Epith Cell Biol (1995) 4:121–130
© 1995 Springer-Verlag London Limited

Epithelial Cell Biology

Original Article

Transitional Differentiation Patterns of Principal and Intercalated Cells during Renal Collecting Duct Development

J. Aigner[1], S. Kloth[1], M. L. Jennings[2] and W. W. Minuth[1]

[1]Department of Anatomy, University of Regensburg, Regensburg, Germany, and [2]Health Science Center, University of Texas, Texas, USA

Abstract. The developing renal collecting duct epithelium of neonatal rabbits exhibits 3 different zones. The ampullary tip epithelium acts as an embryonic inducer and is responsible for the generation of all of the nephron anlagen. It pilots the whole microarchitecture of the kidney. In the ampullary neck epithelium multiple cell divisions cause the elongation of the embryonic collecting duct so that the organ can grow. Finally, the cells in the ampullar shaft transdifferentiate into the functional collecting duct epithelium (CD) consisting of Principal (P) and various kinds of Intercalated (IC) cells. It is unknown by which morphogenic mechanisms the ampullar cells develop into the heterogeneously composed collecting duct epithelium.

Using both morphological and immunohistochemical methods, we investigated the transdifferentiation patterns leading from the ampullar epithelium to the P and IC cells in the neonatal kidney. An electron microscope analysis of the cortico-medullary course of the developing collecting duct revealed that conspicuous morphological alterations start in the neck of the ampulla. The lumen of the neck region is narrowed to a slit. While most of the cells in the ampullar tip exhibit few, short microvilli, the neck cells bear numerous, extremely long microvilli at their apical cell poles. All of the neck cells exhibit the same cytoplasmic staining pattern and the same number of mitochondria. Farther down in the shaft, clearly recognizable P and IC cells are found. Thus, differentiation into P and IC cells starts with a transitional precursor cell type in the ampullar neck.

Perfusion culture experiments with the embryonic collecting duct epithelium made it possible to generate transitional and differentiated cell types for the first time under in vitro conditions. The cultured epithelial cells showed characteristics common to both P and IC cells. Immunohistochemical findings revealed that morphological differentiation starts before the functional properties of P and IC cells can be deteced.

Correspondence and offprint requests to: Will W. Minuth, University of Regensburg, Department of Anatomy, Universtätsstr. 31, D-93053 Regensburg, Germany.

Keywords: Collecting duct; Development; Kidney; P and IC cells; Perfusion culture

Introduction

The development of parenchymal tissues occurs in two consecutive steps. Cell multiplication is needed to obtain a tissue mass large enough to permit volume expansion (Potter 1965; Saxén 1987; Abrahamson 1991; Perantoni et al. 1991). Then the cells differentiate and develop the morphological, physiological and biochemical characteristics known in the adult organ (Holthöfer 1987; Evan et al. 1991; Satlin et al. 1992; Robbillard et al. 1992). While the multiplication of cells is stimulated by a variety of growth factors (Segal and Fine 1989; Vainio et al. 1989; Weller et al. 1991; Chailler et al. 1991; Humes and Cieslinski 1992; Brière and Chailler 1992; Avner et al. 1993), little knowledge is available about the transdifferentiation mechanisms leading from immature to the differentiated cell types (Holthöfer 1987; Satlin et al. 1992).

The renal collecting duct epithelium of neonatal rabbits is an ideal model for investigating the trans-

differentiation mechanisms leading from the embryonic to the differentiated collecting duct epithelium consisting of Principal and Intercalated cells (Kaissling and Kriz 1979; Le Furgey and Tisher 1997; Schuster 1993) (Fig. 1). The ampullary collecting duct epithelium acts as an embryonic inducer which triggers the generation of the nephron anlagen during the whole period of organogenesis (Sorokin and Ekblom 1992). As shown by the expression of collecting-duct-specific proteins (Minuth et al. 1987; Matsumoto et al. 1993; Nouwen et al. 1993; Hanai et al. 1994; Hume et al. 1994; Kim et al. 1994), the ampullary neck epithelium is clearly delimited from the ampullary tip (Kloth et al. 1993). The neck epithelium is the proliferation zone where the tubule elongates and pushes the ampullary tip farther towards the capsula fibrosa. In consecutive steps new generations of nephron anlagen are induced, microvessels reach the differentiation zone, and the organ grows in a centrifugal direction (Neiss 1982; Herzlinger et al. 1993; Kloth et al. 1994). In the shaft the developing Principal (P) cells and Intercalated (IC) cells are found proximal to the neck zone of the collecting duct (Schuster et al. 1986; Koeppen 1987; Emmons and Kurtz 1994; Verlander et al. 1994). Despite intensive research the developmental events in nephron induction, the mitogenic stimuli leading to the elongation of the collecting duct epithelium and the factors triggering the development into functional P and IC cells are unknown (Evan et al. 1991; Kaissling and Kriz 1979; Le Furgey and Tisher 1979).

We investigated the development of P and IC cells during collecting duct differentiation using morphological and immunohistochemical techniques, following the hypothesis that the acquisition of biochemical and physiological functions is correlated with morphological alterations (Dorup and Maunsbach 1982; Fejes-Tóth and Nàray-Fejes-Tóth 1992, 1993; Brière and Magny 1993). Our electron microscopical examination started inside the ampulla and followed the neck zone in a cortico-medullary direction shown to the shaft of the collecting duct, where clearly visible P and IC cells first appear. (Fig. 1). It came as quite a surprise that the developing collecting duct cells displayed common and transitional cell differentiation characteristics within the ampullary neck epithelium before it splits into individual P and IC cell types. We were able not only to observe transitional cell types in the developing kidney, but could also generate them under perfusion culture conditions.

Material and Methods

Preparation of Organ Material

Neonatal New Zealand rabbits were anaesthetized with diethyl ether (Merck, Darmstadt, Germany) and both kidneys were removed aspectically without recovery of the rabbits.

Electron Microscopy

For transmission electron microscopy (TEM) thin pieces of neonatal rabbit kidney cortex and cultured collecting duct epithelia were fixed in Iscove's modified Dulbecco's medium (IMDM, Life Technologies, Eggenstein, Germany) containing 3% glutaraldehyde. Following the initial fixation step the tissue pieces were postfixed with 1% osmium tetroxide in phosphate buffered solution (PBS), dehydrated in a series of alcohols, passed through propylene oxide and embedded in Epon according to methods described earlier (Minuth et al. 1992). Semithin sections were stained with Richardson's solution and examined under the light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate. The specimens were examined with a Zeiss electron microscope 902 (Zeiss, Oberkochen, FRG). For scanning electron microscopy (SEM) pieces of neonatal rabbit kidney cortex or cultured epithelia were fixed in 3% glutaraldehyde, dehydrated in a graded series of ethanols, critical point dried with CO_2 and sputter-coated with gold (Polaron, Watford, GB). The specimens were examined with a Zeiss scanning electron microscope DSM 940 A as described earlier (Aigner et al. 1994).

