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Alternative Splicing of Human Inwardly Rectifying K^+ Channel ROMK1 mRNA

HIDEKI YANO, LOUIS H. PHILIPSON, JAY L. KUGLER, YOSHIHARU TOKUYAMA, ELIZABETH M. DAVIS, MICHELLE M. LE BEAU, DEBORAH J. NELSON, GRAEME I. BELL, and JUN TAKEDA

Howard Hughes Medical Institute (H.Y., G.I.B.) and Departments of Biochemistry and Molecular Biology (H.Y., G.I.B., J.T.), Medicine (L.H.P., Y.T., E.M.D., M.M.L.B., G.I.B.), Neurology (D.N.), and Pharmacology and Physiology (J.K.), The University of Chicago, Chicago, Illinois 60637

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SUMMARY

Recent studies have identified a new family of inwardly rectifying K^+ channels, members of which are known by the acronyms ROMK1, IRK1, and GIRK1. We have isolated cDNAs encoding the human homologue of ROMK1 from an adult kidney cDNA library. The sequences of the human kidney ROMK1 cDNA clones indicated that they were derived from at least two types of mRNAs, human ROMK1A and human ROMK1B, differing in sequence at their 5′ ends. The isolation of the human ROMK1 gene, localized to chromosome band 11q24 by fluorescence *in situ* hybridization, indicated that the different ROMK1 transcripts were generated by alternative splicing. Human ROMK1A mRNA was predicted to encode a protein of 389 amino acids, having 93% identity with the 391-residue rat ROMK1 protein, and expression studies in *Xenopus* oocytes indicated that it encoded a Ba^{2+}-sensitive inwardly rectifying K^+ channel with properties similar to those reported for cloned rat ROMK1. Human ROMK1B mRNA was predicted to encode a protein of 372 amino acids whose sequence was truncated at the amino terminus but otherwise identical to that of the human ROMK1A protein. Translation of human ROMK1B mRNA was predicted to initiate at a codon corresponding to Met-18 of human ROMK1A mRNA. Reverse transcriptase-polymerase chain reaction amplification of human kidney mRNA revealed human ROMK1A and -B transcripts as well as a third type of transcript, human ROMK1C mRNA, which was predicted to encode a protein identical to human ROMK1B. Human ROMK1A, -B, and -C transcripts were identified in kidney, whereas only human ROMK1A mRNA could be detected in pancreatic islets and other tissues in which human ROMK1 was expressed at low levels. Thus, tissue-specific alternative splicing of human ROMK1 mRNA may result in the expression of a family of ROMK1 proteins.

Potassium channel currents are critical to the regulation of electrical activity in both excitable and nonexcitable cells (1–3). The inward rectifier class of K^+ channels regulate the resting potential and membrane excitability in a variety of cell types. Recently an inwardly rectifying K^+ channel (ROMK1) has been cloned from the inner stripe of the outer medulla of rat kidney (4). Analysis of the sequence of the rROMK1 protein suggested a novel molecular architecture, in that there were only two predicted membrane-spanning domains rather than the six domains seen in voltage-, calcium-, and cyclic nucleotide-gated K^+ channels (5). However, the rROMK1 protein showed strong homology with these other K^+ channels in the putative pore-forming H5-like region, suggesting that the ion selectivity pore itself may be well conserved. Two other inwardly rectifying K^+ channels (designated IRK1 and GIRK1) having similar structural organization were cloned from a mouse macrophage cell line (6) and rat heart (7), respectively, indicating that these inwardly rectifying K^+ channels comprised a new superfamily of K^+ channel proteins. Herein, we report the isolation and characterization of hROMK1 cDNA clones and gene and show that tissue-specific alternative splicing generates a family of structurally related transcripts and proteins.

Materials and Methods

General methods. Standard procedures were carried out as described by Sambrook *et al.* (8). Double-strand DNA sequencing was done by the dideoxynucleotide chain-termination procedure (9), after appropriate DNA fragments were subcloned into pGEM-3Z (Promega, Madison, WI).

Isolation of hROMK1 cDNA clones. A probe for hROMK1 was generated using the PCR (10), as follows. An aliquot (~10^7 plaque-forming units) of an adult human kidney cDNA library in λgt10 (11) was amplified using primers 5′-GCACAGTCAAGGTTTATATTCT-TTGTGGACATCTGGA-3′ and 5′-TGGGTCTAGAGATCTTGGCT-

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ABBREVIATIONS: ROMK1; rROMK1, rat ROMK1; hROMK1, human ROMK1; PCR, polymerase chain reaction; sRNA, synthetic mRNA; SDS, sodium dodecyl sulfate; RT, reverse transcriptase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DAPI, 4,6-diamidino-2-phenylindole dihydrochloride; bp, base pair(s); kb, kilobase(s).

