Kidney International, Vol. 41 (1992), pp. 657–664

Development of tubular and glomerular cells of the kidney

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Many of the functions of the kidney are carried out by two cell types, the epithelial and the endothelial cells. Epithelial cells constitute the distal and proximal tubuli, and the visceral (podocytes) and parietal (capsular) epithelium of the glomeruli, while the endothelial cells comprise all vasculature and possibly also give rise to the mesangium of the glomeruli. Several aspects of epithelial cell development have recently been clarified, and over the past ten years new information on angiogenesis of the kidney has become available. These studies have also provided some insight into the nature and biology of the mesangium [1].

During embryogenesis the epithelial cells of most organs of the body form by an extension of a pre-existing epithelial sheet by a process known as branching morphogenesis. The epithelial cells of the collecting ducts and ureter form by such a process [1–4]. The epithelial cells of the kidney tubules and within the glomerulus, however, are exceptions and form by a direct conversion of mesenchyme to epithelium [1, 5, 6]. This latter mode of development renders the kidney an ideal model for studying the establishment of polarity of epithelial cells, a developmentally important event also shared by cells which develop by branching morphogenesis.

Epithelial cell formation in vivo and in vitro

In vivo the stimulus which initiates epithelial cell development is provided by the epithelium of the ureter. At 11 days of gestation in the mouse the ureter begins to grow into the metanephric mesenchyme, initiating epithelial tubule formation. In turn the mesenchyme stimulates further ingrowth and branching of the ureter (Fig. 1). It is at the branching tips of the ureter that the mesenchyme is induced to form epithelium.

At early stages of epithelial tubule formation the inductive ureter and the newly forming tubular epithelium remain separate, enabling isolation of responding and inductive tissues and in vitro growth of the former (Fig. 2). The culture method used is commonly termed the transfilter culture system, and was originally established by Grobstein [2, 5, 6]. It is so called because the metanephric mesenchyme, isolated from 11-day-old mouse embryoes, is dissected free of the ureteric bud and cultured on the top of a filter. In vitro the inductive effect of the ureter can be replaced by a number of embryonic tissues, but the most commonly used is spinal cord [6–8]. The spinal cord is fixed to the opposite side of the filter and is co-cultured with the metanephric mesenchyme for at least 24 hours [9]. After this time the spinal cord may be removed and differentiation of the organ culture will proceed normally. By 36 hours of culture the first signs of epithelial cell formation are evident in the form of areas of condensed mesenchyme. Such condensates also form during in vivo development at the tip of each branch of the ureter. Both in vivo and in vitro, the cells within the condensates polarize to form the epithelial cells which will constitute the tubules of the kidney. In vitro segmentation of the nephron into Bowman's capsule, glomerular podocytes, and distal and proximal tubules is indistinguishable from that which occurs in vivo and is characterized by expression of segment specific markers (Fig. 3) [10].

The transfilter kidney culture model has a number of advantages: The inducing and responding tissues remain separate, and the former can be removed after 24 hours of culture. Development proceeds slowly over a number of days allowing careful analysis of all steps involved, and the tubules which form are indistinguishable from those that form in vivo. Lastly, there is very little, if any, vascular in growth and consequently no complicating third cell type in the case of studies of epithelial cell development. Furthermore, the absence of vasculature from very young embryonic kidneys has made it possible to study angiogenesis in other experimental set ups.

Extracellular matrix in the developing kidney

In the course of renal development, both in vivo and in vitro, significant changes in the extracellular matrix occur. So far, the best studied extracellular matrix protein in this respect is laminin, a major glycoprotein of basement membranes. It has been shown to accumulate at early developmental stages in the forming basement membranes, and antibodies to certain domains of laminin have been the most effective in inhibiting epithelial cell polarization in vitro (Fig. 4) [11].

It is now clear that a number of laminin isoforms exist. The first discovered laminin consists of an A (400 kD), B1 (215 kD) and B2 (205 kD) chain arranged in a cruciform as depicted in Figure 4, and this particular laminin isoform is expressed by many of the developing epithelial cells of the kidney [11–13]. The antibodies that inhibit kidney tubule development in vitro react with the distal part or the long arm of the molecule.

The expression of the A chain and the B chains has been studied in detail in the organ culture model where the mesenchymal cells gradually convert into a new epithelium. It was found that the B chains were expressed by the mesenchymal cells already before the onset of morphogenesis. In contrast,

