The metanephric kidney originates from two distinct embryonic precursor populations. The mesenchymal blastema generates the nephron from glomerulus to distal tubule, whereas the ureter gives rise to the collecting tubule system by branching morphogenesis. The nephron develops by a direct mesenchyme-to-epithelium transition when the ureteric buds invading the mesenchymal blastema induce the transition (9). The stages of this process can be defined in situ and have been separated into the following: induction, determination of the stem cell pathway, condensation, epithelialization, aggregation, cell polarization, and functional expression of the differentiated nephron cell type (reviewed in Ref. 12).

However, functional cell polarization and differentiation, i.e., the processes that lead to the specific absorptive and secretory functions of a nephron segment, have not been evaluated during mesenchyme-to-epithelium transition, most likely because direct access to defined epithelial cells beneath the renal capsule in embryonic organ cultures is limited. In the present work, therefore, the single nephrogenic unit, i.e., the most peripheral dichotomous ureteric branch with its buds and the adherent blastema, was dissected from the embryonic kidney and grown in coculture. The process of mesenchyme-to-epithelium transition in vitro was found to be phenotypically identical to that of the in situ organ culture, with respect to morphology and expression of transcription factors and morphoregulatory proteins.

Changes in plasma membrane ion channel expression during mesenchyme-to-epithelium transition and early tubulogenesis were studied by comparing in vitro pre-epithelial condensed mesenchymal cells with those of the epithelial S-shaped body applying patch-clamp techniques, single-cell RT-PCR, and immunohistochemistry. This work provides first evidence for a profound reorganization of plasma membrane ion channel expression during mesenchyme-to-epithelium transition.

MATERIALS AND METHODS

Mesenchyme-to-epithelium transition in primary culture. All chemicals were from Sigma (Deisenhofen, Germany) unless stated otherwise. Ureteric buds with attached mesenchymal blastema were microdissected from the most peripheral
Nephrogenic mesenchyme-to-epithelium transition in primary culture. A: scheme of the tissue preparation. The outermost branch of the ureteric bud branching tree with adherent mesenchymal blastema was dissected from embryonic mouse kidney (embryonic day E14), explanted on a mouse tail collagen matrix, and cultured for 3–4 days. B–D: light micrograph series of mesenchymeto-epithelium transition in vitro. Condensed mesenchyme (cm) that capped an outgrowing ureteric bud (ub) converted to comma- (cb) and S-shaped body (sb) within the indicated time of culture. E–H: scanning electron micrographs of in vitro mesenchyme-to-epithelium transition stages. Shown are dissected ureteric bud with adherent mesenchymal blastema (E), globular aggregate (F), comma-shaped body (G), and S-shaped body (H). The aggregate (F, bottom) is close to the rudimentary tubular ureteric bud tip (ub). I: schematic redrawing summarizing the developmental changes during nephrogenic mesenchyme-to-epithelium transition in vitro. Ureteric bud cells migrate from the explanted tubular epithelium (far left) to a globular aggregate (a, middle left) and convert to comma- (cb, middle right) and S-shaped body (sb, far right) on top of the ureteric bud cell monolayer. Noninduced blastema cells, by contrast, enter apoptosis, which facilitated the experimental access to condensed mesenchyme, comma-shaped body, and S-shaped body. Bars = 10 μm.

The in vitro development was monitored directly in six independent cultures by light microscopy photo series. Condensed mesenchyme surrounding a ureteric bud (as shown in Figs. 1B, 4A, 4E, 5B, 5G, and 6A) was labeled by the outermost tip of a patch-clamp glass pipette that was fixed to the collagen matrix close to mesenchyme. During culture progress, the marked structures were retrieved easily, and development-dependent transitions of the mesenchyme into globular aggregate, and comma- and S-shaped body within about 2 days of culture were demonstrated (Fig. 1, A–D).

For standard scanning electron microscopy (SEM), ureteric bud-blastema cocultures were fixed according to Ref. 14. From these SEM micrographs (Fig. 1, E–H) and the light micrograph photo series, the in vitro development of ureteric bud- and blastema-derived nephron precursors was deduced as summarized schematically in Fig. 1I.