Histochemistry

The immunoperoxidase method was used to direct the Cl^-/HCO_3^- exchanger on cryosections of collecting duct cells (Jennings et al. 1986, Schuster et al. 1986). Cryosections (8 μm) of neonatal rabbit kidney or cultured epithelia were fixed following a two-step fixation protocol. First the sections were incubated for 30 sec in a solution of 4.2% paraformaldehyde, 16% picric acid, 0.002% cobalt chloride and 0.1% glutaraldehyde (Serva, Heidelberg, Germany) in PBS, pH 7.2. Subsequently, the sections were immersed for 15 min in a solution including all the reagents listed above except glutaraldehyde. Following a washing step (0.1 M Tris-[hydroxymethyl]-aminomethane, 0.8% NaCl, 0.002% Triton X-100 (Pierce, Rockford, USA); pH 7.4) the samples were blocked by incubation in 0.1 M Tris buffer, pH 7.4, 25% fetal calf serum, 1% NaCl, 1% Triton X 100 for 45 min. The anti-Cl^-/HCO_3^- antibody (Jennings et al. 1986) was applied overnight. A biotinylated donkey anti mouse Ig antibody (Dianova, Hamburg, Germany) was diluted 1:600 in blocking solution prior to application. Then the sections were washed and incubated for 30 min in phenylhydrazine solution containing 0.0006% H_2O_2 in order to block endogenous peroxidases. After that an avidin-biotinylated peroxidase-detection complex (ABC-complex) was applied according to the manufacturer's instructions (Vectastain, Vector, Burlingame, USA). The enzyme reaction was started by adding the substrate solution (0.5 mg/ml diaminobenzidine, 0.1 M Tris, pH 7.4, 0.002% cobalt chloride, 0.04% nickel chloride, 0.012% H_2O_2) and stopped by rinsing in washing buffer. The sections were dehydrated in a graded

ethanol series and by a final incubation for 10 min in xylene. Finally the sections were embedded in DePeX (Serva, Heidelberg, Germany).

For PNA binding cryosections were incubated for 40 min with a PNA-rhodamine conjugate (Vector, Burlingame, Vermont, USA) diluted 1:2000 in PBS. The sections were embedded in FITC-guard (Testoc, Chicago, USA) and examined using a Zeiss Axiovert 35 microscope.

Tissue Culture

Cortical explants from the kidneys of newborn New Zealand rabbits were mounted in sterile tissue holder sets (Minuth 1987; Minuth et al. 1992) and placed in 24-well tissue culture plates (Greiner, Nürtingen, Germany). The explants consisted of a piece of capsula fibrosa with adherent collecting duct ampullae, S-shaped bodies and nephrogenic blastema. During the culture of these explants in IMDM containing 25 mM HEPES and 10% fetal bovine serum (Boehringer, Mannheim, Germany) an outgrowth of cells from the collecting duct ampullae was observed. Within 24 h of the initiation of culture the surface of the explant was completely covered by a single-layered collecting duct epithelium. Culture was carried out in a tissue incubator (Heraeus, Hanau, Germany) at 37 °C in a humidified atmosphere containing 5% CO_2/95% air for 24 h.

Perfusion Culture

To adapt culture conditions as closely as possible to the situation within the kidney, the cultured epithelia were exposed to a permanent superfusion of fresh medium from the luminal and basal sides in special culture containers (Munucells and Minutissue GmbH, Starenstraße 2, D-93077 Bad Abbach, Germany). The containers were placed on a 37 °C heating plate and the medium (IMDM/25 mM HEPES) was continuously exchanged at a rate of 1 ml/h with a peristaltic pump. The perfusion cultures with fresh medium was started 24 h after preparing the tissue. Total culture time was 14 days (1 day preculture, 13 days perfusion culture). IMDM/25 mM HEPES was used as the control medium. Aldosterone (1×10^{-7}M, Sigma, Deisenhofen, Germany) was added to the culture medium according to the experimental protocol (Minuth et al. 1993).

Evaluation

More than 10 kidneys from neonatal rabbits were used for the morphological investigation. More than 100 culture epithelia were examined after the perfusion culture experiments. To obtain an objective result each culture experiment was repeated at least three times. Then at least 5 epithelia were analyzed in the experimental series and, again, 30 cryosections were analyzed.

Results

Development of the Collecting Duct within the Kidney

In the neonatal rabbit kidney the collecting duct (Fig. 1) consists of the ampulla with its wide lumen at the tip (Fig. 2a), the ampullary neck with a restricted lumen (Fig. 2b) and the maturing shaft region, where for the first time developing P and IC cells are found (Fig. 3e,f) (Evan et al. 1991; Kloth et al. 1993). Transmission and scanning electron microscopy shows that the ampullary tip epithelium consists of two cell types. The majority of cells showns P cell characteristics with a cilium and short microvilli on the surface (Fig. 2a,b). A small number of cells has a smooth surface (Kloth et al. 1993). In no case do the ampullary cells resemble the IC cells. A new cell type appears at the beginning part of the ampullary neck. The broad lumen still found in the ampullary tip has disappeared and the luminal plasma membranes of the cells touch each other. Opposing cells show extremely long and dense microvillar structures (Fig.

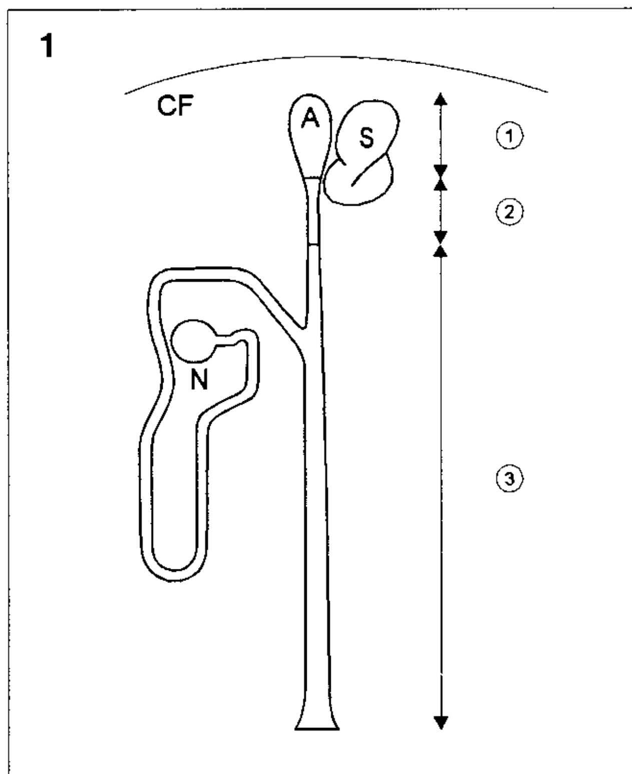


Fig. 1. Schematic illustration of a longitudinal section through the embryonic cortical and maturing medullary part of the neonatal rabbit kidney.

