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Early organogenesis of the kidney

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Abstract. The mammalian permanent kidney consists of three cell lineages of different origin: the epithelial cells of the ureter bud, the mesenchymal cells of the nephric blastema and the endothelial cells of the capillaries. Organogenesis is governed by a cascade of morphogenetic interactions between these cell populations, a reciprocal epithelial-mesenchymal interaction between the branching ureter and the metanephric mesenchyme, homotypic interactions between cells of the tubular anlagen, stimulation of angiogenesis by the differentiating blastema and a mesenchymal – endothelial interaction guiding the migration of the capillary endothelial cells. While the biology of these interactive events is well known, as described in this overview, the molecular mechanisms are less well mapped out.

Key words: Kidney organogenesis – Inductive tissue interactions – Metanephric mesenchyme – Angiogenesis

Introduction

Cytodifferentiation and the spatial assembly of differentiating cells during organogenesis are governed by interactive events between molecules, cells and tissues. The understanding of these regulative forces lays a firm basis for the exploration of the aetiology and pathogenesis of developmental abnormalities. The mammalian permanent kidney, the metanephros, is no exception. The chain of inductive tissue interactions ultimately leading to the maturing kidney can be followed from the early blastula stage [1]. In the present paper, the organogenesis of the metanephros is described from the stage at which the ureter bud reaches the caudal end of the nephric cord, the mesenchymal blastema of the metanephros, to the stage of the assembly of the glomerular basement membrane (GBM). The formation of the permanent kidney results from interactive events between three distinct cell lineages brought together by directed migrations. They are the epithelium of the Wolffian-duct-derived ureter, the mesenchyme of the nephric blastema giving rise to the secretory nephron and the endothelial cell lineage providing the vascular supply of the kidney (Fig. 1). Until recently the fourth component, the nervous system, has, by and large, been ignored in the analysis of early kidney development, yet we have emphasized its role in this process (unpublished data).

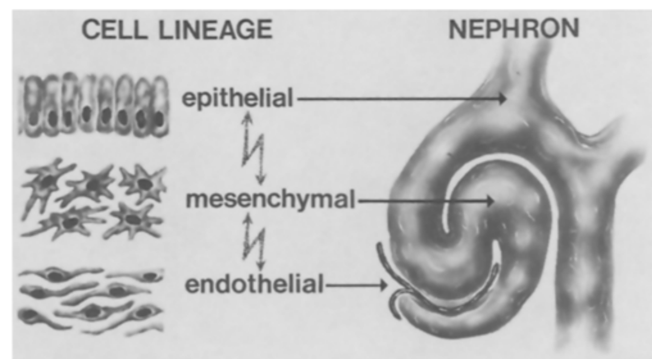

Fig. 1. Schema of the three interacting cell lineages contributing to the formation of the secretory nephron

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The ureter

While invading the metanephric mesenchyme, the ureter enters a programme of regular, dichotomous branching, leading to the formation of the ureter trees, which form the collecting system. The early pattern of this branching can be visualized by several techniques, e. g. microdissection, time-lapse cinematography and whole-mount immunohistology (Fig. 2). Early branching seems to be similar in different species, but the later stages show variations and are best analysed in human embryonic kidneys in which the arcade formation and various attachment sites of the nephrons are well characterized [2, 3].

Growth and branching of the ureter are regulated by the mesenchyme through tissue interaction, and probably by growth factors provided by other tissues [4, 5]. When separated from the mesenchyme and cultivated in isolation, the ureter fails to branch, and similarly, when cultivated with the mesenchyme in chemically defined medium lacking certain growth factors, the branching ceases [6]. The mesenchymal action shows a certain degree of organ-specificity as many heterotypic mesenchymes fail to support normal branching [7].

The mechanisms by which the mesenchyme acts upon the branching ureter have not been explored in the kidney, but detailed information is available on certain other glandular organs. This suggests that the mesenchyme induces changes in the composition and turnover of the epithelial basement membrane at the branching sites [8].