In addition, the SEM micrographs revealed that the in vitro tubulogenic stages lacked a continuous basal lamina (Fig. 1, E–H). Patch-clamp recording and harvesting of cytoplasmic samples into the patch pipette for single-cell RT-PCR, therefore, could be performed without enzymatic treatment. Furthermore, the direct access to the in vitro developmental stages allowed immunostaining of development-specific marker proteins in nonsliced, whole cultures. The methods as applied in the present study are summarized in Fig. 2.

Immunostaining of development-specific marker proteins and of Kir6.1 K+ channels. Immunostaining of Pax-2, WT-1, E-cadherin, and Kir6.1 were performed in whole cocultures of ureteric bud and blastema. For immunostaining of β-catenin, cocultures were grown in flexiPERM chambers on glass slides. After fixation (see below) cultures were stained with hemalaun for 5 min and rinsed with PBS until the tissue could be seen as a blue dot within the background. The
chambers were filled with 3% gelatin on top of the cultures and cooled for about 15 min at 4°C. Thereafter, chambers with cultures that were gelatin-embedded at one side were removed from the glass slides, and gelatin was added to other side of the cultures to prevent destruction of the tissue during the paraffin embedding procedure. Cultures within gelatin blocks were harvested from the chambers by a gentle push, deparaffinized and rehydrated.

Immunostaining of the whole cultures and paraffin sections which were embedded in paraffin, and cut in 2-μm sections from a 2-μm mixture (see below). Cytoplasm was harvested by whole cell recording protocol (see previous reports (14, 19)). Cytoplasm that dialyzed into the patch pipette during whole cell recording was harvested and reverse transcribed. A fragment encoding WT-1 and E-cadherin, or Kir6.1 and KvLQT, respectively, was amplified by 50 cycles of PCR. From the remaining 8 μl cDNA, fragments encoding WT-1 and E-cadherin, or Kir6.1 and KvLQT, respectively, were amplified by 50 cycles of duplex PCRs. The PCR products were precipitated, washed, and diluted in 50 μl H2O. In a second round of nested PCR, each cDNA fragment was reamplified separately by 30–40 cycles using a 0.5-mM primer (Boehringer, Mannheim, Germany). The RNA was reverse transcribed for 1 h at 37°C. From the total volume of about 10 μl cDNA, a 2-μl aliquot was placed in a second tube in some experiments, and both aliquots were stored at −25°C.

RT- and water negative controls (see Figs. 6A and 9A) were performed according to Ref. 14. In addition, aliquots of RT-PCR mixtures were stained with VistraGreen fluorescence dye, visualized by a Fluorescence Phosphor-Imager, and analyzed by sequencing.

Patch-clamp recordings. Patch-clamp recordings were performed at room temperature in voltage clamp mode as described previously (13). Applied voltages refer to the cytoplasmic face of the membrane with respect to the interstitial space. Inward currents, defined as flow of positive charge from the interstitial to the cytoplasmic membrane face, are negative currents and are depicted as downward deflections of the original current traces. Applied voltages were corrected by the liquid junction potential between pipette and bath solution that was set off by the patch clamp amplifier prior to sealing and that was estimated according to Ref. 3.

Cultures were rinsed with NaCl bath solution (in mM: 150 NaCl, 10 d-glucose, 10 HEPES, 5 KCl, 1.6 CaCl2, and 0.8 MgCl2, titrated with NaOH to pH 7.4) and constantly superfused (1 ml/min) with NaCl bath solution or with a solution containing 155 KCl, 10 d-glucose, 10 HEPES, 1.6 CaCl2, and 0.8 MgCl2 (titrated with KOH to pH 7.4).

Patch-clamp experiments were performed using glass pipettes (2–5 MΩ tip resistance) filled with 6.5 Ml of pipette solution (in mM: 110 potassium d-glucosone, 30 KCl, 5 HEPES, 6 MgCl2, and 0.01 Tris) and 0.5 Ml aliquots were used as template. PCR products were stained with VistraGreen fluorescence dye, visualized by a Fluorescence Phosphor-Imager, and analyzed by sequencing.

Since whole cell recordings were used to harvest mRNA for RT-PCR, the pipettes and the Ag/AgCl wire were baked (220°C for 5 h), tubings were rinsed with 0.1% diethyl pyrocarbonate (DEPC) water before use, and 0.1% DEPC was mixed to bath and pipette solutions before autoclaving to avoid degradation of mRNA. In addition, RNase inhibitor (RNasin, 40 U/μl; Promega, Ingelheim, Germany) was added to the pipette solution to a final activity of 0.4 U/μl.

