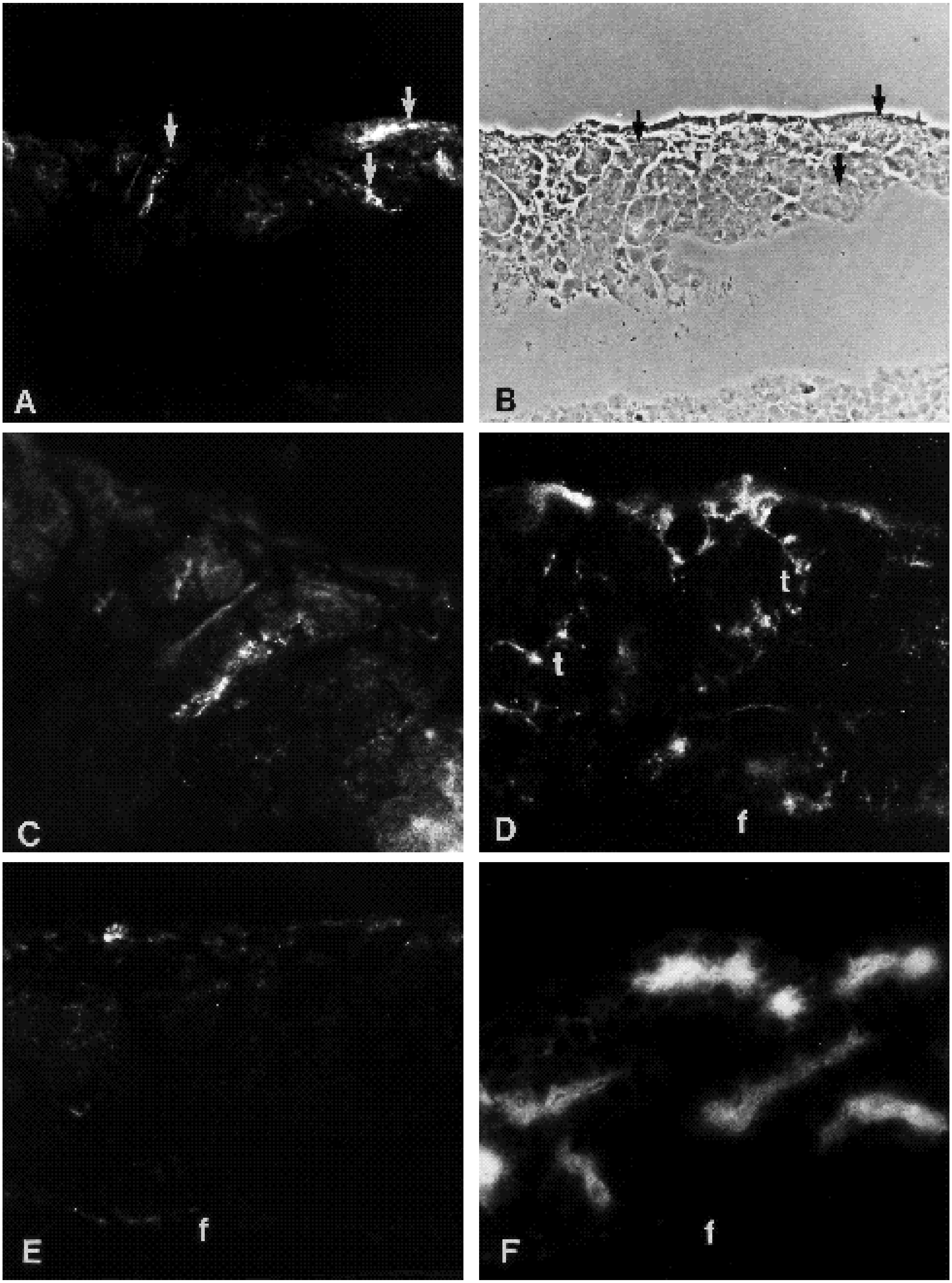
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Fig. 6. Immunohistochemical demonstration of the expression of α_1 gap junction protein in experimentally induced metanephric mesenchymes. (A) A mesenchyme from a 13-day rat embryo was cultured transfilter to the inductor for 4 days and then stained with an antibody to the α_1 GJ protein (arrows). (×200). (B) A phase contrast view of the same tubules (arrows) in the mesenchyme illustrated in (A). (×200). (C) Higher magnification of the tubule seen on the left in the above figures and stained with α_1 GJ antibody. (×900). (D) Tubules (t) expressing the α_1 GJ antigen in an 11-day mouse metanephric mesenchyme induced through a filter (f) for 48 hours and subcultivated for 72 hours. (×750). (E and F) A transfilter-induced mouse metanephric mesenchyme cultivated for 7 days and stained with the α_1 GJ antibody. The result of the immunostaining is negative (E) (×720). A section similar to that in E stained with a brush-border antibody, reacting specifically with the proximal tubules (F) (×720).

ingly, as in vivo, this expression was transient and no α_1-positive cells were detected in mesenchymes cultivated for a total of 7 days.

