# Cellular localization of the potassium channel Kir7.1 in guinea pig and human kidney

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**Cellular localization of the potassium channel Kir7.1 in guinea pig and human kidney.**

*Background.* K$^+$ channels have important functions in the kidney, such as maintenance of the membrane potential, volume regulation, recirculation, and secretion of potassium ions. The aim of this study was to obtain more information on the localization and possible functional role of the inwardly rectifying K$^+$ channel, Kir7.1.

*Methods.* Kir7.1 cDNA (1114 bp) was isolated from guinea pig kidney (gpKir7.1), and its tissue distribution was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR). In addition, a genomic DNA fragment (6153 bp) was isolated from a genomic library. cRNA was expressed in *Xenopus laevis* oocytes for functional studies. Immunohistochemistry and RT-PCR were used to localize Kir7.1 in guinea pig and human kidney.

*Results.* The expression of gpKir7.1 in *Xenopus laevis* oocytes revealed inwardly rectifying K$^+$ currents. The reversal potential was strongly dependent on the extracellular K$^+$ concentration, shifting from $-14$ mV at 96 mmol/L K$^+$ to $-90$ mV at 1 mmol/L K$^+$. gpKir7.1 showed a low affinity for Ba$^{2+}$. Significant expression of gpKir7.1 was found in brain, kidney, and lung, but not in heart, skeletal muscle, liver, or spleen. Immunocytochemical detection in guinea pig identified the gpKir7.1 protein in the basolateral membrane of epithelial cells of the proximal tubule. RT-PCR analysis identified strong gpKir7.1 expression in the proximal tubule and weak expression in glomeruli and thick ascending limb. In isolated human tubule fragments, RT-PCR showed expression in proximal tubule and thick ascending limb.

*Conclusion.* Our results suggest that Kir7.1 may contribute to basolateral K$^+$ recycling in the proximal tubule and in the thick ascending limb.

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Potassium (K$^+$) channels in the kidney are responsible for several important processes. They maintain the negative membrane potential, regulate cell volume, recirculate K$^+$ across the luminal and basolateral membrane, and secrete K$^+$. Each tubular epithelial cell type appears to have a specific set of K$^+$ channels targeted to the basolateral or apical membrane. However, the functional role of the specific characteristics of the K$^+$ channels identified in the kidney is not yet clear. Several of the K$^+$ channels expressed in the kidney belong to the family of inward rectifier K$^+$ channels (Kir channels). Kir1.1 (also named ROMK), a K$^+$ channel showing weak inward rectification, is targeted to the luminal membrane of cortical collecting ducts (CCDs) [1] and is responsible for K$^+$ secretion and, therefore, for the maintenance of K$^+$ homeostasis. This channel has also been found in the luminal membrane of the thick ascending limb (TAL), where it recycles K$^+$ over this membrane, which is essential for the activity of the coupled Na$^+$-2Cl$^-$-K$^+$ transport into these cells [2]. Mutations in the Kir1.1 gene (*KCNJ1*) lead to the antenatal Bartter syndrome, an inherited disease associated with severe renal salt wasting [3, 4]. Other inwardly rectifying K$^+$ channels have been found in the basolateral membrane of the distal tubule (Kir4.1) and in the CCD (Kir2.3) [5, 6]. With Kir4.2 (also named Kir1.3) and Kir5.1, at least two further inwardly rectifying K$^+$ channels are strongly expressed in the kidney [7, 8]. Kir5.1 is expressed in the proximal and distal tubule [8], whereas the cellular localization of Kir4.2 is not yet known.

Recently, a novel inward rectifier channel, Kir7.1, has been described that has rather unique characteristics [9, 10]: It shows very weak inward rectification and has a very shallow dependence on external K$^+$. The expression of Kir7.1 in the brain appears to be restricted to the epithelial cells of the choroid plexus [9, 11]. It is also strongly expressed in various other epithelial cells, for

example, in small intestine or in follicular cells of the thyroid gland. The aim of the present study was to obtain more information on the cellular and subcellular localization and on the possible functional role of the inwardly rectifying $K^+$ channel Kir7.1 in the kidney.

## METHODS

### Cloning of guinea pig Kir7.1

A partial human EST clone (IMAGp998D20569; kindly supplied by the Max-Planck-Institute for Molecular Genetics, Berlin, Germany) was labeled with digoxigenin and used for nonradioactive screening of $5 \times 10^6$ plaque forming units of a λ-FIXII guinea pig genomic library (Stratagene, La Jolla, CA, USA). A single clone was isolated and purified by two further rescreenings. λ-DNA of the purified plaque was isolated with the Lambda Midi Kit (Qiagen, Hilden, Germany). Several restriction fragments were subcloned into pBluescript SK+ vectors, sequenced using an ABI 310 Sequencer (Applied Biosystems, Foster City, CA, USA), and manually assembled. Guinea pig Kir7.1 cDNA was amplified from kidney cDNA using primers located in the 5'- and 3'-nontranslated region, respectively (5'-AGAGAAA TACAGCCTGAG-3' and 5'-CCATTGATACGGTTTA CTCT-3'). PCR was performed using AmpliTaq Gold DNA-Polymerase (Applied Biosystems) at 94°C (denaturation, 30 sec), 50°C (annealing, 30 sec), and 72°C (elongation, 2 min) for 30 cycles. The resulting 1114 bp fragment was cloned into the *Xenopus laevis* oocyte transcription vector pSGEM and completely sequenced. The cDNA sequence and the isolated partial genomic sequence were submitted to GenBank under accession numbers AF200713 and AF200714.