AAAATGGCACCACACAT-3′, corresponding to nucleotides 321–357 and 669–705, respectively, of the rROMK1 cDNA sequence (4). PCR amplification was carried out for 30 cycles of denaturation at 94° for 1 min, annealing at 55° for 1 min, and extension at 72° for 1 min. The PCR products were separated on a 1% low-melting-temperature agarose gel, and fragments in the region of 385 bp were cloned into the *Hinc*II site of pGEM-3Z and sequenced. The sequence of one clone, hROMK-0, had 86% nucleotide sequence identity with the rROMK1 cDNA sequence, suggesting that it represented the human homologue of this sequence. The insert in phROMK-0 was ^{32}P-labeled by nick translation and used as a probe to screen the adult human kidney cDNA library by hybridization. Hybridization conditions were as follows: 50% formamide, 5× SSPE, 10× Denhardt's solution, 1.4% SDS, 100 μg/ml sonicated and denatured salmon testis DNA, and 1×10^6 cpm/ml ^{32}P-labeled probe, at 42° for 15–20 hr. Filters were washed in 0.1× standard saline citrate, 0.1% SDS, at room temperature for 30 min and then at 60° for 30 min and were exposed overnight to X-ray film at −80°, with an intensifying screen.

RNA blotting and RT-PCR. The tissue distribution of hROMK1 mRNA was determined by RNA blotting using a human multiple tissue Northern blot (Clontech, Palo Alto, CA), which included 2 μg of poly(A)$^+$ RNA from heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. The blot was hybridized with ^{32}P-labeled cDNA (nucleotides 1–2489) and washed at a final stringency of 0.1× standard saline citrate, 0.1% SDS, at 60° for 30 min before exposure to X-ray film. For RT-PCR analysis of hROMK1 mRNA expression, cDNA was prepared from 1 μg of total RNA from adult human heart, kidney, liver, spleen, and pancreatic islets, using RT and oligo(dT) priming.

Five nanograms of cDNA were used as a template for PCR. The following primers were used for RT-PCR: A, 5′-CAAGTGGAGATACAAAATGA-3′(nucleotides 470–489); B, 5′-ATATTCTCCACACAGGAGT-3′ (nucleotides 600–619); C, 5′-GCATCAAAGGTGCAGGGACT-3′(nucleotides46–65);D,5′-TGGATACATTTGGATTTCTC-3′ (nucleotides 179–198); and E, 5′-CTTTGGAGACTAGCCTTGCT-3′ (nucleotides 362–381). Human GAPDH mRNA was used as an internal control and was amplified using primers 5′-GTGAACCATGAGAAGTATGA-3′ and 5′-GCCATCACGCCACAGTTTCC-3′, corresponding to nucleotides 463–482 and 638–657, respectively, of the cDNA sequence deposited in GenBank (accession number M33197); these primers span two small introns, of 92 bp and 193 bp, respectively, in the GAPDH gene (12). The PCR was carried out in the presence of [α-^{32}P]dCTP for 30 cycles of denaturation at 95° for 30 sec, annealing at 55° or 60° (hROMK1 cDNA) or at 60° (GAPDH cDNA) for 1 min, and extension at 72° for 30 sec. The products were separated on a 5% polyacrylamide gel, which was dried and exposed to X-ray film.

Expression of hROMK1 in *Xenopus* oocytes. The hROMK1A cDNA clone phROMK-1 (pGEM-3Z vector, nucleotides 1–2489) was linearized with *Sal*I, and run-off sRNA was prepared using T7 RNA polymerase. The hROMK1B channel was constructed for expression in *Xenopus* oocytes as follows: primer A, 5′-GGTACCGTTGACAGAAAGTATGTTC-3′ (nucleotides 284–308, with initial *Kpn*I site), and the reverse primer B, 5′-GTGATTGGCAGAAGGATGG-3′ (nucleotides 581–599), were used with hROMK1A-pGEM template and amplified with PCR conditions of 94° for 1 min, 56° for 30 sec, and 72° for 30 sec, for 30 cycles. The 316-bp PCR product was cloned into the pCR™II vector (Invitrogen, San Diego, CA), sequenced, excised with *Kpn*I and *Eco*RI, and subcloned into hROMK1A (*Eco*RI-*Sac*I fragment)-pBluescript II SK (Stratagene, La Jolla, CA). sRNA was made by *in vitro* transcription with T7 RNA polymerase. Fifty nanoliters of sRNA solution in distilled water, at a concentration of 1 μg/μl, were injected into defolliculated *Xenopus* oocytes, which were incubated at 18° in OR-2 medium (13) containing 5 μg/ml gentamicin (GIBCO, Grand Island, NY) and 2% horse serum. Currents were recorded, using a two-microelectrode voltage clamp (Warner Instruments, Hamden, CT), from oocytes 18 hr to 4 days after injection (14). Microelectrodes filled with 3 M KCl had resistances of 0.5–1.2 MΩ. Initial measurements were made in OR-2 medium without gentamicin or horse serum, followed by perfusion with a high-K$^+$ solution to maximize currents.