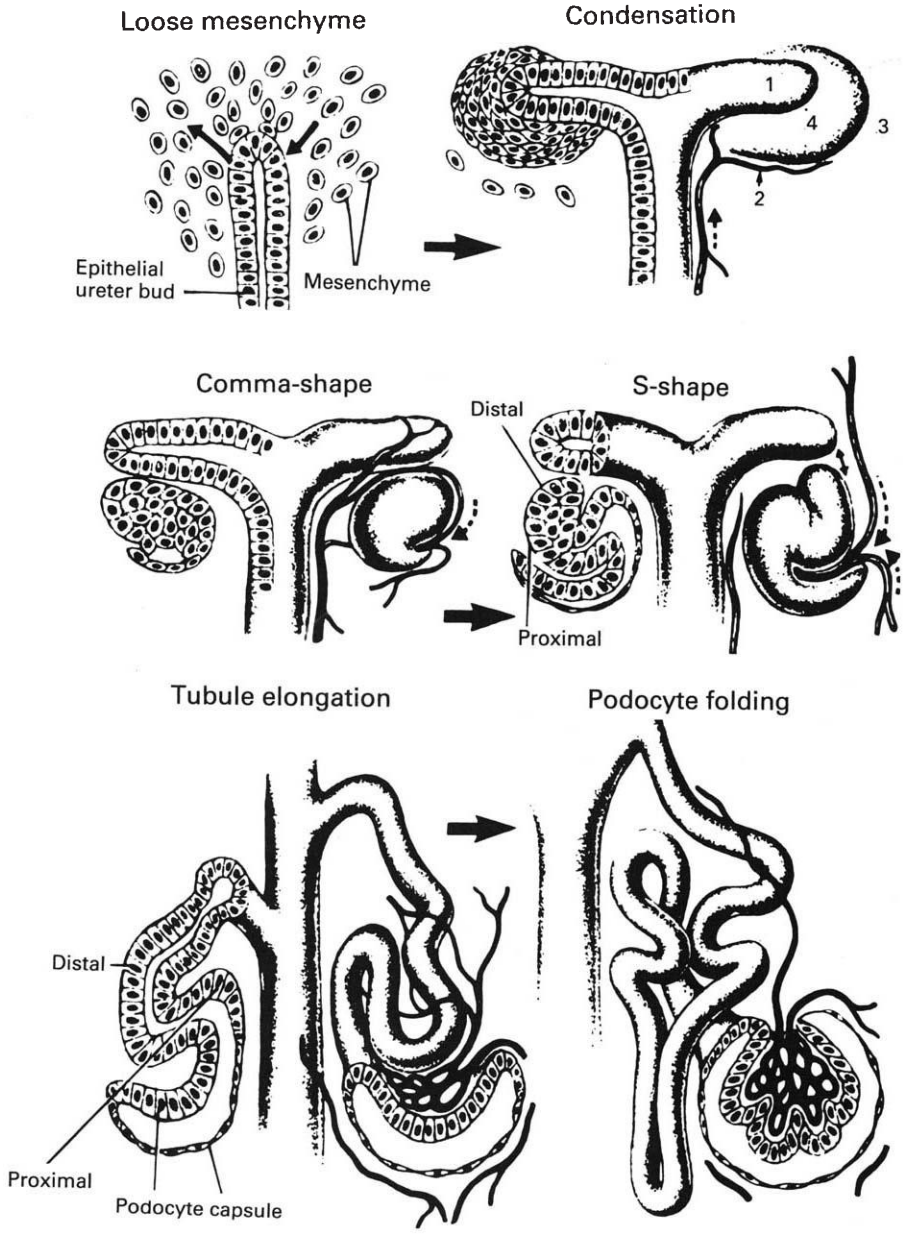

Fig. 1. *Scheme of differentiation of individual nephric units.* The epithelial ureter bud will branch into the mesenchyme as a result of an interaction between the ureter and mesenchyme (indicated by arrows). The stage at the top right shows the different cell lineages that begin to emerge once the ureter epithelium has branched once into the mesenchyme; (1) ureter epithelium, (2) blood vessels, (3) uncondensed mesenchyme that will differentiate into stroma, (4) condensed mesenchyme that will convert into epithelium. The condensed mesenchyme soon develops into a comma-shaped and S-shaped epithelium, and proximal and distal tubules can be distinguished. The glomerulus has also begun to form at the S-shaped stage. Blood vessels can be seen within the developing glomerulus already at the comma-shaped stage. It is possible that the mesengial cells are derived from the endothelial cells but this has not been directly demonstrated. (From ref. 8. Used with copyright permission from Alan R. Liss Publishers).

the A chain could not be detected until the first signs of epithelial cell formation became evident. Thus, it could be shown by immunofluorescence using A chain specific antibodies that A chains appear around mesenchymal cells when they begin to form condensates, the first sign of epithelialization [11]. The uncoordinated expression of the A and B chains, and the specific appearance of the A chain at the onset of epithelial cell development was confirmed by Northern blot data from transfilter cultures (Fig. 5) [13].

Markers for early developmental stages have been shown to be expressed in a fashion quite different from that of the A chains of laminin. The expression of a nuclear proto-oncogene, N-*myc*, increases transiently at 24 hours of culture apparently as an immediate response to induction by the spinal cord. Correspondingly, high levels of N-*myc* mRNA have been localized by in situ hybridization specifically around the lower part of the urteric bud during in vitro development [14].