Single-cell RT-PCR. Development-dependent mRNA expression of the electrophysiologically characterized in vitro nephrogenesis stages was studied by single-cell RT-PCR according to previous reports (14, 19). Cytoplasm that dialyzed into the patch pipette during whole cell recording was harvested by expelling the total pipette volume (6.5 μl) directly into a tube filled with 3.5 μl reverse transcriptase (RT) mixture (see below). Cytoplasm was harvested by whole cell recording mode in 65 cells. Because of either electrical coupling between the cells, or to loss of gigaohm seal during recording, or to an initially insufficient seal, the entire whole cell recording protocol (see Patch-clamp recordings) together with current analysis could be run in 13 cells only.

The RT mixture contained the following: 1 μl dithiothreitol (0.1 mM), 0.1 μl single-strand buffer (5-fold), 0.5 μl SuperScript RT (200 U/μl) (all Life Technologies), 0.5 μl RNase inhibitor (RNasin), 0.5 μl dATP/dCTP/dGTP/dTTP mixture (25 mM each), and 1 μl random hexamer oligonucleotide primer (Boehringer, Mannheim, Germany). The RNA was transcribed for 1 h at 37°C. From the total volume of about 10 μl cDNA, a 2-μl aliquot was placed in a second tube in some experiments, and both aliquots were stored at −25°C.

RT- and water negative controls (see Figs. 6A and 9A) were performed according to Ref. 14. In addition, aliquots of
Fig. 3. Immunostaining of Pax-2, WT-1, E-cadherin, Kir6.1, and β-catenin proteins: flow diagram summarizing in vertical columns the incubation steps and solutions applied for immunofluorescence and immunohistochemistry, respectively. Incubations were carried out at room temperature if not stated otherwise. 3-amino-9-ethylcarbazole (AEC) chromogen yielded a red to brown precipitate. PBS, Ca\(^{2+}\)- and Mg\(^{2+}\)-free phosphate-buffered saline (Seromed); PFA, paraformaldehyde; TBS, Tris-buffered saline (150 mM NaCl, 50 mM Tris \(\times\) HCl, pH 7.5); CB, citrate buffer (10 mM with NaOH to pH 6.0). Twin 20 and Triton X-100 were from Fluka (Buchs, Switzerland), and goat serum was from Vector (Burlingame, CA).
Table 1. Primer sequences used for single-cell RT-PCR

<table>
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<th>Accession Number</th>
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<th>Sequence</th>
<th>Position</th>
<th>Length</th>
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<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
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<td>340 bp</td>
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<td>M55512</td>
<td>WT-1</td>
<td>antisense</td>
<td>5'-CAC GGA AGG CCA TCC CAG TGA-3'</td>
<td>743-723</td>
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<tr>
<td>X06115</td>
<td>E-cadherin</td>
<td>sense (outer)</td>
<td>5'-CTG ATG GCC CAT CCT GCC TCT-3'</td>
<td>2,189-2,207</td>
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<td>Kir6.1</td>
<td>sense (outer)</td>
<td>5'-AGG CCA ATG TCA AAG GGA GCC TCA-3'</td>
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<td></td>
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<tr>
<td>U70068</td>
<td>K_+LQT1</td>
<td>sense (inner)</td>
<td>5'-GAT GGT GCT GGA CTG TCT-3'</td>
<td>2,558-2,576</td>
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second round by “uniplex” PCRs (10 min at 95°C, followed by 30–40 cycles of 45 s at 94°C, 1 min at 56°C, and 1 min at 72°C, and a final extension at 72°C for 7 min) using nested primer pairs (Fig. 3). The PCR mixture (50 μl) contained the following: 5 μl of 10-fold buffer, 3 μl MgCl_2 (25 mM), 0.25 μl AmpliTaq Gold polymerase (5 U/μl), 0.4 μl dATP/dCTP/dGTP/dTTP mixture (each 25 mM, Promega), 0.5 μl each of the two inner primers (10 μM), and 18 μl H_2O.