### Electrophysiological analysis

For the expression in *Xenopus laevis* oocytes, capped run-off poly($A^+$) cRNA transcripts from linearized human Kir7.1 cDNA were synthesized, and approximately 6 ng were injected in defolliculated oocytes. Oocytes were prepared as described previously [12], incubated at 19°C in ND96 solution [96 mmol/L NaCl, 2 mmol/L KCl, 1 mmol/L $MgCl_2$, 1 mmol/L $CaCl_2$, 5 mmol/L 4-(2-hydroxyethyl)-1-piperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.4], supplemented with 100 μg/mL gentamicin and 2.5 mmol/L sodium pyruvate, and assayed at 48 hours postinjection. Two-electrode, voltage-clamp measurements were performed with a Turbo Tec-10 C amplifier (npi; Tamm, Germany), sampled through an EPC9 interface (Heka Electronics, Lambrecht, Germany) using PULSE/PULSEFIT software (Heka) on an Apple Macintosh computer, and data analysis was performed with IGOR software (WaveMetrics, Lake Oswego, WI, USA). For rapid exchange of external solutions, oocytes were placed in a small-volume perfusion chamber with a con-

stant flow of solutions. In the experiments investigating $K^+$ permeability, extracellular $K^+$ and $Na^+$ were mutually exchanged in order to keep the osmolarity constant.

### Tissue distribution of gpKir7.1

Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to test the expression of the *Kir7.1* gene in different tissues (heart ventricle, heart atrium, brain, kidney, lung, liver, spleen, and skeletal muscle) of the guinea pig. RNA from different tissues was isolated using a modified acid guanidinium method [13], and 2 μg of each RNA were reverse transcribed using Superscript II Reverse Transcriptase (GIBCO, Gaithersburg, MD, USA). Two gene-specific and intron-spanning primers were used to amplify a 268 bp fragment (5'-CTTTGGA GACGCAACTCA CCA-3' and 5'-ACACTGGTGAG AGGACTGTG-3'). The PCR was performed using AmpliTaq Gold DNA-Polymerase (Applied Biosystems) at 94°C (denaturation, 30 sec), 55°C (annealing, 30 sec), and 72°C (elongation, 1 min) for 35 cycles. In addition, the expression of the housekeeping enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was determined to check successful reverse transcription as described previously [14]. PCR products were analyzed on a 4% Nusieve agarose gel, and the specificity of the PCR product was verified by direct sequencing with the flanking primers.

### Isolation of human and guinea pig nephron segments and RT-PCR analysis of selected tubules

Human and guinea pig nephron segments were isolated using the procedure described previously for rat and rabbit kidney [15, 16]. Human nephron segments were isolated from healthy cortical kidney pieces of patients (with their written consent) undergoing tumor nephrectomy. Selected tubules of a total length of 200 mm or glomeruli (400 pieces) were lyzed in a 4 mol/L guanidinium chloride buffer, and total RNA was isolated using the RNeasy-kit (Qiagen). Isolated total RNA was incubated with 10 U DNase I (Promega, Heidelberg, Germany) at 37°C for one hour to digest isolated traces of genomic DNA. RNA and DNase I were then separated by an additional cleaning step using a new RNeasy column. cDNA first-strand synthesis was performed in a total reaction volume of 30 μL containing 5 μg total RNA, 10 mmol/L dNTP-Mix, 1 nmol/L p(dT)$_{10}$ nucleotide primer (Roche Diagnostics GmbH, Mannheim, Germany), and 200 U MMLV reverse transcriptase (Promega). One thirtieth of each cDNA first-strand reaction mixture was then subjected to a 50 μL PCR reaction using 20 pmol of each primer (5'-GAATGGTGATTTG GAACTAGA-3' and 5'-GCATAACTGGCTGGGTG TA-3', amplifying a 886 bp product from human Kir7.1) and 1 U of Taq DNA polymerase (Qiagen). Reaction conditions were as follows: 3 minutes at 94°C, 30 seconds

at 53°C, and 1 minute at 72°C, 1 cycle; 30 seconds at 94°C, 30 seconds at 53°C, and 1 minutes at 72°C, 30 cycles; 30 seconds at 94°C, 30 seconds at 53°C, and 10 minutes at 72°C, 1 cycle.

For guinea pig tubule segments, the One-Step PCR method (Qiagen) and intron-spanning primers (discussed previously in this article) were used. Reverse transcription (50°C for 30 min and 95°C for 15 min) and PCR were performed in one reaction tube. Reaction conditions for PCR were as follows: 30 seconds at 94°C, 30 seconds at 61°C, and 1 minute at 72°C, 35 cycles, and 9 minutes at 72°C, 1 cycle. PCR reaction products were analyzed by agarose gel electrophoresis. All positive signals obtained from PCR experiments with human and guinea pig cDNA were directly sequenced. Human and guinea pig GAPDH expression was used as a positive control for the PCR.

### Antiserum against recombinant Kir7.1

Polyclonal antibodies against the intracellular carboxy terminal portions of Kir7.1 (amino acids 293 to 360) were raised in two four- to five-month-old New Zealand white rabbits by immunization with fusion proteins derived from pGEX expression system following standard protocols [17]. Cross reactivities against the fusion part of the molecule were removed as described previously [18]. Cross reactivities against the other Kir channel subunits were removed by sequential adsorption to nitrocellulose strips loaded with the cross-reacting antigen [19]. Such monospecific antisera were finally affinity purified using nitrocellulose immobilized Kir7.1-GST protein as specific antigen. A titer of the purified antibody was determined by direct enzyme-linked immunosorbent assays (ELISAs). Affinity-purified antibodies were stored in aliquots at −20°C until further use. To verify the specificity of Kir7.1 antibodies, cell extracts of COS-7 cells transfected with hKir7.1-pcDNA3 or with the pcDNA3 vector only were analyzed by Western blotting as described previously [20].