Taken together, these studies suggest that embryonic mesenchymal cells in the kidney produce laminin variants lacking the A chain, and that the cells begin to produce increased amounts of A chain when they begin to differentiate into epithelium. The conversion process is certainly very complicated and includes an early activation of many transcription factors and other nuclear proteins. However, we do not yet know whether the expression of nuclear proteins such as N-*myc* are directly controlling the expression of any of the genes for the basement

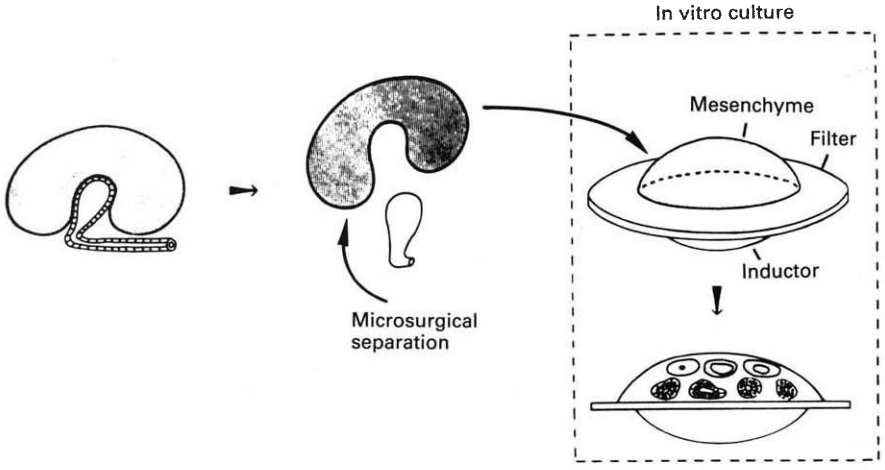

Fig. 2. *Transfilter culture model for studies of kidney development.* Metanephric mesenchymes from 11-day-old mouse embryos are dissected free of the epithelial ureter bud and cultured on top of a filter with an inductor tissue fixed to the opposite side of the filter (right). By 36 hours of culture the first signs of epithelial cell development are apparent, characterized by the presence of mesenchymal condensates similar to those which develop in vivo at the tips of the branching ureter. At 96 hours of the vitro culture tubules indistinguishable to those forming in vivo are present. Further details are in references [1, 5–9]. [Used with copyright permission from Academic Press Inc. (London) Ltd.]

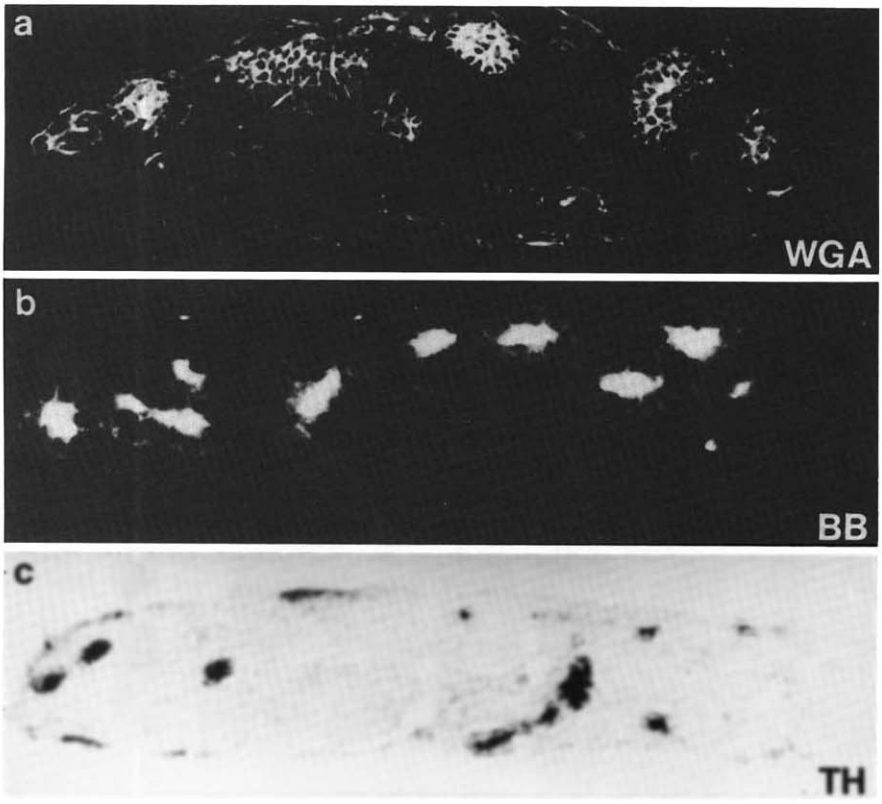

Fig. 3. *In vitro segmentation of the nephron in the transfilter culture.* Glomerular podocytes which react with the lectin wheat germ agglutinin (WGA) form large clusters in transfilter cultures (a); proximal tubules are revealed by antibodies against brush border antigen (BB) (b); and distal tubules are revealed by antibodies against Tamm-Horsfall glycoprotein (TH) (c). (Reprinted from ref. 10. Used with permission from Academic Press Inc. (London) Ltd.)

membrane components, or whether they have completely other functions.