Twelve microliters of each PCR reaction was separated by electrophoresis in a nondenaturating 5% acrylamide gel. PCR product bands were stained with a fluorescence DNA dye (VistraGreen, Amersham) and visualized with ImageQuant Software on a Storm fluorophorimager (both from Molecular Dynamics, Krefeld, Germany). Remaining PCR products of the positive samples were pooled and purified, and identity of the amplified fragment was confirmed by sequencing (SequServe, Vaterstetten, Germany).

RESULTS

Protein expression of the transcription factor Pax-2 and the Wilms’ tumor suppressor WT-1 was analyzed by immunofluorescence in metanephrogenic cultures to characterize the in vitro developmental stages of the nephron lineage. These stages as defined by light microscopy to represent mesenchymal blastema, condensed mesenchyme, globular aggregate, comma-shaped body, and S-shaped body differed in their WT-1 and Pax-2 protein expression.

WT-1 protein was apparent neither in ureteric bud tubules nor monolayer, nor in blastemal cells (Fig. 4, A and B). Marked WT-1-specific staining that was confined to the nuclei occurred in all cells of condensed mesenchyme (Fig. 4A), globular aggregate (Fig. 4B), and comma-shaped body (Fig. 4C). In S-shaped body, staining was restricted to the long (proximal) arm of the S (Fig. 4D; see below), suggestive of upregulation of WT-1 protein upon condensation and of downregulation in most cells during comma-to-S conversion.

In contrast to WT-1, rudimentary ureteric bud tubules (Fig. 4E) and blastema cell populations (Fig. 4G), as well as condensed mesenchymal cells (Fig. 4E), exhibited nuclear immunostaining for Pax-2. This staining was not apparent in globular aggregate (Fig. 4F), comma-shaped body (Fig. 4G), and S-shaped body (not shown), suggesting a rapid downregulation of Pax-2 protein before globular aggregation. In addition, nuclei of ureteric bud monolayer (Fig. 4H) were Pax-2 protein positive, similar to those of rudimentary ureteric bud tubules and of the early nephron lineage stages.

Progress of mesenchyme-to-epithelium transition during nephrogenesis was monitored by E-cadherin and β-catenin immunohistochemistry. Cell adhesion molecule E-cadherin-specific immunostaining of the plasma membrane occurred in ureteric bud monolayer between adjacent cells but not in blastema (Fig. 5A), confirming the mesenchymal phenotype of the latter. Condensed mesenchyme (Fig. 5B), globular aggregate (Fig. 5C), and S-shaped body (Fig. 5D), similar to the ureteric bud, exhibited plasma membrane-localized staining, suggesting that E-cadherin-mediated cell adhesion starts upon condensation of the mesenchyme.

To further analyze the cell organization of the nascent epithelial cells, protein expression of the plasma membrane-to-cytoskeleton linker β-catenin was studied in paraffin sections. Weak plasma membrane-associated β-catenin immunostaining was apparent in condensed mesenchyme, further suggesting advanced epitheliogenesis in the condensed state (Fig. 5G). Globular aggregate, ureteric bud monolayer (Fig. 5H), and S-shaped body (Fig. 5I) showed regular sharp staining on both sides of the lateral cell-cell junction. Blastema cells, by contrast, were β-catenin negative, emphasizing their mesenchymal phenotype. Negative controls for E-cadherin and β-catenin immunohistochemistry were not immunoreactive (Fig. 5, E, F, and J).

To study the changes in plasma membrane ion conductance during mesenchyme-to-epithelium transition, patch-clamp whole cell recordings were performed
in two developmental stages, in the preepithelial condensed mesenchyme (Fig. 6A) and in the proximal S-shaped body (Fig. 6B). Specifically, recordings were made from the region where the long, proximal arm became the first bend of the S and where a lumen clearly was visible, suggesting that this region represented the first stage of the nephron lineage that was organized in a tubular epithelium (Fig. 6, C and D).

In cytosol that dialyzed into the recording pipette during whole cell recording, WT-1 and E-cadherin mRNA expression was analyzed by single-cell RT-PCR to directly identify the nephrogenic fate of the recorded cells (Fig. 6E). To ascertain that the single-cell RT-PCR method worked with similar yields in condensed mesenchyme and S-shaped-body cells, a cDNA fragment specific for the housekeeper GAPDH was amplified further from aliquots of the cytosol samples. After one round of PCR (see MATERIALS AND METHODS and Fig. 2), about 40% of the cytosol samples from both developmental stages were positive for the housekeeper GAPDH, suggesting similar efficiency in applying the single-cell RT-PCR method to condensed mesenchyme and S-shaped-body cells (Fig. 6F).