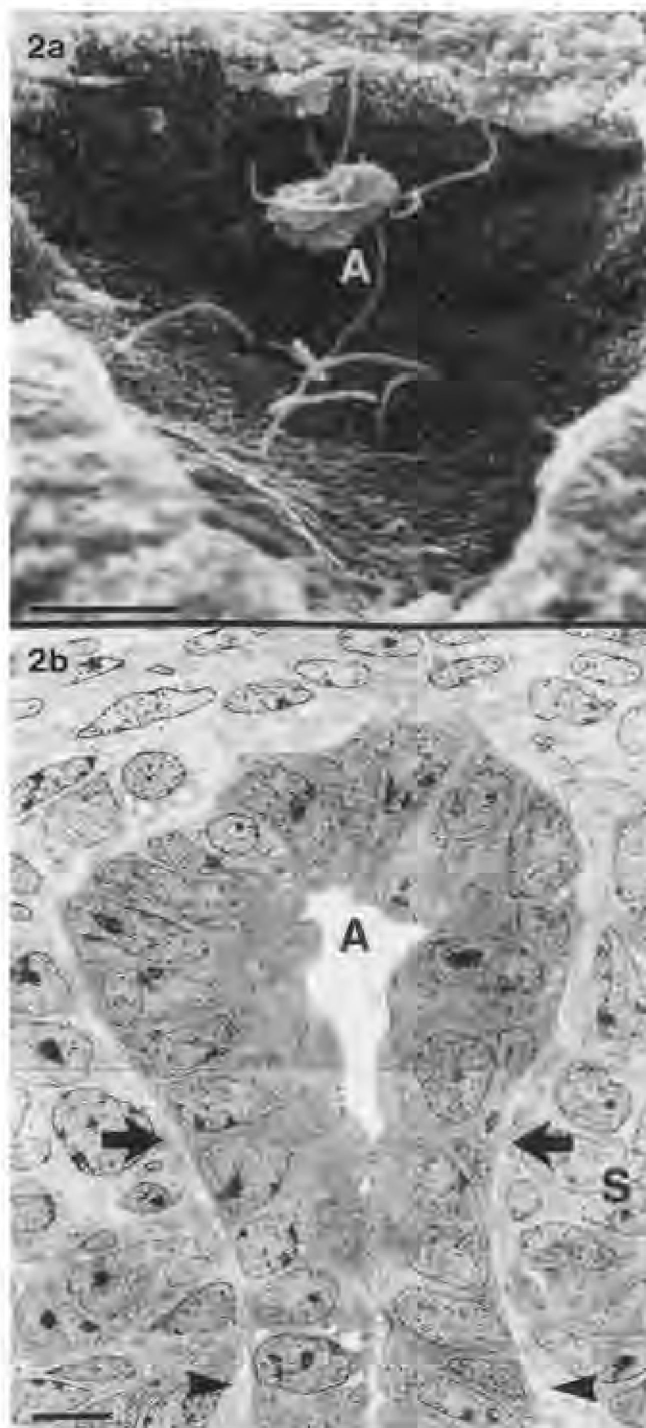


Fig. 2a,b. Scanning (a) and transmission (b) electron microscopy through the ampulla of the developing collecting duct. A wide lumen is found in the ampullary tip (A), cf. zone 1 in Fig. 1. At the neck region the lumen of the collecting duct is narrowed to a slit. (➔) and (◄) mark the beginning and ending of the neck region, cf. zone 2 in Fig. 1. These cells are shown in Fig. 3 at a higher magnification. (S) S-shaped body. *bar=10µm*

3a,b). Clearly visible cytoplasmic staining differences between light P and dark IC cells as observed in the adult kidney (Evan et al. 1991; Kaissling and Kriz 1979) are not observed at this site. About 50 µm farther down in the medullar direction the lumen of the shaft is widened and the development of two distinct cell types becomes visible (Fig. 3 c–f). One type of cell bears numerous and long microvilli (Fig. 3c,e), the other has only a few, short microvilli with a single cilium (Fig. 3d,f). These two cell types cannot be distinguised according to the number of mitochondria nor according to their dark cytoplasmic staining as the adult IC cells can. Beyond this differentiation zone clearly visible light P and dark IC cells can be recognized.

Generation of Transitional Differentiation Stages under In Vitro Conditions

In the maturing renal collecting duct of newborn rabbits cilia-bearing cells were shown in combination with numerous and long microvillar structures resembling the β-type IC cells (Minuth et al. 1993, Aigner et al. 1994). As far as we know, this cell type is transient and is only found during cell differentiation in the developing kidney (Evan et al. 1991). Thus we were interested in finding out if such transient cell stages could be generated under culture conditions (Figs. 4–7). Renal collecting duct epithelia were mounted in a holder set (Fig. 4a) and placed in a perfusion culture container (Fig. 4b). The container was connected via silicone tubes to 2 glass bottles. A peristaltic pump transported the medium through the container (Fig. 4c).

The cultured collecting duct epithelia show a fully unexpected pattern of cell composition (Figs 5,6) dissimilar to both the ampullary tip (Fig. 2) and the neck region (Fig. 3a,b). Transmission electron microscopy reveals cells with light- and dark-staining cytoplasm (Fig. 5a,b,d). Dark cells with few mitochondria (Fig. 5a) are found along with dark cells with numerous mitochondria (Fig. 5d). Light cells with few mitochondria (Fig. 5b) are observed as well as light cells containing numerous mitochondria (Fig. 5f). In addition, even light cells with long microvilli (Fig. 5b,e), normally a feature of differentiated β-type IC cells, are seen beside dark cells with long (Fig. 5c) and short (Fig. 5d) microvilli.

A further result was that the majority of cultured collecting duct cells exhibits a single cilium at the apical surface (Fig. 6). A cilium in combination with a few short microvilli is normally a sign of adult P cells (Aigner et al. 1994; Herter et al. 1993). However, our experiments demonstrate that the cilium is also found in combination with numerous, long microvilli (Fig. 6a,c) and in conjunction with few (Fig. 6d) numerous (Fig. 6b,c) mitochondria.