Role of integrin receptors in epithelial cell polarization

Since antibodies against certain domains of laminin (E8,E3) could perturb kidney tubule development, it was of interest to study the surface receptors that could mediate the effect of laminin. An integrin receptor with the subunit composition $\alpha 6\beta 1$ has been shown to specifically interact with the E8 fragment of laminin [15, 16], and the possibility that this receptor could be involved in development of kidney epithelial cells was therefore studied. An antibody, known as GoH3, specifically blocks cell binding to laminin fragment E8 by reacting with the $\alpha 6$ integrin subunit [15, 16]. Using this antibody we could show that there is an extensive co-localization of the A chain of laminin and the $\alpha 6$ subunit in the developing kidney. Moreover, the $\alpha 6$ subunit seems to co-appear with the A chain of laminin during conversion of the mesenchyme to epithelium (Fig. 6). In vitro, the GoH3 antibody significantly perturbed kidney tubulogenesis, suggesting that

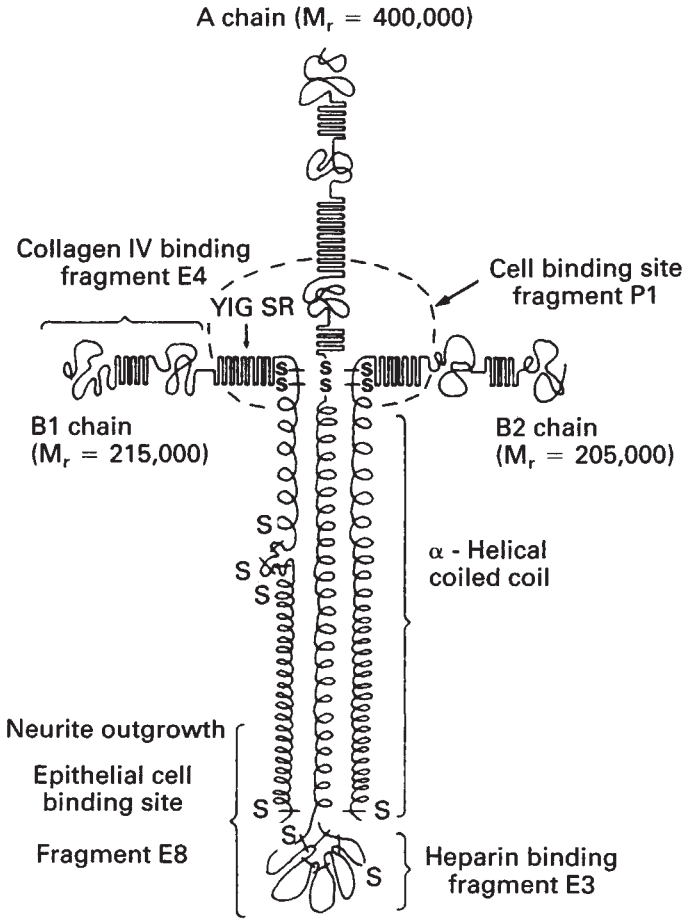

Fig. 4. *A model of the laminin isoform of developing kidney epithelial cells.* Laminin is composed of three chains, A, B1 and B2, arranged in a cruciform. The E3 fragment constitutes the end most portion of the central long arm of the molecule and contains only A chain sequences. Fragment E8, containing an epithelial cell binding site, occurs adjacent to E3 and contains both A and B chain sequences. (From ref. 12. Used with permission.)

indeed the polarizing activity of laminin lies within the E8 fragment and is mediated by an integrin-type receptor possessing the $\alpha 6$ subunit (Fig. 7) [17]. It is still unclear whether the domains binding the receptor are from the B or A chains of laminin, and one possibility is that the binding requires all three chains. The laminin isoform lacking the A chain, found at the early stages of epithelial cell development, could thus have quite different binding capacities to integrins and consequently also distinct functions.