Extracellular negative controls did not yield GAPDH-, WT-1-, or E-cadherin-specific PCR products (Fig. 6, E and F). Thus specific PCR products amplified from the cytosol samples were not due to mRNA spilled into the extracellular space as might occur, for instance, by cell death.

Whole cell currents of preepithelial condensed mesenchyme cells and of tubular epithelial S-shaped body cells differed markedly (Fig. 7, A and B). Condensed mesenchyme cells when recorded with potassium gluconate/KCl pipette and NaCl bath solution exhibited large outward whole cell currents at depolarizing, but only small inward currents at hyperpolarizing voltages. Upon depolarizing voltage steps to greater than or equal to +50 mV, outward currents activated time dependently in five of a total of seven cells with a mean time constant of \( T = 44 \pm 10 \) ms at +90 mV voltage (Fig. 7A, left). This depolarization-dependent, slow-activating current fraction rectified outwardly. Replacement of bath NaCl by KCl (Fig. 7A, right) elicited an increase in inward currents suggestive of a \( K^+ \)-selective fractional whole cell \( K^+ \) conductance (\( G_K \)). Mean current-voltage (I/V) relation of capacitance-
normalized whole cell currents (NaCl bath, potassium gluconate/KCl pipette solution) exhibited weak outward rectification and a conductance of $G = 6.7 \pm 1.3$ nS/10 pF ($n = 7$) as calculated for the outward currents (Fig. 7C).

The reversal potential ($V_{\text{rev}}$) of the $I/V$ curve ($V_{\text{rev}} = -40 \pm 7$ mV) was between the equilibrium potential ($E$) for Cl ($E_{\text{Cl}} = -36$ mV) and $E_K (-85$ mV). Replacement of bath NaCl by KCl in paired experiments shifted $V_{\text{rev}}$ by $+29$ mV along the change of $E_K (E_K = +3$ mV) to $V_{\text{rev}} = -10 \pm 3$ mV (Fig. 7C), further demonstrating a fractional $G_K$ (Fig. 7C). A fractional $G_{\text{Cl}}$ was indicated by deflection of $V_{\text{rev}}$ in KCl bath solution from $E_K (+3$ mV) as well as from the equilibrium potential for nonselective cations (NSC; $E_{\text{NSC}} = +3$ mV) toward $E_{\text{Cl}}$ by about $-13$ mV. This fractional $G_{\text{Cl}}$, in addition to $G_K$, mainly contributed to the whole cell conductance of condensed mesenchyme cells. A further contribution of other cation conductances, e.g., of a nonselective cation conductance ($G_{\text{NSC}}$) to whole cell conductance could not be deduced from these experiments (Fig. 7C).

Proximal S-shaped-body cells, by contrast, did not show slowly activating whole cell outward currents. Replacement of bath NaCl by KCl evoked large inward currents suggestive of a very high fractional $G_K$ (Fig. 7B). The mean $I/V$ curve when recorded in NaCl bath (potassium gluconate/KCl pipette solution) did not rectify and exhibited a slope of $G = 4 \pm 1.2$ nS/10 pF ($n = 6$) and a $V_{\text{rev}}$ at $E_K (V_{\text{rev}} = -82 \pm 6$ mV). Upon replacement of bath NaCl by KCl, $V_{\text{rev}}$ shifted with $E_K (V_{\text{rev}} = -1 \pm 1$ mV), indicating that whole cell conductance in proximal S-shaped-body cells was almost exclusively generated by $G_K$ (Fig. 7D).

$G_K$ was further characterized in whole cell tail currents of both developmental stages (Fig. 7, E and F). Tail currents were analyzed in NaCl bath solution (potassium gluconate/KCl pipette solution) after repolarizing the membrane potential from voltages between $-110$ and $+90$ mV to $-10$ mV holding potential. Three condensed mesenchyme cells that had relatively high fractional $G_K$ exhibited fast inactivating tail currents (Fig. 7E, left). The amplitude of the tail currents increased with the degree of hyperpolarization of the preceding square pulse, consistent with a hyperpolarization-activated current that inactivated upon repolarization. This current was $K^+$ selective since replacement of NaCl by KCl in the bath with consequent shift of $E_K$ close to $-10$ mV holding potential abolished the tail currents (Fig. 7E, right).