### Immunohistochemistry

Six adult Hartley-Dunkin guinea pigs of either sex were killed by inhalation of fluothane, followed by transcardiac perfusion with rinsing solution, and subsequently, with phosphate-buffered formaldehyde (PBS; 2%)/saturated picric acid (15%). Tissues were dissected, cryoprotected, frozen, cut with a cryostat (Leica, Bensheim, Germany), and processed for routine indirect immunofluorescence as described in detail elsewhere [21]. The rabbit polyclonal antiserum was applied overnight at room temperature at a dilution of 1:200, and bound antibodies were visualized by incubation with fluorescein isothiocyanate-conjugated anti-rabbit Ig raised in goat (1:400; Diagnostic International, Schriesheim, Germany) for one hour. Sections were coverslipped in carbonate-buffered glycerol (pH 8.6) and evaluated by epifluorescence microscopy (BX 60; Olympus, Hamburg, Germany). Controls included (*1*) omission of primary antiserum, (*2*) replacement of primary antiserum by normal rabbit serum, and (*3*) preabsorption (overnight at 4°C) of the primary antiserum with the antigen used for immunization at a concentration of 40 mg/mL.

## RESULTS

### Molecular cloning

The inward rectifier channel Kir7.1 is strongly expressed in human kidney, as has been shown previously by Northern blot analysis [9, 11]. The aim of our work was to analyze the cellular and subcellular localization of the Kir7.1 channel protein in guinea pig and human kidney in order to get more information on its possible function. First, a guinea pig genomic library was screened and we isolated a genomic clone of 6153 bp containing the partial Kir7.1 gene (*KCNJ13*), including two thirds of the coding region split by a 2094 bp intron (GenBank accession number AF200714; Fig. 1B). A comparison of guinea pig and human genomic structure revealed that the position and length of the intron are strongly conserved, although sequence conservation of the intron is low. To get the full-length guinea pig ortholog of Kir7.1, the coding region was amplified by RT-PCR. The isolated 1114 bp fragment showed an open reading frame of 1080 bp encoding a 360 amino acid protein (GenBank accession number AF200713; Fig. 1A). A sequence comparison showed strong homology to human (93% identity) and rat (90% identity) Kir7.1 channels, but only low homology (<36% identity) to other Kir channels, unambiguously identifying the open reading frame as the guinea pig ortholog of Kir7.1 (gpKir7.1).

### Electrophysiological properties of gpKir7.1

When expressed in *Xenopus laevis* oocytes and measured with the two-electrode voltage clamp, gpKir7.1 gave rise to large inward currents with similar unusual properties as recently determined for rat Kir7.1 channels [9]. The dependence of the membrane conductance attributable to the opening of gpKir7.1 channels on the external $K^+$ concentration ($[K^+]_e$) was studied by varying $[K^+]_e$ from 1 to 96 mmol/L. Voltage-step and ramp protocols revealed a very shallow dependence of gpKir7.1 conductance on $[K^+]_e$ (Fig. 2 A, B). In other inward rectifier channels, the $K^+$ conductance is roughly proportional to the square root of $[K^+]_e$. The shape of the I-V relationship was also strikingly different from other, more classic Kir channels. At hyperpolarizing potentials, the slope conductances were nonsaturating and showed a unique curvature. At more depolarized potentials, gpKir7.1 carried a substantial outward $K^+$ flux, especially

**A**

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GATAGAGAAATACAGCCTGAGATGGAAAGCAGTAACTGTAAAGTTATTACTCCTCTCCTCAGTCAAAGACATCGA
                            M  E  S  S  N  C  K  V  I  T  P  L  L  S  Q  R  H  R

AGGATGGTCACCAAAGATGGCCATAGCACCCTTCAAACGGATGGCGCTCCAAGAGGCCTTGTGTATCTTCGGGAT
R  M  V  T  K  D  G  H  S  T  L  Q  T  D  G  A  P  R  G  L  V  Y  L  R  D

GCATGGGGGAACCCTAATAGACATGCGCTGGCGCTGGGTGATGCTAGTCTTTTCTGCCTCTTTCGTTCTCCACTGG
A  W  G  T  L  I  D  M  R  W  R  W  V  M  L  V  F  S  A  S  F  V  L  H  W

CTTGTCTTTGCAGTGCTCTGGTACGTTCTAGCCGAGATGAATGGTGATCTGGAACTAGATCACGATGCTCCACCT
L  V  F  A  V  L  W  Y  V  L  A  E  M  N  G  D  L  E  L  D  H  D  A  P  P

GAAAACCACACTATCTGTGTCAAGTACATCACCAGTTTCACAGCTGCATTTTCCTTCTCTTTGGAGACGCAACTC
E  N  H  I  I  C  V  K  Y  I  T  S  F  T  A  A  F  S  F  S  L  E  T  Q  L

ACCATTGGTTATGGTACCATGTTCCCCAGTGGTGACTGTCCAAGTGCAATCGCCTTACTTGCCATACAAATGCTC
T  I  G  Y  G  T  M  F  P  S  G  D  C  P  S  A  I  A  L  L  A  I  Q  M  L

CTAGGCCTCATGCTAGAGGCTTTTATCACAGGTGCCTTTGTGGCGAAGATAGCCCGCCCAAAGAATCGAGCCTTT
L  G  L  M  L  E  A  F  I  T  G* A  F  V  A  K  I  A  R  P  K  N  R  A  F