Laminin isoforms in basement membranes of the kidney

Laminin isoforms lacking the A chain are apparently found not only in embryonic mesenchyme but also in some kidney basement membranes. Immunofluorescence studies have revealed that the $\alpha 6$ integrin subunit and the laminin chain co-localized on epithelial structures, but this is not the case for all cell types within the kidney. In particular, the endothelial cells of blood vessels express the $\alpha 6$ subunit but no staining for the laminin A chain occurs at these sites [13, 17]. However, B chains can be detected in these basement membranes. One possibility is that the A chain epitopes are merely masked by other matrix components and are therefore not seen by immunofluorescence. Another possibility is that there are alternatives to the 400 kD A chain of laminin [18], and indeed one such isoform, merosin, has been described [19, 20]. However, no alternative to the 400 kD A chain has been reported to occur in kidney endothelial cell basement membranes. To date only a B1 chain variant known as S-laminin has been positively identified in glomeruli and larger blood vessels of rat kidneys [21]. It is unlikely that S-laminin compensates for the absence of the A chain in endothelial basement membranes. However, the recent discovery of novel laminin chains suggests the existence of other variants which still await identification. The studies on the A chain of laminin and S-laminin have thus convincingly shown that basement membranes in the kidney are molecularly heterogenous, but the extent of the heterogeneity is still not known in detail. The laminin variants lacking the known A chain probably do not interact with the $\alpha 6\beta 1$ integrin, and may have distinct functions. In this respect, it is of great interest that the different segments of the nephron express distinct integrin types already at the S-shaped tubule stage of development [22]. Some of these integrins probably interact with other basement membrane components such as type IV collagen [23], but a few could also have affinity to the laminin variants. One could speculate that the segmentation of the nephron in part could be driven by the differential capacity of each developing cell type to bind to the basement membrane.

Origin of glomerular endothelial and mesangial cells

The in vitro organ culture studies have, without doubt, established that cells of the metanephric mesenchyme have the capacity to convert into epithelial cells. However, it appears that they lack the ability to convert into endothelial cells. In the transfilter cultures of the metanephric mesenchyme the glomeruli that form lack endothelial cells, rather they are composed of multicellular clusters of epithelial podocytes [24–26]. Such studies raised the possibility that the endothelial cells and mesangial cells were not derived from the mesenchyme. Observations of in vivo development suggested that endothelial cells of the kidney originated from external blood vessels which grew into the developing kidney [26]. The blood vessels could be visualized by antibodies to various basement membrane components and combined with the in vitro data this suggested that each endothelial cell was derived from a precursor endothelial cell rather than from the metanephric mesenchyme. To exclude the possibility that the growing tip of the blood vessel is somehow recruiting adjacent mesenchymal cells to become a part of the endothelium, experiments in the chorion allantoic membrane (CAM) of quail eggs have been performed [reviewed in 27]. The CAM system is a very good model system to study blood vessel formation because quail cells have a nucleolar marker that makes it easy to distinguish between quail and donor endothelial cells. Avascular kidney rudiments from mice were grafted onto the quail CAM. It was found that the kidneys

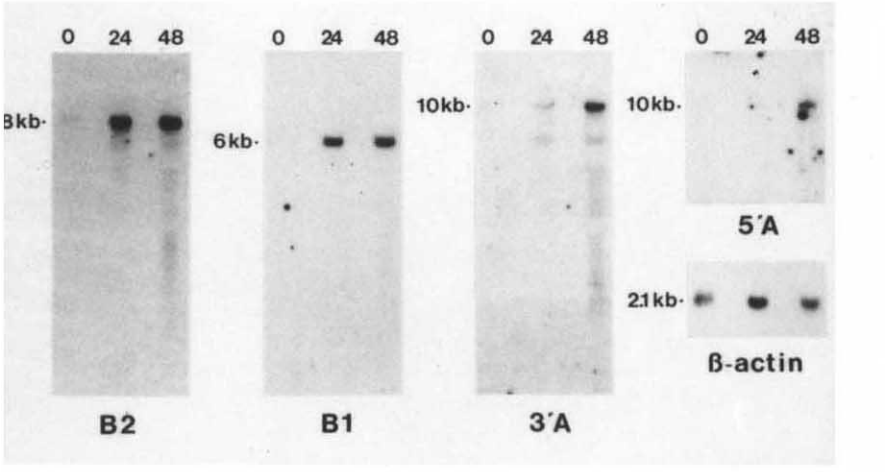

Fig. 5. *Uncoordinated expression of laminin A and B (B1 and B2) chains mRNA during in vitro conversion of mesenchyme to epithelium.* Northern hybridization for laminin A, B1 and B2 mRNA of total RNA isolated from metanephric mesenchyme of 11-day-old embryos (lane 0), and mesenchymes co-cultured with embryonic spinal cord for 24 hours and 48 hours. Ten micrograms of total RNA was loaded onto each lane, and the same filter was sequentially hybridized with radioactively labelled cDNA fragments specific for laminin B1 and B2, the 3′ and 5′ ends of laminin A chain, and for β-actin. The 6 kb mRNA for the B1 and B2 chains of laminin are already well expressed at 24 hours of culture, while the 10 kb A chain mRNA is only barely detectable. With the appearance of polarized epithelial cells at 48 hours of culture the mRNA for laminin A chain increases substantially. (From ref. 13. Used with copyright permission from *Cell Press*.)