The mesenchyme

Throughout its history, the mesenchyme of the metanephric blastema has already been predetermined and developed a "kidney bias". This is

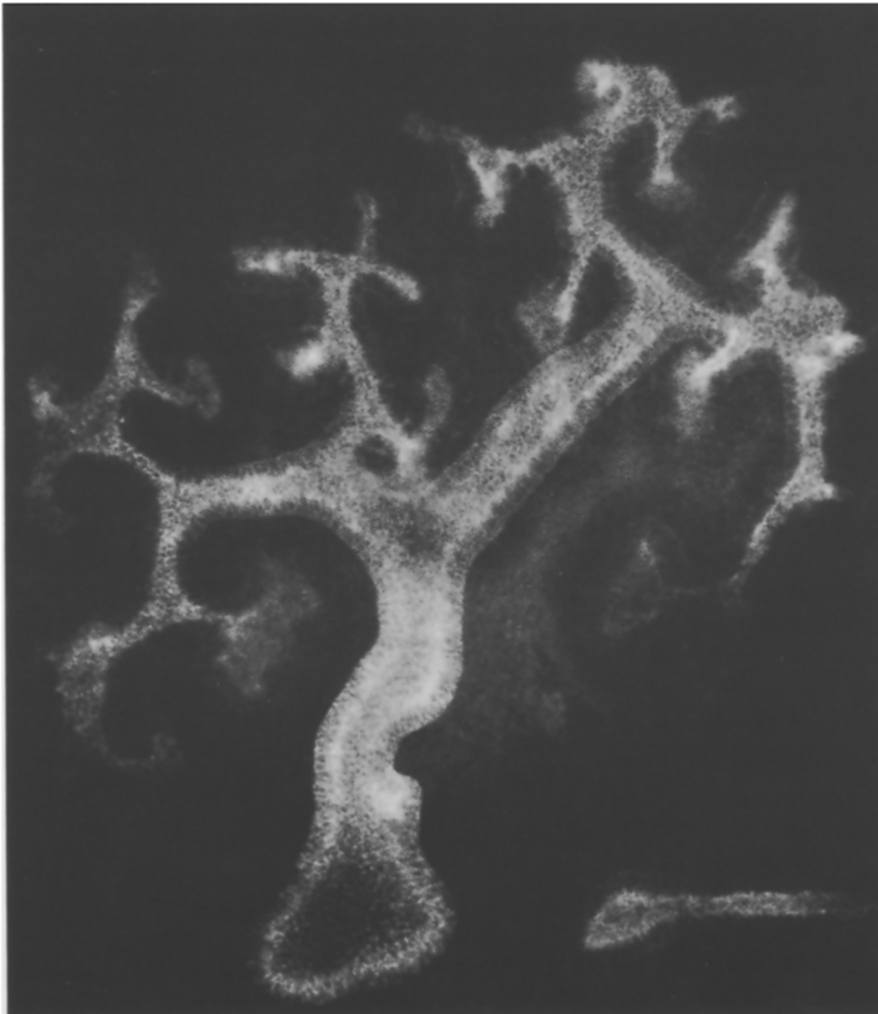


Fig. 2. Fluorescence micrograph illustrating the branching pattern of the ureter (collecting ducts) in an embryonic mouse kidney. Visualized with a monoclonal antibody against cytokeratin not reacting with the epithelium of the secretory nephron

shown by two kinds of experiments involving heterotypic combinations; a variety of tissues will trigger tubule formation when combined to the metanephric mesenchyme [7] but only the metanephric blastema will respond to these inductors by tubule formation [9].

By most criteria, the metanephric mesenchyme, prior to the ingrowth of the ureter consists of a homogeneous cell population, but recent studies (unpublished observations) suggest a certain heterogeneity in the constitution of the extracellular matrix and in the lectin-binding properties of the mesenchymal cells. Immunohistological examination of laminin showed an uneven distribution in the mesenchyme, and similarly, staining with a large repertoire of fluorochrome-conjugated lectins revealed at least two cell types. Whether the heterogeneity reflects different cell lineages with different developmental options and fates is not yet known, but we have shown that a short induction pulse can convert the mesenchymal cells into different types of cells of the secretory nephron.

Epithelial-mesenchymal interactions

The separation experiments mentioned above [7] as well as many previous observations in vivo [10] show that the interaction between the ureter epithelium and the surrounding mesenchyme is reciprocal, because a trigger provided by the ureter is a prerequisite for the epithelialization and morphogenesis of the mesenchyme. This particular step in kidney development has been thoroughly analysed in vitro, especially by the use of the transfilter technique developed by Grobstein [11] and modified by our group [12]. The main results and conclusions are briefly summarized.