TCAATTCGCTTTACTGACTTAGCAGTGGTAGCTCACAGAGATGGCAAACCTAATCTTATCTTTCAAGTGGCCAAC
S  I  R  F  T  D  L  A  V  V  A  H  R  D  G  K  P  N  L  I  F  Q  V  A  N

ATTCGACACAGTCCTCTCACCAGTGTCCGGGTCTCCGCTGTTCTCTATCAGGAAAGAGAAAACGGCCAACTGCAC
T  R  H  S  P  L  T  S  V  R  V  S  A  V  L  Y  Q  E  R  E  N  G  Q  L  H

CAGACCAGCGTGGACTTTCACCTTGATGGCATCAGTTCAGAGGAATGTCCGTTCTTTATCTTTCCACTAACCTAC
Q  T  S  V  D  F  H  L  D  G  I  S  S  E  E  C  P  F  F  I  F  P  L  T  Y

TACCATTCCATTACACCATCAAGTCCTCTGGTCACTCTGCTGCAGCATGAAAATCCTCCCCACTTTGAATTAGTT
Y  H  S  I  T  P  S  S  P  L  V  T  L  L  Q  H  E  N  P  P  H  F  E  L  V

GTATTCCTCTCAGCAATGCAGGAAGGCACTGGAGAAATCTGCCAAAGGAGGACATCCTACCTACCATCAGAGATC
V  F  L  S  G  M  Q  E  G  T  G  E  I  C  Q  R  R  T  S  Y  L  P  S  E  I

ATGTTACATCATTGTTTTGCATCTCTGCTGACCCGAGGTTCCAAAGGTGAATATAAAGTCAAGATGGAGAATTTT
M  L  H  H  C  F  A  S  L  L  T  R  G  S  K  G  E  Y  K  V  K  M  E  N  F

GACAAGACTGTTCCTGAACTTCCAACTCCTCTGGTGTCTAAGAGCCCACACAGGACTGACCTGGATATCCGTATC
D  K  T  V  P  E  L  P  T  P  L  V  S  K  S  P  H  R  T  D  L  D  I  R  I

AATGGACAAAGCATTGACAATTTTCAGATCTCGGAAACAGGACTGACAGAGTAAACCGTATCAATGG
N  G  Q  S  I  D  N  F  Q  I  S  E  T  G  L  T  E  *
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**B**

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                              Intron
GCTTTTATCACAGgtaatttgtctttgtt...2094bp...gtggtttctaatttagGTGCCTTTGTGGCGAAG


              ...GCTTTTATCACAGGTGCCTTTGTGGCGAAG...
                 A  F  I  T  G* A  F  V  A  K
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**Fig. 1. Sequence analysis of gpKir7.1.** (*A*) cDNA and amino acid sequence of gpKir7.1. The codon of glycine-154 (labeled with an asterisk) is split by an intron. Transmembrane regions (frames) and pore region (dotted frame) are highlighted. (*B*) Genomic sequence of the guinea pig Kir7.1 gene (*KCNJ13*) around the splice sites (bold). Coding regions are shown in capital letters, and the intron sequence is in small letters.

at low [K$^+$]$_e$ (Fig. 2B). However, the zero-current potentials closely coincided with $E_K$, as predicted from the Nernst equation, and followed [K$^+$]$_e$ with a slope of approximately 54 mV per decade, indicative of a highly selective K$^+$ channel (Fig. 2C). The sensitivity of gpKir7.1 to the K$^+$ channel blocker Ba$^{2+}$ was exceptionally low with a calculated $K_i$ of 750 μmol/L, which is 25 to 150 times higher than for most other Kir channels (Fig. 2D). Furthermore, the block by external Ba$^{2+}$ ions showed a very weak voltage dependence, which is in marked contrast to other Kir channels.

**Tissue distribution and cellular localization of Kir7.1**

To clarify the tissue distribution of Kir7.1 in guinea pig, we amplified a specific fragment of the gpKir7.1 by RT-PCR. Intron-spanning primers were used to avoid contamination with genomic DNA. As a positive control, the expression of the housekeeping enzyme GAPDH was determined. Of the tissues tested, only brain, kidney, and lung showed a significant gpKir7.1 expression; no expression could be detected in heart (ventricle and atrium), liver, spleen, and skeletal muscle (Fig. 3).