Morphological Differentiation is the First Step toward Functional Maturation

To investigate the time-dependent correlation of morphological and functional maturation the last series of experiments was designed to reveal the degree of differentiation by scanning electron microscopy and

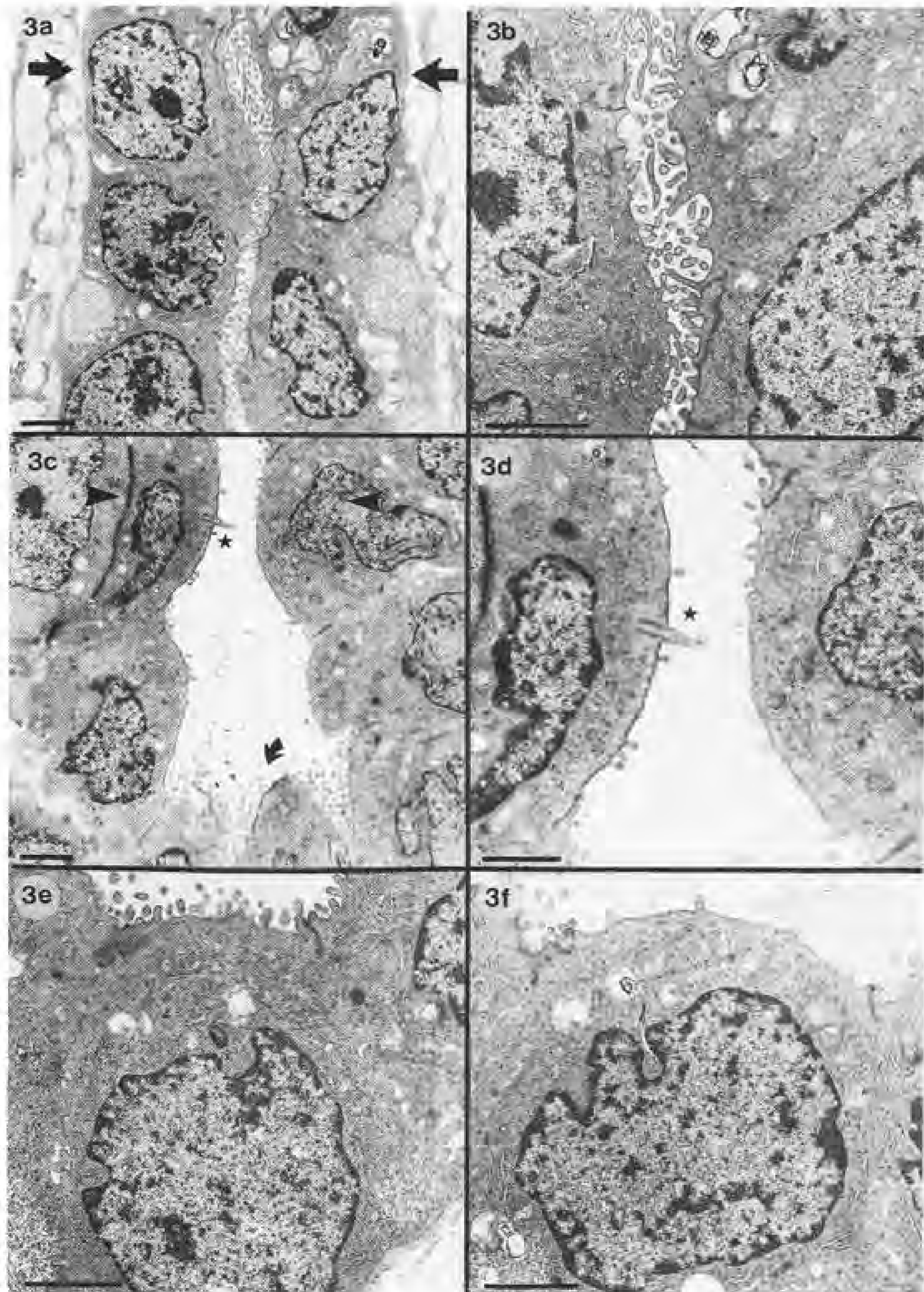
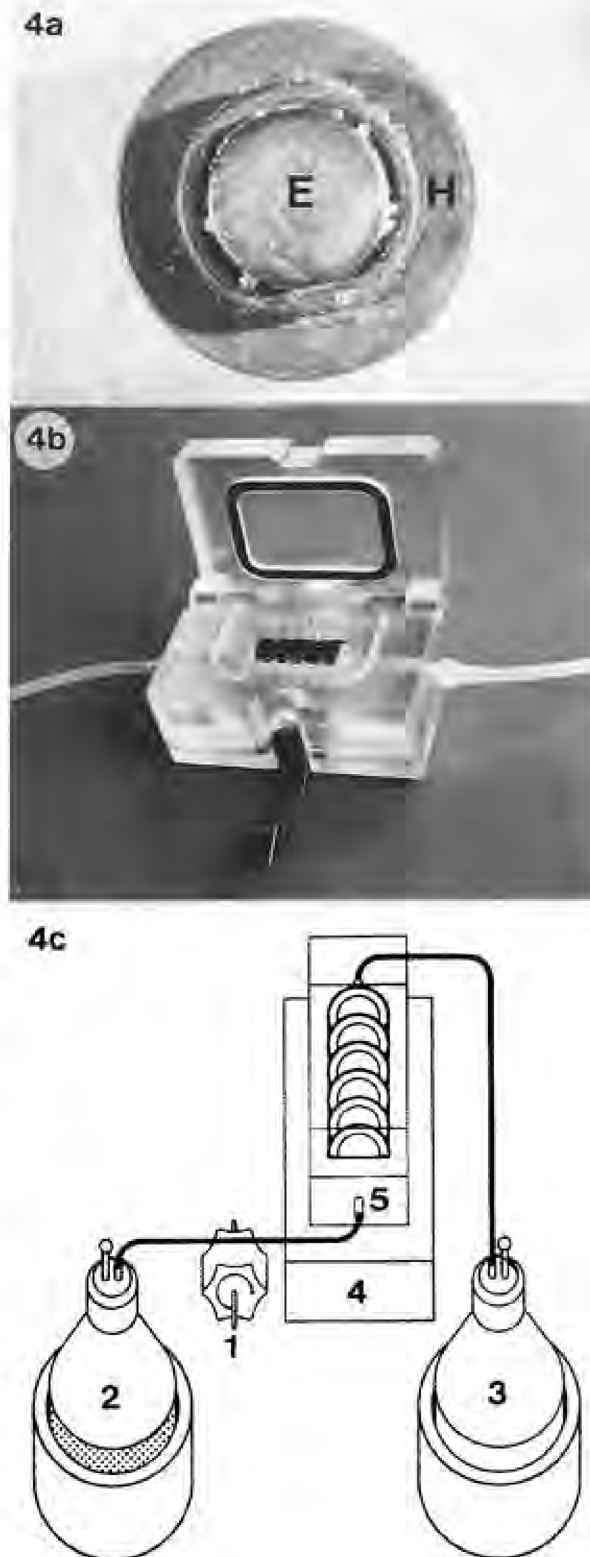