Tail currents of three proximal S-shaped-body cells, by contrast, activated fast ($T$ in the range of 1 ms) upon repolarization from hyperpolarizing square pulses (Fig. 7F, left) and inactivated subsequently with larger time constants (not shown). These tail currents were...
extinguished when bath NaCl was replaced by KCl, indicating their K⁺ selectivity (Fig. 7F, right).

In addition to the differences in K⁺-selective whole cell tail currents, condensed mesenchyme and proximal S-shaped body cells differed in their K⁺ channel activity (Fig. 8, A–E). In three condensed mesenchyme cells, channels with a mean unitary conductance of 35 pS, as determined at 0 mV voltage with NaCl in the bath and potassium gluconate/KCl in the pipette, were identified in outside-out excised patches (Fig. 8, A and B). The $V_{\text{rev}}$ of the current amplitude extrapolated by linear regression to $-75 \pm 5$ mV voltage, indicating K⁺ selectivity. In a single cell-attached experiment (potassium gluconate/KCl pipette solution), channels with characteristics identical to the 35-pS channel exhibited inwardly rectifying $I/V$ curves (Fig. 8B). BaCl₂ (1 mM) when added to the bath solution reversibly blocked the 35-pS K⁺ channel in outside-out patches (Fig. 8C).

Fig. 7. Functional expression of plasma membrane ion conductances in condensed mesenchyme and proximal S-shaped body cells. A and B: original whole cell current traces of a condensed mesenchyme cell (A) and a proximal S-shaped-body cell (B) recorded with the patch-clamp, voltage-clamp technique in NaCl (left) and KCl (right) bath solution (potassium gluconate/KCl pipette buffer). Currents were evoked by 11 successive square pulses from $-10$-mV holding potential to voltages between $-110$, $-70$, $-30$, $+10$, $+50$, and $+90$ mV square pulse and after repolarization to $-10$-mV holding potential.

Fig. 6. WT-1 and E-cadherin mRNA expression during nephrogenic mesenchyme-to-epithelium transition. A and B: light micrographs of condensed mesenchyme (A) and of a proximal S-shaped body cell (B) during patch-clamp, whole cell recording. C and D: light micrograph and redrawing of an S-shaped body grown in vitro showing its microarchitecture. The long (proximal) arm of the S expressed a lumen, and the short (distal) arm had fused with the rudimentary ureteric bud trunk, which remained tubular in this particular culture (cm, condensed mesenchyme; p, patch-clamp recording pipette; sb, S-shaped body; ub, ureteric bud). E: mRNA expression analyzed by single-cell RT-PCR. PCR products specific for WT-1, E-cadherin, and GAPDH, respectively, amplified from single cytosol samples were separated by polyacrylamide gel electrophoresis, then stained by VistraGreen DNA dye, and fluorescence intensity was imaged in pseudo gray tones. Individual cytosol samples ($n = 8$) from 4 proximal S-shaped body cells (sb 1–4) and 4 condensed mesenchyme cells (cm 1–4), as well as 4 extracellular controls (e 1–4), an RT², and a H₂O control are shown. For extracellular controls, medium solution aspirated into the patch pipette close to the cells was processed identically to the cytosol samples; bp, base pairs of the size marker, as indicated. F: summary of the experiments in E. Fractions are presented as n/m of PCR-positive experiments, where n is the number of cytosol (cm, sb) and extracellular samples (e) yielding WT-1-, E-cadherin-, and GAPDH-specific PCR products, respectively, and m is the total number of samples.
To identify K\(^+\) channel molecules in both developmental stages, mRNA expression of the K\(_{LQT}\), a voltage-activated K\(^+\) channel involved in epithelial Cl secretion, and Kir6.1, an inward rectifier which is highly expressed in the nephrogenic zone of embryonic kidney (unpublished observation), was investigated by single-cell RT-PCR. In addition, Kir6.1 protein expression was studied immunohistochemically.

K\(_{LQT}\) mRNA was detected in about 60% and 75% of condensed mesenchyme and proximal S-shaped body cells, respectively, suggesting similar mRNA abundance in both developmental stages (Fig. 9, A and B). Kir6.1 mRNA, by contrast, was identified in only 2 of 12 condensed mesenchyme cells but not in proximal S-shaped-body cells (Fig. 9, A and B).