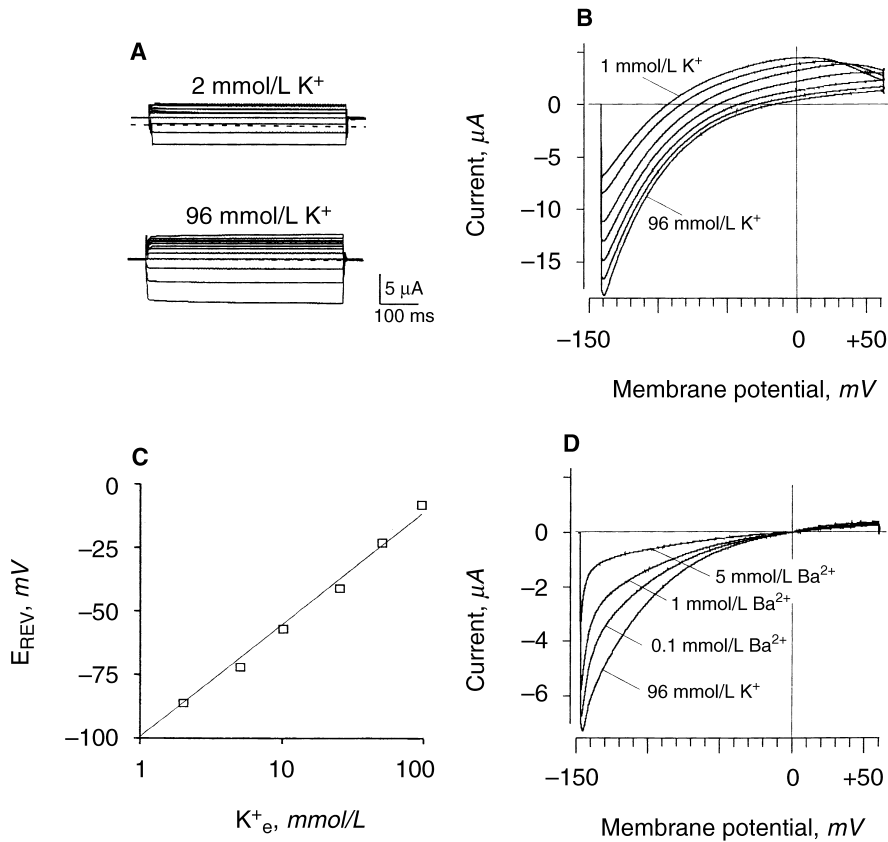To clarify the cellular localization of Kir7.1 in guinea

**Fig. 2. Characterization of macroscopic gpKir7.1 inwardly rectifying currents in *Xenopus laevis* oocytes.** (*A*) Current response of an oocyte expressing gpKir7.1 to brief voltage steps from a holding potential of $V_h = 0$ mV to potentials between 80 mV and −140 mV. The dotted line indicates zero current. The external $K^+$ concentration was 1 or 96 mmol/L as indicated. (*B*) gpKir7.1 currents in response to fast voltage ramp show outward currents and shift of reversal potentials with different external $K^+$ concentrations; $[K^+]_e$ was 1, 2, 5, 10, 25, 50, or 96 mmol/L. (*C*) Zero-current potentials (reversal potentials, $E_{REV}$) of gpKir7.1, which are in close agreement with $E_K$, are plotted vs. $[K^+]_e$ on a semilogarithmic scale. The solid line is a linear regression fit to the data. (*D*) Current responses to voltage ramps of two seconds duration between −150 mV and 60 mV show the inhibition of Kir7.1 by 0.1, 1, and 5 mmol/L $Ba^{2+}$, respectively.
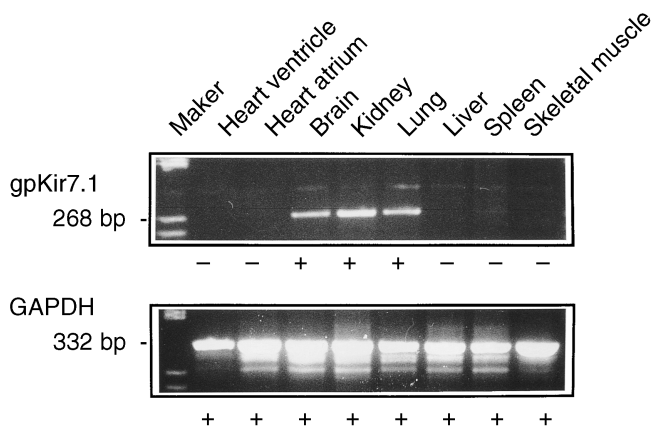


**Fig. 3. Tissue distribution of Kir7.1 in guinea pig determined by RT-PCR with intron-spanning primers.** A 268 bp fragment was amplified and visualized on a 4% Nusieve agarose gel (upper panel). The housekeeping enzyme GAPDH expression (332 bp fragment) was used as a control (lower panel). A pBR322/*HaeIII* marker is shown on the left.

pig kidney, different tubular segments and glomeruli were isolated and analyzed by RT-PCR. Robust expression of Kir7.1 was found in proximal tubules (PTs). Weak signals could be detected in glomeruli (GLO) and TAL, and no signals were found in CDs (Fig. 4, lower panel). A similar RT-PCR approach was used to analyze the ex-

pression of Kir7.1 in human kidney (Fig. 4). The expression of human Kir7.1 was exclusively found in the PT and in the TAL. No PCR products were obtained in isolated glomeruli and CDs.