As mentioned above, induction of the metanephric mesenchyme is merely permissive and acts upon predetermined cells. The triggering stimulus is of relatively short duration, as in our experimental conditions a 24-h "pulse" is sufficient to programme the mesenchymal cells to differentiate into at least three distinct types: the epithelial podocytes, the epithelial cells of the distal tubules expressing the Tamm-Horsfall glycoprotein, and the brush-border-carrying cells of the proximal tubules [13, 14]. The interaction between the inductor and the mesenchyme occurs only between cells that are in close association ("contact") [15–17], and the induction of differentiation is a function of the duration and extent of the intercellular contacts [18]. Thus far, induction has been obtained only with living cells, and attempts to use cell-free extracts, membrane preparations or specified compounds have failed [5].

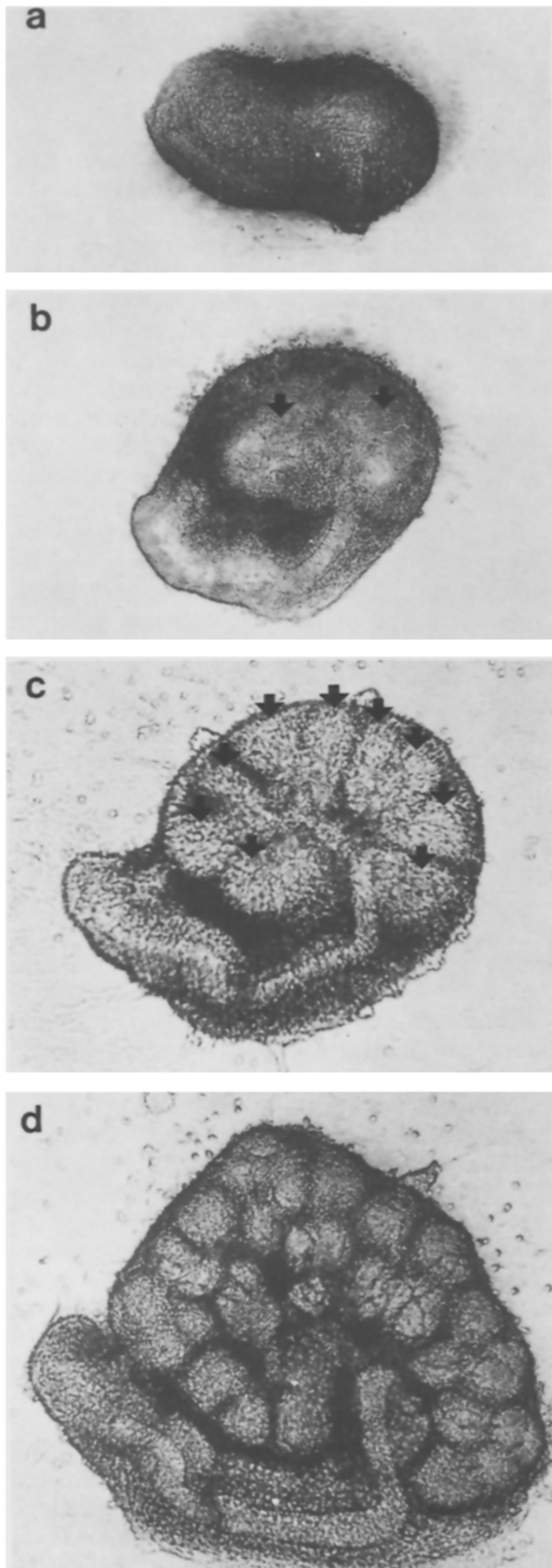
Response of the mesenchyme

Morphological, immunohistological and biochemical studies have revealed a cascade of early differentiative events in the mesenchyme after its exposure to an inductor. These processes have been seen either in whole kidney anlagen around the inductively active tips of the ureter or in vitro when the mesenchyme has been experimentally brought into contact with an inductor, usually a piece of embryonic spinal cord. Temporal correlation of the various events is possible by using the transfilter technique when both the exposure of and the subsequent changes in the mesenchyme can be precisely timed [13, 14].