The low yield of the Kir6.1 PCR was not due to a low sensitivity of the PCR reaction since Kir6.1- and K\(_{LQT}\)-specific fragments were amplified with similar sensitivity from sequentially diluted cDNAs of whole cultures (i.e., mesenchyme-derived stages and ureteric bud epithelium) using the single cell PCR protocol (duplex combined with nested uniplex PCR; not shown). Rather, the inability to amplify Kir6.1-specific fragments from proximal S-shaped body cells might reflect a downregulation of Kir6.1 mRNA expression between both stages.

Similar downregulation of Kir6.1 was observed at the protein level. Kir6.1 protein was detected in the plasma membrane of condensed mesenchyme (Fig. 9C) and globular aggregate (Fig. 9D), but immunostaining was only weak or absent in that of S-shaped-body cells (Fig. 9E). In rudimentary tubules (Fig. 9D) and monolayers of the ureteric bud (Fig. 9F), Kir6.1 protein stained in the apical and lateral membrane and to a lesser extent in the basal membrane. Control cultures incubated with antigen-saturated antibodies yielded no staining (Fig. 9, G and H), indicating the specificity of the Kir6.1 immunohistochemistry.

Figure 10 summarizes the principal findings of the present study.

DISCUSSION

The present study has applied electrophysiological and RT-PCR methods for the first time to single cells passing through metanephrogenic mesenchyme-to-epithelium transition in vitro. The developmental stages of this in vitro model, i.e., mesenchymal blastema, condensed mesenchyme, globular aggregate, comma-, and S-shaped body, were defined by their characteristic morphology and by the expression of Pax-2, WT-1, E-cadherin, and β-catenin.

Previous studies in vivo and on kidney rudiments in organ culture have shown that WT-1 which is weakly expressed in uninduced blastema cells is upregulated after induction in condensing cells, and expression continues through the comma- and S-shaped stage, while high levels in the terminal nephron persist in podocytes only (2, 8, 24, 25). The Pax-2 gene, by contrast, is activated in the mesenchymal blastema in response to induction and is subsequently downregu-

In the serosal membrane of proximal S-shaped body cells, by contrast, 35-pS K\(^+\) channels were not apparent. A further type of K\(^+\)-selective channel was detected in the serosal outside-out patch of a proximal S-shaped-body cell (Fig. 8D). This type exhibited fast activation upon repolarization from hyperpolarizing voltages followed by a slow inactivation (Fig. 8D, inset), very similar to the kinetics of the macroscopic tail currents of the proximal S-shaped-body cells (Fig. 7F). The \(I/V\) curve of this channel type as recorded in outside-out and cell-attached mode was linear with a low conductance of ~10 pS (Fig. 8E).
lated rapidly at or soon after the S-shaped body stage (7, 8, 26). The in vitro model of the present study showed comparable time courses of protein expression and repression of both genes with an identical sequence. In addition, the time course of transition in the present study, from condensed mesenchyme morphology to the early tubular epithelium of the S-shaped body (1–2 days of culture; see Fig. 1, B and C), was similar to that occurring in renal rudiments in organ cultures (10), indicating the validity of in vitro model used in the present study.

Condensed mesenchyme, which formed epithelium-like arrays capping the ureteric bud tip (e.g., in Fig. 6A), expressed E-cadherin and β-catenin proteins. E-cadherin plays a crucial role in early epithelial polarization by mediating cell-cell adherens junctions, assembling basolateral plasma membrane-cytoskeleton complexes that involve β-catenin, and by integration and retention of Na\(^+\)-K\(^+\)-ATPase in these complexes (21, 23, 28). The E-cadherin expression in the condensed mesenchyme stage of the present study suggests that this E-cadherin upregulation is a very early event in mesenchyme-to-epithelium transition.

Expression changes of plasma membrane ion channels during this process were studied by comparing whole cell currents of an early, preepithelial (condensed mesenchyme cells) with those of a late developmental stage (tubular epithelium of the proximal S-shaped body). Both stages expressed WT-1 and E-cadherin mRNA as determined by single-cell RT-PCR, thus providing further proof for the nephrogenic commitment of the electrophysiologically characterized cells.