As in the rat, immunostaining with the β_1 and β_2 GJ antibodies yielded invariably negative results (Fig. 6E) despite the presence of well- developed proximal tubules expressing the brush-border antigen (Fig. 6F).

Discussion

This study examined the expression of mRNA and protein from three gap junction (GJ) genes during murine kidney development. Two non-overlapping patterns of expression were observed. The mRNA for the α_1 GJ protein was already prevalent in the mouse kidney by embryonic day 13, and it continued to be expressed throughout embryonic development. Immunofluorescence microscopy showed that this GJ protein was expressed as early as day 12 in the metanephric kidney, as also recently reported by Yancey et al. (1992). The α_1 GJ protein becomes localized to the transient S-shaped bodies produced by the mesenchymal aggregates.

The expression of the mRNAs and antigens for both β_1 and β_2 GJ proteins follow similar patterns. By S1 nuclease protection assay, this mRNA is detected around day 17 of mouse development, and soon thereafter the protein can be localized to a subset of proximal tubules as verified with the brush border (mouse) and the dipeptidylpeptidase (rat) antigens. By birth, the kidney already expresses an adult pattern wherein all proximal tubules can be decorated by the two gap junction antibodies. This is consistent with the mRNA data, which provides evidence for an increased expression of the mRNAs after their initial appearance during late intrauterine development.

The different expression of the α and β GJ proteins during nephrogenesis suggests that they have different functions. The transient expression of the α_1 GJ protein in vivo coincides with a unique stage of development of the secretory nephron. At this stage, the primitive nephric vesicle undergoes a transformation into the S-shaped body, an event involving cleft formation and invagination followed by differential cytodifferentiation of the main segments (Jokelainen, 1963; Saxén and Wartiovaara, 1966). In the experimental in vitro model system, the appearance of the α_1 GJ antigen coincides temporally with this crucial step of tubulogenesis although the shaping process remains incomplete. In such transfilter cultures where development was followed after a short induction pulse, the antigen was not expressed. This may lend further support to the morphogenetic role of the α_1 GJ protein. Gossens and Unsworth (1972) have provided experimental evidence for a two-step process in tubule induction: an initial, epithelializing stimulus is followed by further interactions between the epithelium of the renal vesicle and the mesenchymal stroma leading to the shaping and coiling of the tubule. This second step might be impaired in our short-term induction pulse experiments in which the uninduced mesenchyme is soon lost in prolonged cultures. When the segments of the secretory nephron have segregated (in vivo and in vitro), the α_1 GJ protein is downregulated. All of this suggests that the protein is involved in a specific stage of development, a suggestion consistent with many previous experimental results and observations on various systems. The findings in the kidney might be analogous to that described by Yancey et al. (1992) in the developing limb bud, where α_1 GJ protein was seen to interconnect the polarizing cells within the apical ectodermal ridge rather than in the epithelial-mesenchymal interphase. The role of gap junctional communication during development has been shown in snail mesoderm formation (de Laat et al., 1980), in mammalian oocyte maturation (Anderson and Albertini, 1976; Gilula et al., 1978), in preimplantation mouse embryos (Lo and Gilula, 1979), and also suggested by observations on mutant *Drosophila* (Jurnisch et al., 1990). The transient expression of this GJ antigen and its appearance in the avascular, nonfunctional isolated mesenchymes in vitro speak against its functioning in the physiological processes of the newborn or mature kidney.

The expression of the mRNAs and antigens for the β_1 and β_2 GJ proteins follows a pattern different from that above, and it suggests a different function. Both appear rather late during development in vivo, and the proteins can be localized first to a subset of maturing proximal tubules. Around birth, apparently all proximal tubules express both β proteins. In vitro, despite the prolonged culture period and the appearance of well-developed, non-functioning proximal tubules, the two β GJ proteins were not detected. Both findings can be best interpreted as suggesting that the β_1 and β_2 GJ proteins are not directly involved in the process of tubulogenesis, but rather are connected to proximal tubule function.

The genes for these GJ proteins show different patterns of regulation. This has also been observed for other embryonic organs (Nishi et al., 1991). In the uterus, the day prior to parturition is characterized by a dramatic increase in α_1 gene expression and a corresponding decrease in β_2 expression (Risek et al., 1990). However, in heart and liver, no changes in GJ protein gene transcription are seen around parturition. Thus, different GJ proteins can be regulated differently within the same organ, and the same GJ gene can be regulated differently in different organs in the same organism.

This study of GJ proteins and their transcripts demonstrates that different GJ proteins are utilized in different portions of the renal nephron. As a biological model, the developing kidney offers a good opportunity to analyze the formation of physiological compartments during the development of a complex mammalian organ. The major aspects of renal development are well characterized, and the nephron forms an array of distinct anatomical and physiological compartments. The formation of these physiological units may be structured by GJs which are used to compartmentalize information and to inform cells of their neighbors.

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