The first morphological sign of the onset of tubulogenesis is a condensation of the loose mesenchyme at the inductor/mesenchyme interphase. Both in vivo and in vitro the maximal depth of this condensed area is approximately 200 μm [19]. In whole kidney rudiments cultivated in vitro the subsequent development of the primary condensate can be followed in time-lapse cinematography [20, 21]: with continuous growth and branching of the ureter bud, its tips move apart, the primary condensate is gradually split into halves along the equatorial line between the tips until the first generation of pretubular aggregates are segregated (Fig. 3).

Concomitant with the early morphogenesis, definite changes in the composition of the extracellular matrix of the mesenchyme can be detected by immunohistology [22–26]. The interstitial proteins fibronectin, collagen type I and type III are lost from the condensed areas and replaced by an enhanced synthesis of a set of epithelial-type proteins, collagen type IV and type V, laminin, heparan sulphate proteoglycan, and entactin. The latter group of compounds first becomes distinct as randomly distributed dots and is subsequently confined to the periphery of the aggregate, where the epithelial proteins contribute to the basement membrane. Parallel to the above changes in the extracellular matrix and the early condensation of the mesenchyme, stimulation of DNA synthesis can be monitored as a threefold increase in the incorporation of thymidine [27].

Causal relationships between the early molecular changes in the metanephric mesenchyme and differentiation of its cells can only be speculated upon until the primary action mechanism of the inductor is known. Direct observations of the



condensation and splitting of the condensate suggest increased intercellular adhesiveness of the mesenchymal cells, also detected as increased resistance towards dissociative treatments and as a cessation of the random motility of cells within the aggregates [20, 21]. This hypothesis predicts an enhanced synthesis of some adhesive compounds. Such molecules are found in many embryonic tissues which have been shown to exert "form-shaping" actions [28].

Aggregation of the cells is further facilitated by the disappearance (degradation) of the interstitial proteins, which allows the cells to make more intimate contacts, and by the increased proliferation, leading to an increased cell density within the condensates. The enhanced synthesis and deposition of the epithelial proteins (laminin, collagen type IV) may experience developmental roles slightly later, e. g. after the initial condensation. These components then accumulate at the periphery of the pretubular aggregate and constitute the basement membrane to which cells become attached. This scaffold function maintaining the epithelial organization of cells has been shown in a great variety of normal and neoplastic tissues.

Formation of the nephron

Detailed descriptions of the early stages of nephron formation have been given by Jokelainen [29] Potter [2] and Saxén [5], and some stages are illustrated in Fig. 4. Following aggregation, the mesenchymal cells acquire an elongated, epithelial shape by increasing their mutual contact surface at the expense of the less adhesive heterotypic contact area between the aggregated cells and the uninduced stroma. The first sign of a baso-apical polarization within the aggregate now detected is an accumulation of the basement membrane material on the base of the cells and an opening of a central lumen [30]. Subsequently, two slits open in the comma-shaped aggregate, creating the typical S-shape of an early nephron. Like the early aggregation process, this formation of the S-shaped body has been attributed to a gradually increasing adhesion within the aggregate, but evidence for this hypothesis has remained circumstantial [21].

Fig. 3a–d. Micrographs of the early stages of developing mouse metanephros cultivated in vitro. Note the primary condensate (*arrows* in **b** and **c**) and its gradual splitting into first generation pretubular aggregates. **a** Day 0, **b** day 1, **c** day 2, **d** day 3 of cultivation