Fig. 3a–f. Transmission electron microscopy of the ampullar neck and beginning shaft zone of the maturing collecting duct. Cf. zone 2 and beginning zone 3 in Fig. 1. **a,b.** The lumen of the ampullar neck is only a slit. All of the cells show long, densely packed microvilli on the luminal surface. **c,d.** Clearly visible signs of P and IC cell development are seen in the beginning shaft zone. The lumen here is wider than at the neck. At this point two cell types are visible: cells with numerous, long microvilli (➷) and cells with few, short microvilli in conjunction with a cilium (★). **e,f.** Two different collecting duct cells can be recognized not by cytoplasmic staining, but by the luminal microvillar structures: a developing IC cell with numerous, long microvilli (e) and a cell with few, short microvilli (f) resembling a maturing P cell. **a.** (➔) marks the region demonstrated in Fig. 2b. *bar=1μm*



immunohistochemistry in perfusion-cultured collecting duct epithelia and in the neonatal kidney. Besides typical P cell characteristics two types of IC cell features are found in the cultured epithelia. The one type reveals long, densely packed microvilli at the luminal cell pole and resembled the β-type IC cell (Fig. 7a). The other cell type shows irregular microplicae at the cell surface, which is described as the typical feature for α-type IC cells (Fig. 7b). Labelling of cultured epithelia with PNA (Fig. 7c) and analysis by back-scattered electron microscopy (Herter et al. 1993) reveals that only cells with long, dense microvilli exhibit an intense reaction with the lectin. It indicates that a typical feature of the β-type IC cells that is observed in the neonatal kidney (Fig. 7e) is also developed under perfusion culture conditions. The microplicae cell type resembling the α-type IC cell is negative for PNA labelling (Fig. 7b). Immunohistochemical labelling with the anti-Cl^-/HCO_3^- antibody does not give a positive reaction on the cultured epithelia (Fig. 7d). It demonstrates that typical morphological features of α-type IC cells are visible before cell-type-specific proteins such as the Cl^-/HCO_3^- exchanger are expressed.

Discussion

A New Cell Type in the Ampullary Neck of the Developing Collecting Duct

The mechanisms of transdifferentiation leading from the embryonic nephron to the heterogeneously composed collecting duct epithelium of the adult kidney are unknown. Cytodifferentiation into P and IC cells starts in the ampullary neck epithelium (Fig. 3). In earlier investigations this zone was recognized to be only the source of collecting duct elongation during development (Kaissling and Kriz 1979; Neiss 1982; Dorup and Maunsbach 1982). In the present investigation we obtained evidence that the ampullary neck is an important site of collection duct cytodifferentiation. The tubular lumen of the neck (Fig. 3a,b) is much smaller in diameter than at the ampullary tip (Fig. 2a,b) or the more matured (Fig. 3c–f) collecting duct epithelium in the following shaft. The luminal plasma membrane of the neck cells shows numerous extremely long microvilli (Fig. 3a,b), while the cells of the ampullary tip exhibit few, short microvilli (Fig. 2a,b). The appearance of numerous, long microvilli on all the neck cells happens abruptly (Fig. 2b), just as it is observed in Malphigian tubules (Skaer 1992). The staining profile of the cyto-

←

Fig. 4a–c. Perfusion culture experiments with embryonic renal collecting duct cells. **a.** The collecting duct epithelia (E) were mounted in a holder set (H). **b**, which is placed in a special container for perfusion culture. **c.** The container is connected via silicone tubes to media bottles. A peristaltic pump transports the medium at a rate of 1 ml/h. The perfusion container is placed on a warming plate providing a constant temperature of 37 °C for the cells. 1-peristaltic pump; 2-medium bottle; 3-medium waste bottle; 4-warming plate; 5-perfusion container.