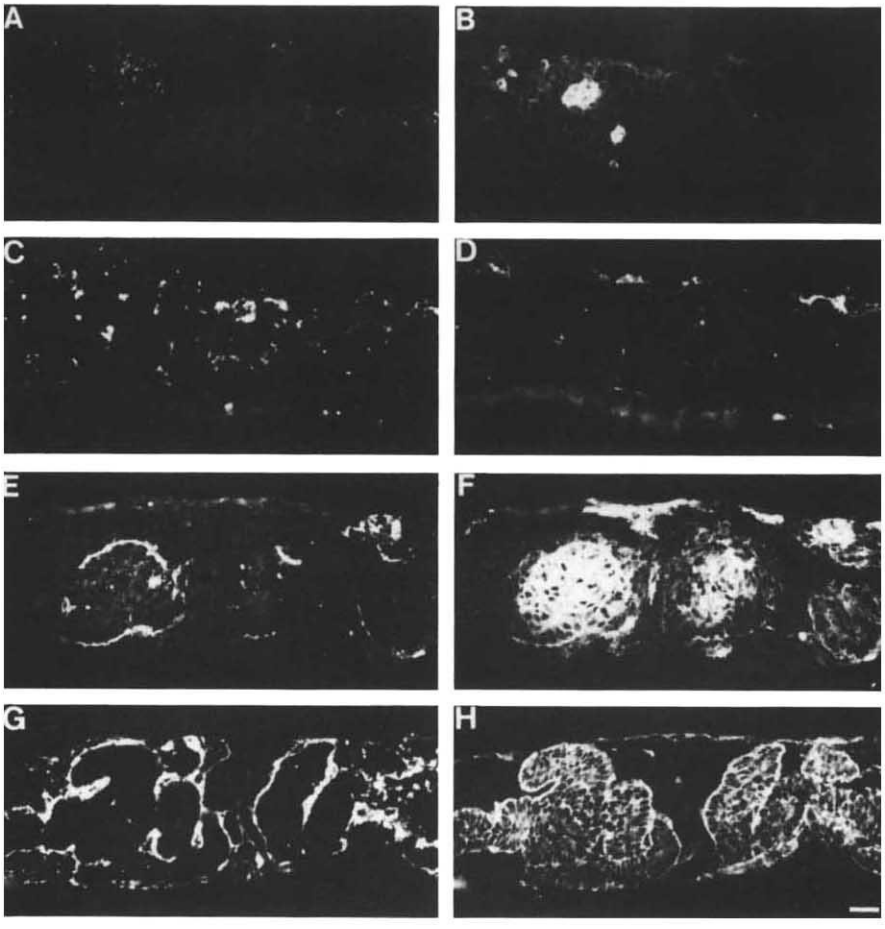

Fig. 6. *Co-appearance of laminin A chain (left) and the integrin α6 subunit (right) during the conversion of mesenchyme to epithelium in vitro.* Metanephric mesenchyme was cultured for 24 hours without (A, B), or with spinal cord as an inducer tissue (C, D). No staining for A chain (A) or the α6 subunit (B) was apparent in the uninduced mesenchyme and the expression of the α6 in (B) is restricted to blood vessels. In mesenchyme co-cultured for 24 hours with spinal cord, weak expression of A chain (C) and the α6 subunit (D) was noted throughout the mesenchyme. After 36 hours of culture, cellular condensates formed that were bordered by laminin A chain (E), while the surfaces of most cells constituting the condensates expressed the α6 subunit (F). At 72 hours of culture, polarized epithelial cells forming epithelial sheets are present. Note that the A chain is restricted to the basal extracellular matrix of these sheets (G) and that the α6 subunit is expressed on the entire cell surface of each epithelial cell with a slight enrichment basally. (Reproduced from the *Journal of Cell Biology*, ref. 17, with copyright permission from the Rockefeller University Press.)

became richly vascularized with endothelial cells carrying the quail nucleolar marker [28]. This was also the case for the glomerular endothelial cells (Fig. 8). These studies strongly suggest that the endothelial cells of all parts of the kidney are derived from the blood vessels that grow into the kidney during embryogenesis. It is possible that the developing endothelial cells secrete angiogenesis factors that stimulate this process [29].