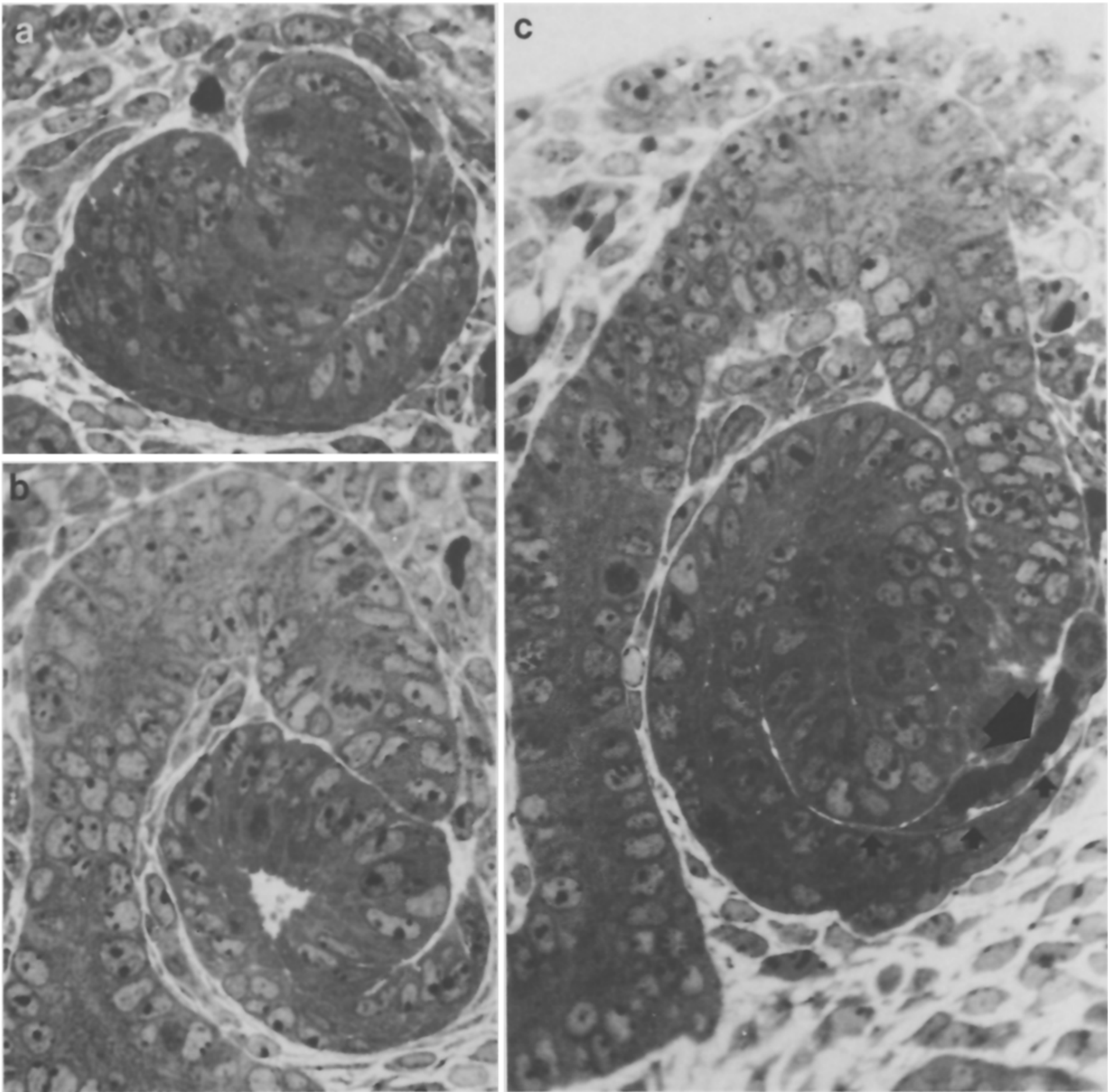


Fig. 4a–c. Micrographs illustrating the formation and shaping of the S-shaped body. **a** First indication of the formation of the two slits into the nephric aggregate. **b** Deepening of the lower, preglomerular crevice. No connection yet to the collecting duct. **c** First capillary endothelial cells *(arrow)* in the crevice and connection to the collecting duct system

Vascularization of the nephron

The first sign of nephron vascularization has been described as the appearance of endothelial-like cells within the lower, preglomerular crevice of the S-shaped body [29]. The hypothesis that these cells give rise to the glomerular capillary loop has been the subject of some controversy. While some authors consider the vascular elements to be derived from mesenchymal cells that have migrated into the crevice or been trapped therein, others conclude that these are true endothelial cells of outside origin [31].

Recent experimental results with kidney grafts on avian chorioallantoic membrane (CAM) support the second alternative. Mouse embryonic, avascular kidney rudiments or mesenchymal explants first induced in vitro were grafted on avian CAM. The grafts soon became richly vascularized

by the sprouting capillaries of the CAM, and, unlike in vitro, the glomeruli of the whole kidney grafts showed a vascular component. To explore further the origin of the glomerular endothelium, mouse kidney grafts on quail CAM were examined. Since the cells of the quail embryos carry a nuclear marker [32], these could be identified in the mouse kidney grafts, and the host origin of the glomerular endothelial cells could be verified [33].