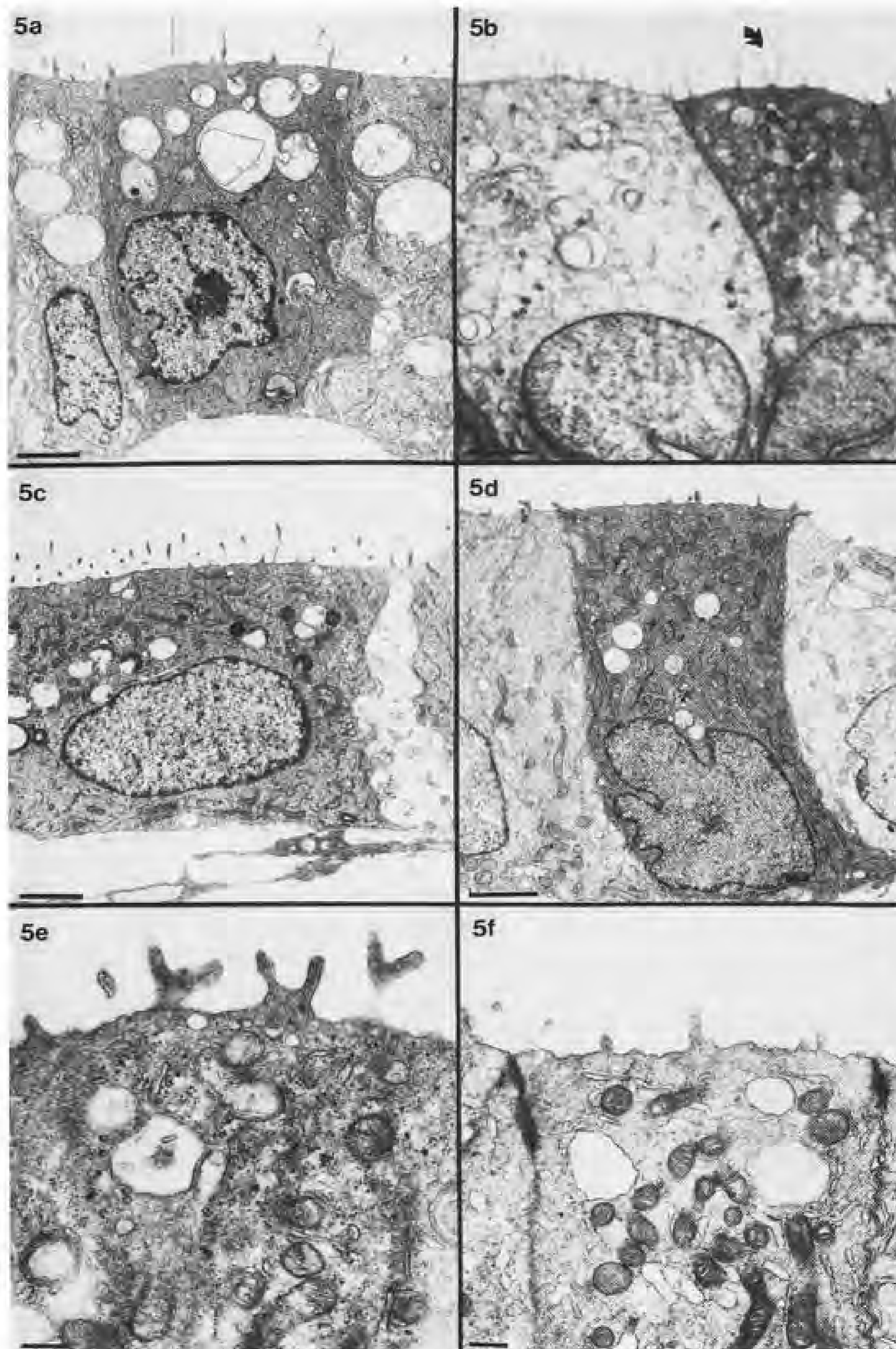


Fig. 5a–f. Transmission electron microscopy of developing collecting duct epithelial cell in perfusion culture. Multiple transitional differentiation patterns are visible. **a–d.** Dark cells were found beside light cells. Dark cells with few mitochondria (**a**) were present beside dark cells with numerous mitochondria (**b,c,d** ➘) resembling the IC cells. Dark cells with few short microvilli (**d**) occurred beside dark cells with numerous long microvilli (**b,c**). In contrast, light cells with few long microvilli and a small number of mitochondria (**e**) were present beside light cells with few, short microvilli but numerous mitochondria (**f**). *bar=1μm*

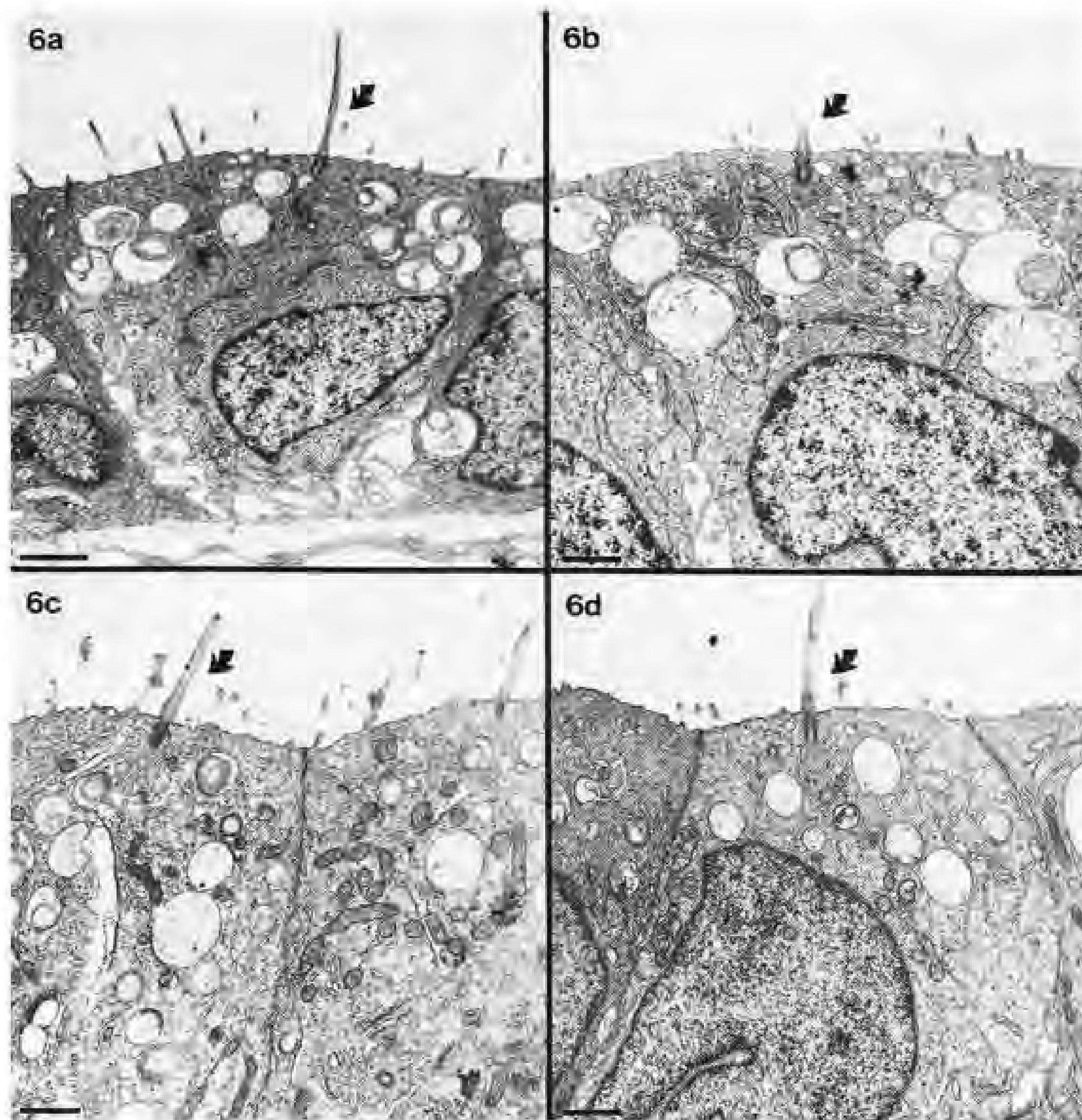


Fig. 6a–d. Phenotypes of cilia-bearing cells within cultured collecting duct epithelia. All of the cells exhibit a cilium (➚). **a,b**. The cilium is present on dark (**a**) as well as on light (**c**) cells bearing numerous long microvilli. **b,d**. The cilium is also found on light cells with few, short microvilli resembling P cells. *bar=1μm*

plasm and the number of mitochondria does not vary within the individial cells as it is known to do in differentiated collecting duct cells. To date we are unable to determine the molecular signals which tell the cells to transdifferentiate. However, our morphological and histochemical data indicate that this signal reaches all of the cells of the ampullary neck at the same time and that it provokes the same type of luminal differentiation in all of the involved cells.

Fluent Transitions between P and IC Cells are Observed In Vivo and under In Vitro Conditions

It is an unsolved question whether P and IC cells derive from a common precursor or from separate cell lines (Evan et al. 1991; Herzlinger et al. 1993; Fejes-Tóth and Náray-Fejes-Tóth 1992; Jamous et al. 1995). As previously shown in the developing kidney, most of the ampullary cells bear a cilium with few microvilli at the luminal cell pole (Kloth et al. 1993). Only few cells show a smooth luminal surface. Cilia are found under in vitro conditions in maturing P cells (Fig. 6b,d) and, astonishingly, in maturing IC cells (Fig. 6a,c). The cilia-bearing P cells can be clearly identified on the basis of their few short microvilli, while the cilia-bearing IC cells show numerous long microvilli (Fig. 3c). In the maturing collecting duct the same cell type *without* a cilium is present (Fig. 3e), representing the β-type IC cell (Herter et al. 1993). Thus, the developing β-type IC cell arises in the ampullary neck epithelium and for an undetermined period of time bears a cilium which is lost during functional maturation.