## Immunocytochemical localization of Kir7.1 channels in the guinea pig kidney

To investigate the subcellular expression of the Kir7.1 protein in the kidney, a specific antiserum against a C-terminal part of human Kir7.1 was raised in rabbits. In Western blots probed with these Kir7.1 antibodies, a signal of approximately 50 kD was detected in COS-7 cells transfected with Kir7.1 but not in mock-transfected cells (Fig. 5), which confirmed the specificity of the antibody. Immunocytochemical studies were carried out in isolated guinea pig kidney. Intense, specific Kir7.1 immunolabeling was observed for cells of the PT (Fig. 6). The weaker labeling of intercalated cells of the CD (Fig. 6B) was still present after preabsorption of the antiserum with its corresponding antigen and, therefore, was considered nonspecific. Kir7.1 immunolabeling of tubular cells started abruptly at the glomerulus, whereas the cells of Bowman's capsule were negative (Fig. 6C). Immunolabeling of epithelial cells was restricted to the basolateral membrane, including the basal folds, while the apical
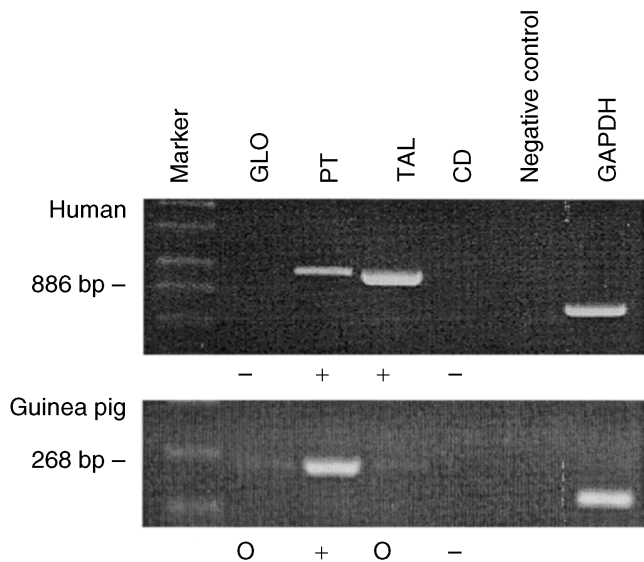
**Fig. 4. Polymerase chain reaction (PCR) products of the Kir7.1 K⁺ channel in isolated human and guinea pig glomeruli and tubules.** Lane 1, PCR DNA marker (Biometra, Göttingen, Germany). Lane 2, glomeruli (GLO). Lane 3, proximal tubules (PT). Lane 4, thick ascending limbs (TAL). Lane 5, collecting ducts (CD). Lane 6, negative control. Lane 7, GAPDH, positive control. Note that mRNA for Kir7.1 could be amplified in human isolated PTs and TALs and in guinea pig glomeruli, isolated PTs, and TALs. (+) Strong expression. (o) Weak expression. (−) No expression. All PCR products were sequenced.

membrane facing the tubular lumen was not labeled by the antiserum (Fig. 6D).

## DISCUSSION

A number of different K⁺ channels are expressed in various segments of the nephron at specific locations. Each of them is likely to be involved in specific functions, such as (*1*) maintenance of the negative membrane potential necessary for Na⁺-driven transport; (*2*) regulation of cell volume; and (*3*) recirculation of K⁺ across the luminal or the basolateral membrane. In the CCD, K⁺ is secreted, which plays an important role in K⁺ homeostasis. In the PT, K⁺ is reabsorbed, but the major part of reabsorption is thought to occur via paracellular pathways [22, 23]. Nevertheless, several K⁺ channels have been identified in epithelial cells of the PT, either by molecular biological or by electrophysiological techniques, which may also make a substantial contribution to net transepithelial K⁺ transport. In the luminal membrane of the PT, three different K⁺ channels have been found so far: (*1*) a cGMP-inhibited, depolarization-activated K⁺ channel that transports K⁺ as well as NH₄⁺, and is most likely responsible for the maintenance of a negative potential across the luminal membrane [24]; (*2*) a Ca²⁺-dependent "maxi" K⁺ channel, which is known to be involved in the volume regulation of kidney cells [25, 26]; and (*3*) a member of the tandem pore domain
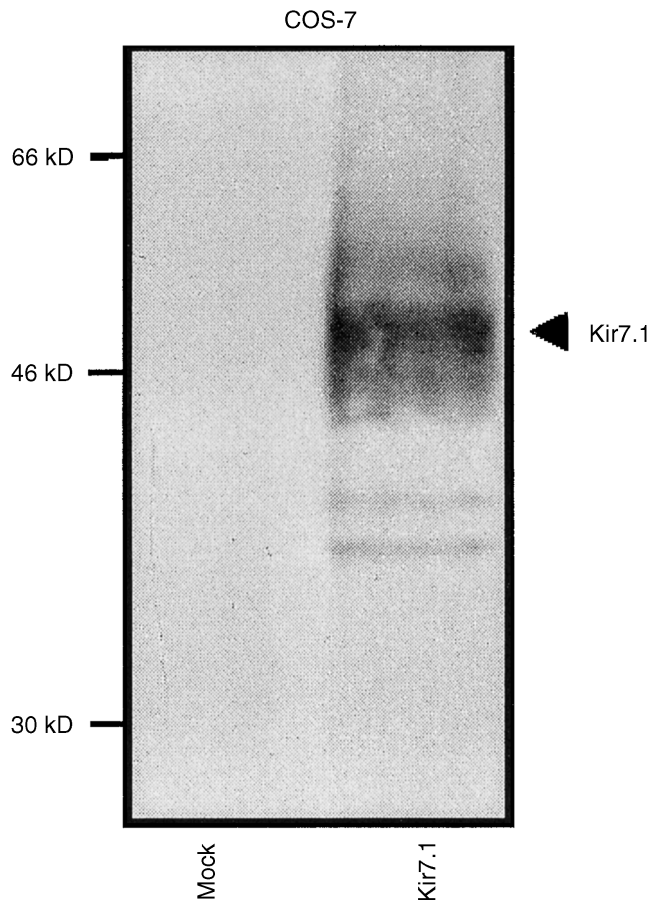


**Fig. 5. Western blot of COS-7 cell extracts transfected with hKir7.1-pcDNA3 (right lane) or the vector only (left lane).** The blot was probed with rabbit anti-Kir7.1 (1:2000), and a band of approximately 50 kD was detected only in hKir7.1 transfected cells. Bands between 30 and 46 kD may result from proteolytic degradation.

K⁺ channel family, TWIK-1 [27], in which the function remains to be elucidated. In the basolateral membrane of the PT, an inwardly rectifying adenosine 5′-triphosphate (ATP)-sensitive K⁺ channel has been characterized electrophysiologically [28, 29]. This channel is thought to be necessary for pump-leak coupling to the Na⁺,K⁺-ATPase [11, 28, 30].