Uninduced mesenchymes as well as undifferentiated areas of induced explants remained avascular [33]. Thus, it is clear that induction leads to the acquisition of an angiogenesis-stimulating action of the nephric mesenchyme, and recently Risau and Ekblom [34] have isolated and characterized a growth factor carrying this activity from mouse embryonic kidneys.

The origin of the GBM was explored in chimeric murine/avian glomeruli by immunohistology, applying species-specific antibodies against constituents of the basement membrane. The results showed that the epithelial podocytes and the vascular endothelial cells both contributed to the GBM [35]. Its dual origin was confirmed more recently by Abrahamson [36], who by immunoelectron microscopy detected intracellular laminin in both the podocytes and the endothelial cells of newborn rat kidneys.

Formation of the GBM in the normal and chimeric glomeruli thus represents an interaction between molecules from two sources. As shown by the antigenic differences, the basement membrane compounds are not identical in the mouse epithelial cells and the chick endothelial cells. This leads to a molecular mismatch, which results in the formation of an abnormal GBM with incomplete fusion of its layers [37]. Interestingly, this defective GBM resembles that found in Alport's syndrome.

Guided migration of the capillaries

Homing of the capillary endothelial cells into the crevice of the S-shaped bodies must be spatially and temporally strictly regulated to ensure the formation of a normal glomerulus and its GBM. The migration of the capillaries and the distribution of the CAM-derived endothelial cells was explored in whole kidney grafts in immunohistology by the use of antibodies against quail endothelial cells (Fig. 5). Double treatment with these anti-