Fig. 9. Molecular expression of K\(^+\) channels during nephrogenic mesenchyme-to-epithelium transition. A: Kir6.1 and KvLQT mRNA in condensed mesenchyme and proximal S-shaped body cells. cDNA fragments specific for KvLQT (top) and Kir6.1 (bottom) were amplified by single-cell RT-PCR from cytosol (sb 1–4, cm 1–4) and extracellular samples (e 1–4) and from an RT− and a H\(_2\)O control, and PCR products were separated by polyacrylamide gel electrophoresis. B: summary of the experiments in A. Fractions are presented as n/m of PCR-positive samples (see legend to Fig. 6, E and F). C–F: Kir6.1 protein expression of condensed mesenchyme (C), of tubular ureteric bud and of globular aggregate (D), of S-shaped body (E), and of ureteric bud monolayer (F). Light micrographs of whole cultures stained immunohistochemically (pseudo dark-field imaging; bright staining indicates antibody binding). G and H: background staining of condensed mesenchyme (G) and ureteric bud monolayer (H) in control cultures. For these negative controls, the Kir6.1 antibody was saturated with specific antigen prior to incubation. a, Globular aggregate; cm, condensed mesenchyme; sb, S-shaped body; ub, ureteric bud. Bars = 20 μm.

Fig. 10. Summary of changes in expression patterns during mesenchyme-to-epithelium transition. Stages of transition are in top, corresponding to vertical lines. Shaded horizontal bars indicate relative expression of proteins. Frames indicate mRNA expression, and solid squares indicate current expression.
Whole cell currents of condensed mesenchyme and proximal S-shaped-body cells differed in conductance, reversal potential, and K⁺-selective tail currents. In addition, differing K⁺ channel types were apparent in outside-out patches of both developmental stages (summarized in Fig. 10). Whole cell currents in condensed mesenchyme cells, by the high fractional Cl⁻ currents, the depolarization- and time-dependently activating current component, and by the low reversal potential resemble very closely those of embryonic ureteric bud cells (17), the second primordium of the kidney. These current properties were absent in the proximal S-shaped-body cells (present study) and in the descendants of the ureteric bud cells, the collecting duct epithelium (13, 17).

Furthermore, in the present study, condensed mesenchyme but not proximal S-shaped body cells expressed Kir6.1 K⁺ channel mRNA and protein. Much like condensed mesenchyme, ureteric bud epithelium expresses Kir6.1 mRNA and protein that is downregulated during development of the collecting duct (Ref. 6 and unpublished observations). This further suggests that the same types of embryonic ion channels are expressed in branching morphogenesis of the collecting duct and mesenchyme-to-epithelium transition, which are simultaneously downregulated in both primordia-derived nascent nephron epithelia.

In the present study, Kᵢ,LQT mRNA was abundant in condensed mesenchyme and in proximal S-shaped body cells. Kᵢ,LQT interacts with IsK protein to form a low-conductance, cAMP-stimulated Kᵢ channel (5). These channels are expressed in the basolateral membrane of cells at the colonic crypt base where they maintain the driving force for Cl⁻ during cAMP-stimulated colonic Cl⁻ secretion (30). The expression of secretory-type channel mRNA during mesenchyme-to-epithelium transition might suggest that similar secretory processes occur during tubulogenesis of the mesenchyme-derived nephron segments.

During branching morphogenesis of lung and collecting duct epithelium, such Cl⁻ secretion is suggested by the development-specific expression of secretory Cl⁻ channels (15, 16, 22). In embryonic lung, a morphogenic action of secretion by the developing epithelium has been demonstrated directly (1). Therefore, Cl⁻ secretion might be a common feature of tubulogenesis and could contribute to build up and maintain the lumen of the embryonic tubular epithelium against tissue pressure. Luminal volume expansion might induce proliferation and longitudinal growth of the tubular epithelium. It is of interest in this context that a reactivation of probably embryonic programs of vectorial transport by mature renal epithelia is involved in the pathogenesis of renal cysts (27).

In conclusion, dissected metanephrogenic units consisting of ureteric buds with adherent mesenchymal blastema, explanted in primary culture, provide an in vitro model that allows experimental access to the cell physiology of metanephrogenic mesenchyme-to-epithelium transition. During this process, a set of embryonic ion channels in the plasma membrane of condensed mesenchyme is replaced by other channel types that might be involved in secretory processes of the nascent epithelium.

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