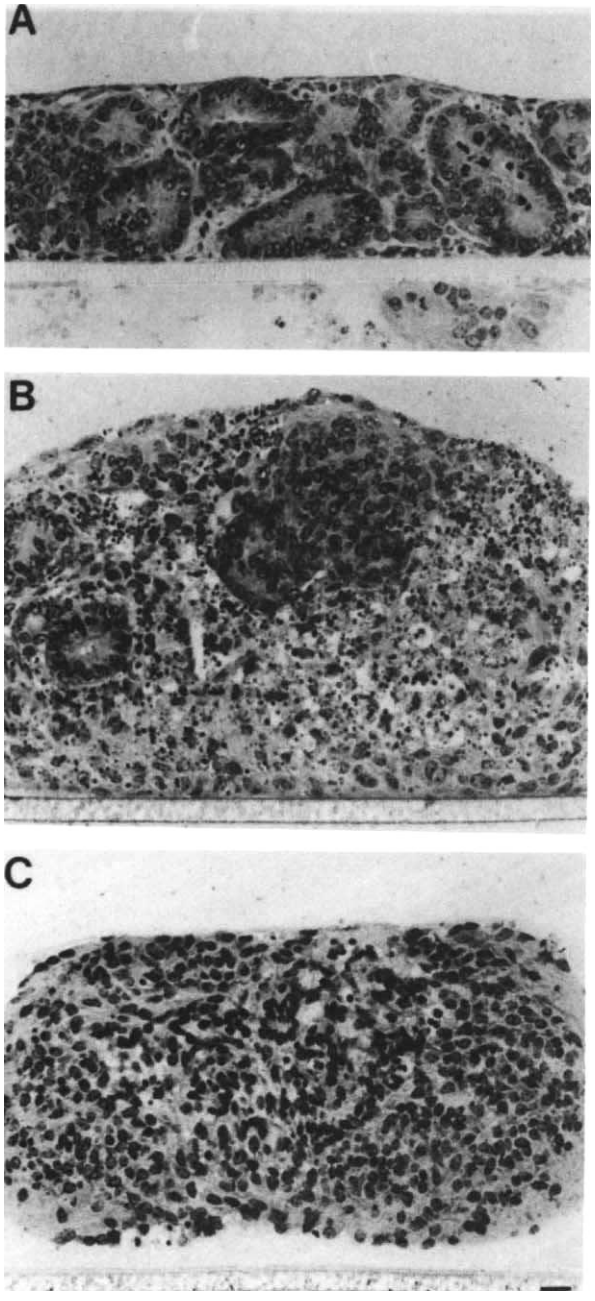

Fig. 7. *Perturbation of epithelial cell development by antibodies against the α6 subunit.* Histological sections of mesenchyme cultured in the presence of either antibodies against a polysialic acid unit (A) or antibodies against the α6 subunit (B, C). Mesenchyme was grown in transfilter culture for 24 hours with spinal cord that was subsequently removed, and the cells cultured a further 48 hours before processing for histology. Note the well formed tubules in control cultures (A) and partial (B) or complete (C) inhibition of tubulogenesis with antibodies against the α6 subunit. Some of the more organized areas in (B) may be clusters of podocytes rather than tubular cells. Note that cultures treated with antibodies against the α6 subunit show increased thickness. (Reproduced from the *Journal of Cell Biology*, ref. 17, with copyright permission from the Rockefeller University Press.)

The studies on kidney angiogenesis have obvious implications for our understanding of the development of the glomerular basement membrane (GBM). The GBM separates the endothelial cells from the epithelial podocytes and acts as the main filtering membrane of the kidney [30]. There is now good evidence that this basement membrane is derived from both the podocytes and the endothelial cells within the glomerulus. If kidneys are treated with guanidinium hydrochloride the GBM splits into two separate basement membranes [31]. More direct evidence for a dual cellular origin of the GBM was obtained by analyzing the chimeric kidneys which contained mouse podocytes and avian-type endothelial cells [32]. Species-specific antibodies either against type IV collagen or laminin were used in immunohistology and revealed that both cell types produce GBM material (Fig. 8). It has now become clear that the GBM in many respects is molecularly distinct from tubular basement membranes. It will be of great interest to study whether the molecules specific for the GBM are produced by the endothelial cells or the podocytes.

An important third cell type of the glomerulus, the mesangial cell, is closely associated with the endothelial cell. An obvious question is whether this cell type is derived from the metanephric mesenchyme or from the endothelial cell lineage. The rather intracapillary location of the cell in the glomerulus at the adult stage is not necessarily proof of an endothelial origin. However, some of the embryological studies described above suggest this possibility. In the in vitro cultures the glomerular-like bodies contain podocytes, but seem to lack both endothelial and mesangial cells [25, 26]. In the chimeric kidney, the mesangial area that can be seen at the early developmental stages contains cells with the quail nucleolar marker (Fig. 8). Moreover, the mesangial area in such chimeric glomeruli has basement membrane material of host rather than donor cell origin (Fig. 8) [32, 33]. From such studies it has been tentatively concluded that the mesangial cells are derived from the endothelial cell lineage rather than from the mesenchyme (Fig. 9). However it has not, in any of the studies, been directly shown that the cells found in the mesangial area express markers that unequivocally identify these cells as true mesangial cells. It therefore cannot be excluded that the cells found in the mesengial area are endothelial cells that later would have assumed a location closer to the GBM. Further studies are thus needed to clarify the ontogeny of the mesangial cells.