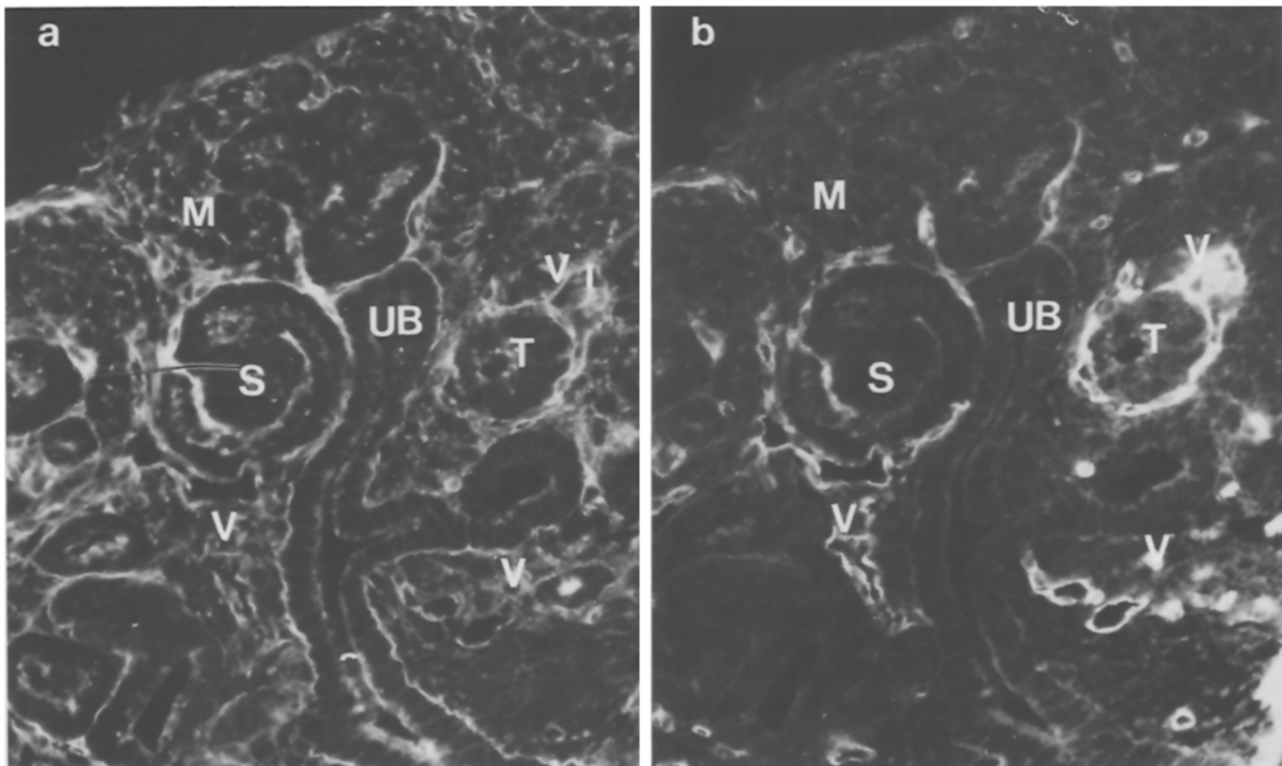


Fig. 5 a, b. Fluorescence micrographs of a section of a metanephric kidney anlage grafted for 4 days on quail chorioallantoic membrane. **a** A section treated with fluorochrome-conjugated wheat germ agglutinin demonstrating the architecture of the kidney. **b** The same section treated with an antiserum against quail endothelial cells. *V*=vessel, *T*=tubule, *M*=mesenchyme, *S*=S-shaped body, *UB*=ureter bud

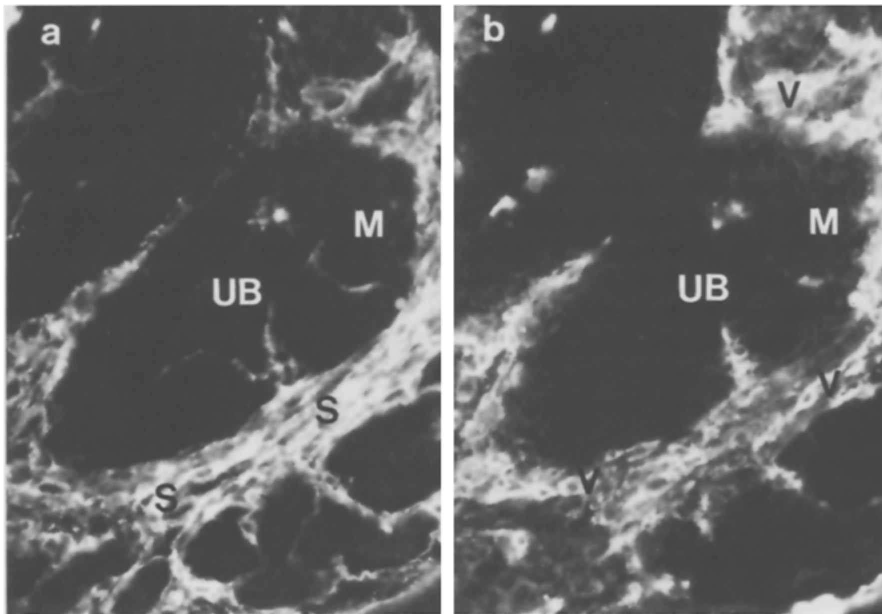


Fig. 6a, b. Fluorescence micrograph demonstrating the codistribution of fibronectin and host vessels within a CAM-grafted mouse kidney. **a** A section treated with an antiserum against fibronectin. **b** The same section reacted with an antiserum against quail endothelial cells. *V*=vessels, *M*=undifferentiated mesenchyme, *UB*=ureter bud, *S*=stroma

bodies and antisera against distinct components of the extracellular matrix showed a clear codistribution between the capillaries and the stromal fibronectin (Fig. 6). Hence, it has been hypothesized that fibronectin could be the adhesive molecule providing the directive clues for the migrating endothelial cells [38].

In conclusion, the observations and experimental results summarized here suggest that the decisive regulatory component in the developing metanephric kidney is the ureter. Stimulated by the mesenchyme to implement its inherent branching programme, the ureter determines the time and site of appearance of the secretory nephrons; by inducing changes in the extracellular matrix, it creates the paths for the migrating endothelial cells ultimately trapped in the glomerular crevice. Moreover, stimulation of the capillary ingrowth is due to an angiogenic factor synthesized as a consequence of induction by the ureter. Many steps in this chain of events still remain molecularly unexplained, but the model system has been reasonably well mapped out at the cell and tissue levels, and it constitutes a good basis for further analyses of the molecular events behind induction and early differentiation in the kidney, both during normal and impaired organogenesis.

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