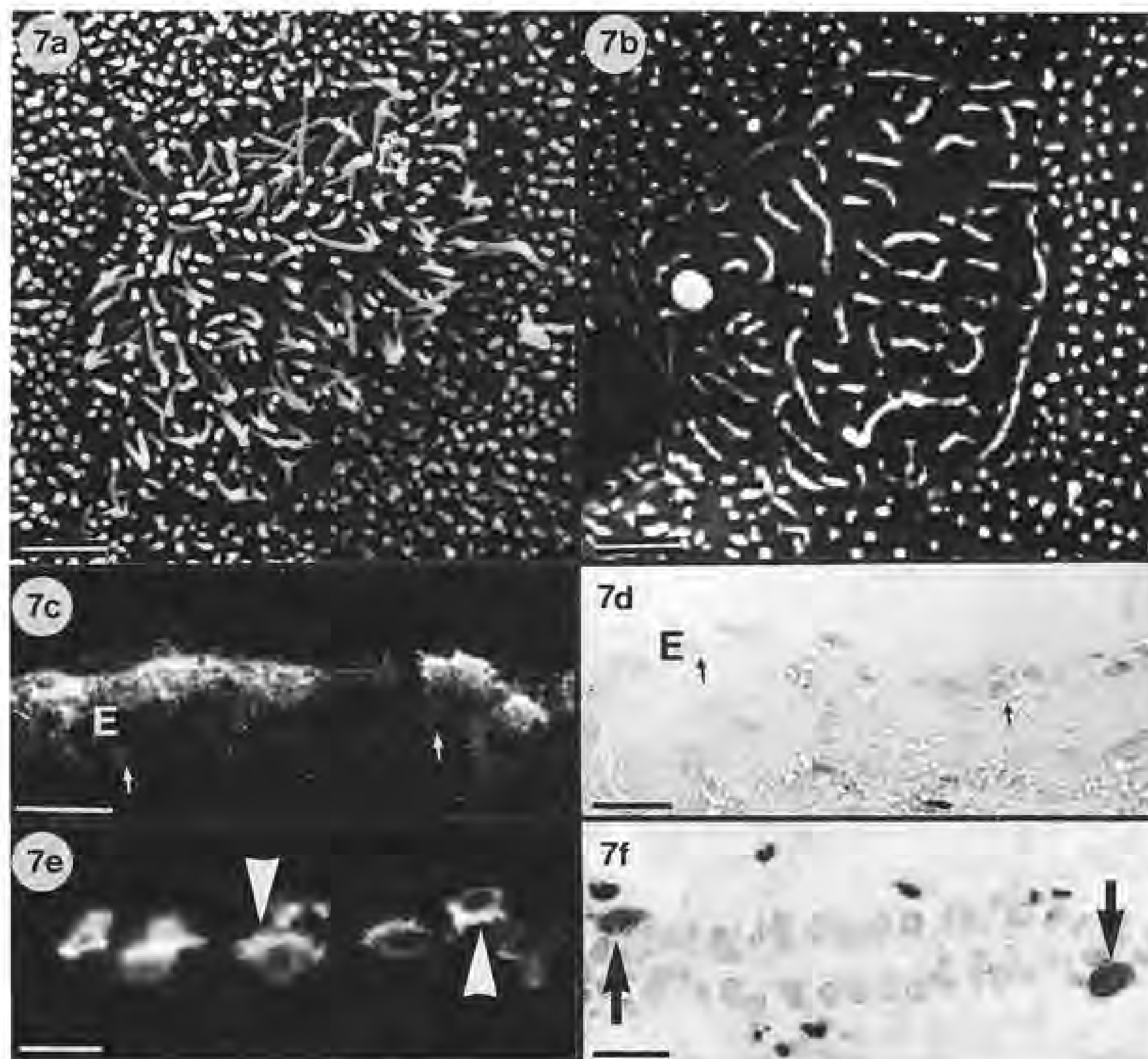


Fig. 7a–f. Development of IC cell characteristics. **a,b**. Scanning electron microscopy of a cultured β-type IC cell with numerous microvilli (**a**) and an α-type IC cell with the typical microplicae (**b**). **c,d**. While the cultured collecting duct epithelial cells (E) develop the typical PNA binding (**c**), the Cl^-/HCO_3^- exchanger typical for α-IC cells is not expressed on the cultured epithelium (**d**) (→; basal plasma membrane). **e**. In the neonatal kidney (**e**) PNA binding on the β-IC cells has developed (►). (**f**). The Cl^-/HCO_3^- exchanger is found only in a diffuse pattern in a small number of cells (➔). Unlike the functional collecting duct cells in the adult kidney, it is not typically expressed on the basolateral cell site. **7a,b**: *bar=2μm*; **7c,d,e,f**: *bar=20μm*

Perfusion culture experiments show that even transient types of collecting duct cells can be generated, which have characteristics common to both P and IC cells (Figs 4,5,6) (Minuth et al. 1993; Herter et al. 1993). A greater number of mitochondria than P cells in combination with numerous long microvilli is evidence for the differentiation of the β-type of IC cells (Aigner et al. 1994). The presence of a cilium on both cell types indicates that P cells and β-type IC cells may have a common origin (Jamous et al. 1995). In combination with an earlier investigation (Minuth et al. 1993) such transitional stages of developing renal collecting duct cells could be demonstrated for the first time within the kidney (Fig. 3) and under organotypical culture conditions (Figs 5,6). The cell culture model now makes it possible to test morphogenic factors according their capability to trigger the development of P and IC cells in the renal collecting duct.

Acknowledgements. The skillful technical assistance of Mrs Marion Kubitza and Ms Elfriede Eckert is gratefully acknowledged. This investigation was supported by the Deutsche Forschungsgemeinschaft (Mi 331-4-1).

References

Abrahamson DR (1991) Glomerulogenesis in the developing kidney. Sem in Nephrol 11:375–389

Aigner J, Kloth S, Kubitza M, Kashgarian M, Dermietzel R, Minuth WW (1994) Maturation of renal collecting duct cells in vivo and under perifusion culture. Epith Cell Biol 3:70–78