We have identified a second K⁺ channel, Kir7.1, in the basolateral membrane of the PT and in the TAL. The electrophysiological properties of gpKir7.1 described here are in good agreement with the data reported previously for human Kir7.1 [9, 10]. The most striking characteristic of the Kir7.1 channel is the very low dependence of K⁺ permeation on external K⁺. In other inwardly rectifying K⁺ channels, a reduction of the external K⁺ concentration from 100 to 4 mmol/L is associated with a decrease in conductance of approximately 80%, whereas in the case of Kir7.1, this is associated with a decrease of only approximately 30% [9]. Thus, in comparison with other inwardly rectifying K⁺ channels, Kir7.1 allows for a rela-
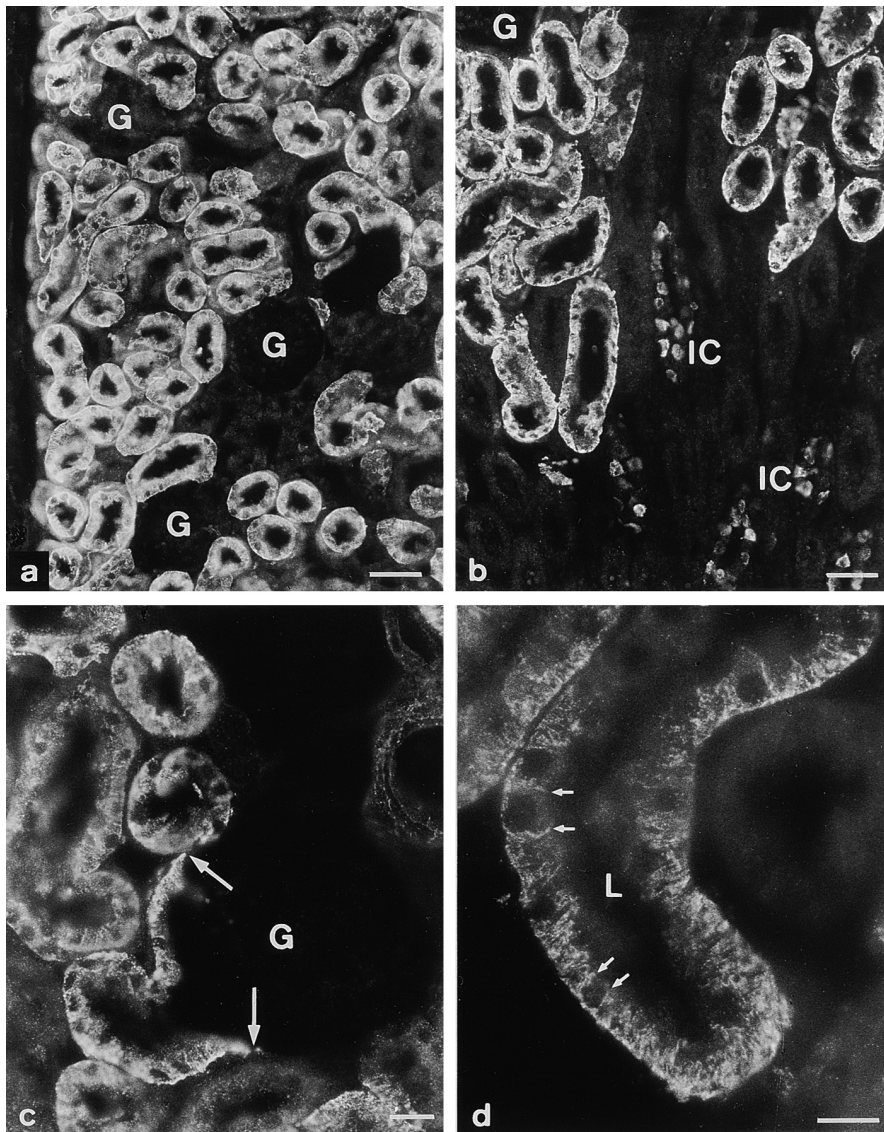
**Fig. 6. Immunocytochemistry of guinea pig kidney.** (*A*) Kir7.1 immunoreactivity is seen in proximal tubules (PTs) but not in glomeruli (G). (*B*) Corticomedullary border: single cells, probably intercalated cells (IC) of collecting ducts (CDs), are weakly labeled. This labeling persisted after preabsorption (data not shown) and, hence, is considered as nonspecific. (*C*) While the flattened capsular cells of Bowman's capsule are negative, epithelial immunolabeling starts abruptly with the tubular epithelial cells (*arrows*). G, glomerulus. (*D*) Higher magnification of a PT reveals basolateral membrane immunolabeling that ends shortly before the plasma membrane reaches the luminal cell surface, indicated by arrows at two individual cells. L, tubular lumen. Bar represents 50 μm in A and B and 20 μm in C and D.

tively large K$^+$ efflux at low external K$^+$ concentrations. Furthermore, at physiological external K$^+$ concentrations, the weak inward rectification observed with symmetrical K$^+$ concentrations is converted to an almost linear current-voltage relationship between $-100$ and 0 mV. Thus, Kir7.1 appears to be particularly well suited to mediate K$^+$ efflux.