In the chimeric kidneys composed of avian endothelial cells and mouse epithelial cells, all three cell types within the glomerulus seemed to express basement membrane components. This is in line with previous morphological studies showing that the mesangial matrix is basement membrane-like. Yet, it is likely that there are molecular differences between mesangial matrix and the GBM. With the availability of specific probes to study the different basement membrane components it will now be possible to compare the molecular composition of the matrices produced by each of three major cell types. It is reasonable to predict that each cell type produces a distinct set of matrix proteins. A more detailed description of these matrices will certainly be useful for our understanding of the mesangial cells during development, in the adult kidney, and in various disease conditions which affect the glomerulus.

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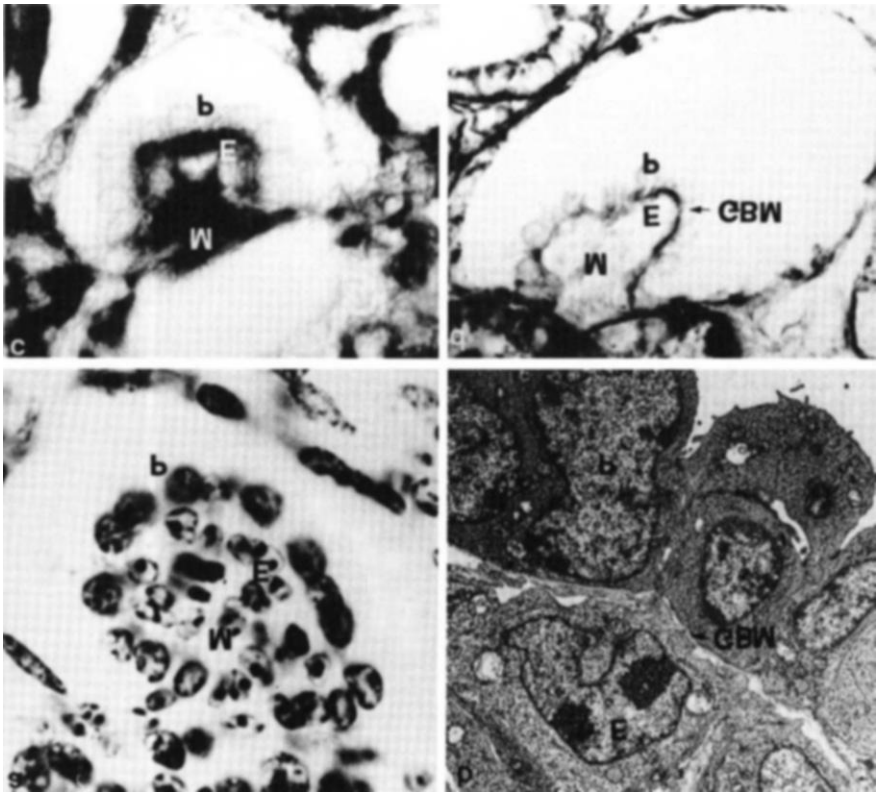

Fig. 8. *Characterization of interspecies hybrid glomeruli.* (a) In the mouse kidney grown on quail chorioallantoic membrane, quail endothelium has grown into the mouse glomerulus. Both endothelial cells (E) and cells probably forming mesangium (M) have a quail nucleolar marker, whereas the podocytes (P) are of mouse origin. (b) Electron microscopy of the hybrid glomeruli shows the glomerular basement membrane (GBM) between the cells. (c) In the hybrid glomeruli the extracellular matrix of the mesangial area and endothelial area react positively with antibodies against avian type IV collagen. (d) Antibodies against mouse collagen type IV do not react with the mesangium or endothelium of hybrid glomeruli, but the GBM is positive. (Reprinted from ref. 28. Used with copyright permission from Academic Press Inc. (London) Ltd.)

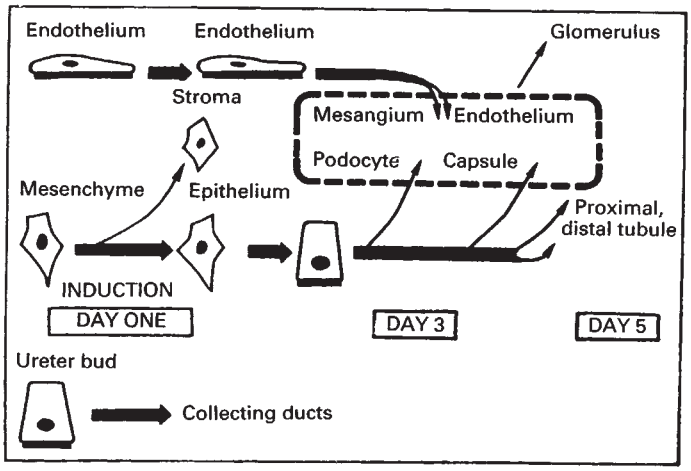

Fig. 9. *Cell lineages during renal development.* Initially three major cell lineages can be distinguished in the developing kidney, but each will give rise to a number of different cell types. In the scheme it is proposed that the mesangial cells are formed from the endothelial cell lineage, but no direct evidence for this possibility is available. (Reprinted from ref. 24. Used with permission.)

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