Avner ED, Sweeney WE, Jones WK, Harris HW (1993) Osteogenic

protein-1 (OP-1) is an inhibitor of growth and differentiation in the developing metanephros. (abstract) J Am Soc Nephrol 4:461
Brière N, Chailler P (1992) Minimal growth factor requirements of human fetal kidney in serum- and glucose-free culture. BioFactors 4:55–61
Brière N, Magny P (1993) Scanning electron microscopic observations of human fetal kidney maturing in vivo and in serum-free organ culture. Anat Rec 7:461–474
Chailler P, Ferrari J, Brière N (1991) Fetal mouse kidney maturation in vitro: coordinated influences of epidermal growth factor, transferrin and hydrocortisone. Anat Embryol 184:319–329
Dorup J, Maunsbach AB (1982) The ultrastructural development of distal nephron segments in the human fetal kidney. Anat Embryol 164:19–41
Emmons C, Kutz I (1994) Functional characterization of three intercalated cell subtypes in the rabbit outer cortical collecting duct. J Clin Invest 93:417–423
Evan AP, Satlin LM, Gattone II VH, Connors B, Schwartz GJ (1991) Postnatal maturation of rabbit renal collecting duct. II. Morphological observations. Am J Physiol 261:F91–F107
Fejes-Tóth G, Náray-Fejes-Tóth A (1992) Differentiation of renal β-intercalated cells to α-intercalated and principal cells in culture. Proc Natl Acad Sci, USA 89:5487–5491
Fejes-Tóth G, Náray-Fejes-Tóth A (1993) Differentiation of intercalated cells in culture. Pediatr Nephrol 7:780–784
Hanai T, Usuda N, Morita T, Nagata T (1994) Light microscopic lectin histochemistry in aging mouse kidney: study of composital changes in glycokonjugates. J Histochem Cytochem 42:897–906
Herter P, Laube G, Gronczewski J, Minuth WW (1993) Silver enhanced colloidal gold labelling of rabbit kidney collecting duct cell surfaces imaged by scanning electron microscopy. J Micros 171:107–115
Herzlinger D, Abramson R, Cohen D (1993) Phenotypic conversions in renal development. J Cell Sci (Supplement) 17:61–64
Holthöfer H (1987) Ontogeny of cell type-specific enzyme reactivities in kidney collecting ducts. Pediatr Res 22:504–508
Hume R, Bell JF, Hellas A, Burchell A (1994) Immunohistochemical localisation of glucose-6-phosphatase in developing human kidney. Histochemistry 101:413–417
Humes HD, Cieslinski DA (1992) Interaction between growth factors and retinoic acid in the induction of kidney tubulogenesis in tissue culture. Exp Cell Res 20:8–15
Jamous M, Bidet M, Tauc M, Koechlin N, Gastineau M, Wanstok F, Poujeol P (1995) In young primary cultures of rabbit kidney cortical collecting ducts intercalated cells originate from principal or undifferentiated cells. Eur J Cell Biol 66:192–199
Jennings ML, Anderson MP, Monaghan R (1986) Monoclonal antibodies against human erythrocyte band 3 protein: localization of proteolytic cleavage sites and stilbene disulfonate-binding lysine residues. J Biol Chem 261:9002–9010
Kaissling B, Kriz W (1979) Structural analysis of the rabbit kidney. Adv Anat Embryol Cell Biol 56:1–123
Kim J, Tisher CC, Madsen KM (1994) Differentiation of intercalated cells in developing rat kidney: an immunohistochemical study. Am J Physiol 266:F977–F990
Kloth S, Aigner J, Brandt E, Moll R, Minuth WW (1993) Histochemical markers reveal an unexpected heterogenous composition of the renal embryonic collecting duct epithelium. Kidney Int 44:527–536
Kloth S, Aigner J, Schmidbauer A, Minuth WW (1994) Interrelationship of renal vascular development and nephrogenesis. Cell Tissue Res 277:247–257
Koeppen BM (1987) Electrophysiological identification of principal and intercalated cells in the rabbit outer medullary collecting duct. Pflügers Arch 409:138–14
Le Furgey A, Tisher CC (1979) Morphology of rabbit collecting duct. Am J Anat 155:111–124
Matsumoto T, Fejes-Tóth G, Schwartz GJ (1993) Developmental expression of acid-base-related proteins in the rabbit kidney. Pediatr Nephrol 7:792–797
Minuth WW (1987) Neonatal rabbit kidney cortex in culture as tool for the study of collecting duct formation and nephron differentiation. Differentiation 36:12–22
Minuth WW, Dermietzel R, Kloth S, Hennerkes B (1992) A new method culturing renal cells under permanent superfusion and producing a luminal-basal medium gradient. Kidney Int 41:215–219
Minuth WW, Fietzek W, Kloth S, Aigner J, Herter P, Röckl W, Kubitza M, Stöckl G, Dermietzel R (1993) Aldosterone modulates PNA binding cell isoforms within renal collecting duct epithelium. Kidney Int 44:537–544
Minuth WW, Gross P, Gilbert P, Kashgarian M (1987) Expression of the α-subunit of Na/K-ATPase in renal collecting duct epithelium during development. Kidney Int 31:1104–1112
Neiss WF (1982) Morphogenesis and histogenesis of the connecting tubule in the rat kidney. Anat Embryol 165:81–95
Nouwen EJ, Dauwe S, van der Biest I, De Broe ME (1993) Stage- and segment-specific expression of cell-adhesion molecules N-CAM, A-CAM and L-CAM in the kidney. Kidney Int 44:147–158
Perantoni AO, Williams CL, Lewellyn AL (1991) Growth and branching morphogenesis of rat collecting duct anlagen in the absence of metanephrogenic mesenchyme. Differentiation 48:107–113
Potter EL (1965) Development of the human glomerulus. Arch Path 80:241–255
Robbillard JE, Smith FG, Segar JL, Guillery EN, Jose PA (1992) Mechanisms regulating renal sodium excretion during development. Pediatr Nephrol 6:205–213
Satlin LM, Matsumoto T, Schwartz GJ (1992) Postnatal maturation of rabbit renal collecting duct. III. Peanut lectin binding intercalated cells. Am J Physiol 262:F199–F208
Saxén L (1987) Organogenesis of the Kidney. (Development and Cell Biology Series (Vol. 19), Barlow PW, Green PB, Wylie CC (eds)), Cambridge, Cambridge University Press
Schuster VL (1993) Function and regulation of collecting duct intercalated cells. Annu Rev Physiol 55:267–288
Schuster VL, Bonsib SM, Jennings ML (1986) Two types of collecting duct mitochondria rich (intercalated) cells: Lectin and band 3 cytochemistry. Am J Physiol 251:C347–C355
Segal R, Fine LG (1989) Polypeptide growth factors and the kidney. Kidney Int 36 (Suppl 27):2–10
Skaer H (1992) Development of the insect Malphigian tubule. In: Fleming TP (ed) Epithelial organization and development. Chapman and Hall, London, pp 191–218
Sorokin L, Ekblom P (1992) Development of tubular and glomerular cells of the kidney. Kidney Int 41:657–664
Vainio S, Lehtonen E, Jalkanen M, Bernfield M, Saxén L (1989) Epithelial-mesenchymal interactions regulate the stage-specific expression of a cell surface proteoglycan, syndecan, in the developing kidney. Dev Biol 134:382–391
Verlander JW, Madsen KM, Stone DK, Tisher CC (1994) Ultrastructural localization of H+ATPase in rabbit cortical collecing duct. J Am Soc Nephrol 4:1546–1557
Weller A, Sorokin L, Illgen EM, 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

Accepted for publication 24 June 1996