The maintenance of unidirectional Na$^+$ transport across epithelia requires K$^+$ recycling across the membrane in which the Na$^+$,K$^+$-ATPase is localized. Interestingly, a recent study has shown that Kir7.1 is colocalized with the Na$^+$,K$^+$-ATPase in thyroid follicular cells, intestinal epithelial cells, and epithelial cells of the choroid plexus [11]. In thyroid follicular cells and intestinal epithelial cells, both Kir7.1 and Na$^+$,K$^+$-ATPase are on the basolateral side, whereas in choroid plexus, both are on the luminal side. All of these epithelia are actively involved

in ion transport. Thus, it is tempting to speculate that in the basolateral membrane of the PT, where expression of Na$^+$,K$^+$-ATPase is very strong, Kir7.1 and the Na$^+$,K$^+$-ATPase may form a functional unit providing for unidirectional Na$^+$ transport associated with K$^+$ recycling.

Our immunocytochemical data suggest that the Kir7.1 channel proteins are evenly distributed over the entire basolateral membrane, including the intercellular clefts. This finding creates obvious difficulties for the classic picture of K$^+$ reabsorption in the PT, which is assumed to take place mainly via paracellular pathways [23]. In a widely accepted model, Weinstein tried to account for the large K$^+$ reabsorption in the PT by assuming that a net K$^+$ uptake into the tubular epithelial cell occurs in a restricted space at the lateral clefts via the Na$^+$,K$^+$-ATPase and a net K$^+$ efflux occurs at the basal membrane [23, 31]. This is difficult to reconcile with the appar-
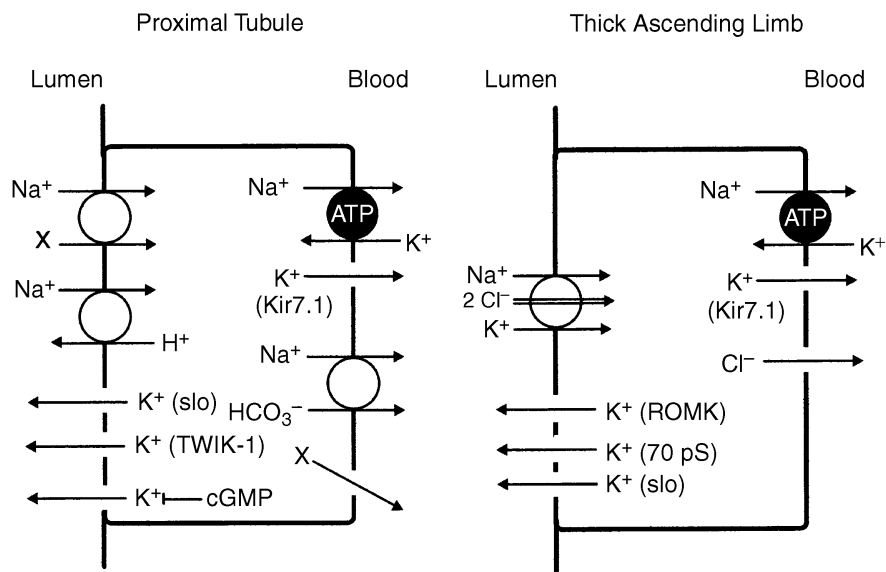
**Fig. 7. Simplified scheme of mechanisms contributing to K$^+$ transport in the proximal tubule (PT) and the thick ascending limb (TAL).** X = glucose, amino acids, PO$_4^-$, SO$_4^-$.

ently homogenous distribution of Kir7.1 channels across the entire basolateral membrane (Fig. 6D). Clearly, further research is required to elucidate the molecular and cellular mechanisms underlying transepithelial K$^+$ transport in the PT. A scheme of the components known to contribute to K$^+$ transport in the PT is shown in Figure 7.

Our RT-PCR experiments on tubular fragments of human and guinea pig kidney showed a significant expression of Kir7.1 in the PT and in the TAL. In contrast, our immunocytochemical analysis in guinea pig kidney provided no evidence for expression in TAL. However, it should be noted that the sensitivity of the RT-PCR analysis is usually higher than that of immunohistochemical approaches and that expression of the Kir7.1 protein in guinea pig TAL cannot be excluded on the basis of the immunocytochemical data. Taken together, our results suggest that Kir7.1 is expressed at a low level in human and guinea pig TAL.

The polar distribution of K$^+$ channels in the TAL is similar to that of the PT (Fig. 7). In the luminal membrane, three different K$^+$ channels have been found, Kir1.1 (ROMK), the "maxi" K$^+$ channel (slo), and a 70 pS channel that has not yet been cloned [32–37]. Kir1.1 and the 70 pS channel provide for K$^+$ recirculation across the luminal membrane, and the Ca$^{2+}$-dependent maxi K$^+$ channel may be involved in volume regulation. Our RT-PCR results are consistent with the idea that in human TAL, Kir7.1 plays a similar role as in the PT; that is, it serves to recirculate K$^+$ ions across the basolateral membrane.

In conclusion, we have cloned the inwardly rectifying Kir7.1 K$^+$ channel from guinea pig kidney and demonstrated its localization in the basolateral membrane of the PT. We have shown that in human kidney Kir7.1 is

expressed in the PT and in the TAL. In the guinea pig, Kir7.1 mRNA can be found in the PT, the TAL, and the glomeruli. Our immunocytochemical data suggest that the channel is targeted to in the basolateral membrane of the PT. The localization and the electrophysiological properties of Kir7.1 suggest that this channel may be involved in basolateral K$^+$ recycling in the proximal tubule and the thick ascending limb.

## ACKNOWLEDGMENTS

## APPENDIX

Abbreviations used in this article are: CCD, cortical collecting duct; CD, collecting duct; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; gpKir7.1, guinea pig ortholog of Kir7.1; HEPES, 4-(2-hydroxyethyl)-1-piperazine-N′-2-ethanesulphonic acid; RT-PCR, reverse transcription-polymerase chain reaction; PT, proximal tubule; TAL, thick ascending limb.

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