Isolation of the hROMK1 gene. A human genomic library in λFIX™II (catalogue number 946203; Stratagene) was screened by hybridization with the 2.4-kb ^{32}P-labeled insert from phROMK-1, as described above. To isolate the region containing exons 1 and 2, the inverse PCR method (15) was used. Briefly, 0.5 μg of human DNA was partially digested with *Sau*3AI, self-ligated, and PCR amplified with primers for exons 1 and 2, 5′-ACCAGATTCTCGATGTGAGC-3′ and 5′-ATTTGGATTTCTCAACATTT-3′, corresponding to nucleotides 67–86 and 179–198 (Fig. 1A), respectively. Amplification was carried out for 30 cycles of denaturation at 94° for 1 min, annealing at 50° for 1 min, and extension at 72° for 1.5 min. The PCR products were separated on a 1% low-melting point agarose gel, extracted from the gel, and reamplified under the conditions described above. The inverse PCR products were ligated into the *Hinc*II site of pGEM-3Z and sequenced. The intron 1 sequence present between the two primers in exons 1 and 2 was determined by subsequent PCR amplification of this region with primers in the 3′ flanking intron and 5′ untranslated region.

Chromosomal localization of the hROMK1 gene. The chromosomal location of the hROMK1 gene (gene symbol, *KCNJ1*) was determined by PCR amplification of DNA from a panel of reduced rodent/human somatic cell hybrids containing different human chromosomes National Institute of General Medical Sciences (NIGMS) Human/Rodent Somatic Cell Hybrid Mapping Panel 2; Coriell Institute for Medical Research, Camden, NJ). Briefly, 50 ng of genomic DNA were amplified using primers 5′-AGATGAACCAACCAATCAAT-3′ and 5′-GACATAGTAACATTCTGACT-3′, corresponding to nucleotides 1898–1917 and 2298–2317 (Fig. 1A), respectively, for 30 cycles of denaturation at 95° for 1 min, annealing at 55° for 1 min, and extension at 72° for 1 min. The PCR products were separated by 1% agarose gel electrophoresis and visualized by staining with ethidium bromide. Fluorescence *in situ* chromosomal hybridization was performed as described previously (16). Human metaphase cells were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. The hROMK1 genomic clone λhgROMK-1, containing a 18-kb insert, was labeled with biotin by nick translation using biotin-11-dUTP (Enzo Diagnostics, New York, NY). Hybridization was detected with fluorescein-conjugated avidin (Vector Laboratories, Burlingame, CA). Chromosomes were identified by staining with DAPI.

Results and Discussion

Isolation of hROMK1 cDNA clones. Using primers based on the sequence of the rROMK1 cDNA (4) and an adult human kidney cDNA template, a 311-bp fragment was amplified by PCR. The sequence of this PCR product, hROMK-0, showed 86% nucleotide identity with rROMK1 cDNA, suggesting that it corresponded to part of the hROMK1 mRNA. An adult human kidney cDNA library was screened by hybridization using the ROMK1-like PCR product as a probe. Eight clones were identified and three, λhROMK-l, -5, and -8, were sequenced. Overlapping segments of λhROMK-1 and λhROMK-5 generated a composite hROMK1A cDNA sequence of 2489 bp.[1] The longest open reading frame in this sequence begins at nucleotide 252, assuming that translation is initiated at this methionine codon, and has the potential to encode a protein of 389 amino acids (hROMK1A, M_r 44,615) (Fig. 1A); this reading frame is immediately preceded by an in-frame translation termination codon. The hROMK1A cDNA sequence also contains four ATG codons in the putative 5′ untranslated region (Fig.

[1] The nucleotide sequence reported in this paper has been submitted to the GenBank/EMBL Data Bank, with accession number U03884.

A

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ATCAACAGGGCCTCGGGTACCCTCACCTAGCATATCCAAACTCTTGCATCAAAGGTGCAGGGACTTGCTCACATCGAGAATCTGGTTGCTTTCTTGGAGACCAAGAAAATGAGTTTTTG  119

TTTCTACATTTACTCCAGCAATCCATGAG GACTTTATAGGAATTTTGCACCATTCTGAATGGATACATTTGGATTTCTCAACATTTGTTCAGCTTCCTAATGACTGTTGTGACAATTG  237
                            ↑
       (Met Gly Ala Ser Glu Arg Ser Val Phe Arg Val Leu)        Ala             Arg          20              Arg     Phe
           Met Pro Thr Val Tyr Leu Cys Ser Glu Gln Ile Arg Val Leu Thr Glu Ser Met Phe Lys His Leu Arg Lys Trp Val
CTCTATACCAGTGA  ATG CCA ACT GTT TAT CTC TGC TCT GAA CAG ATC AGG GTG TTG ACA GAA AGT ATG TTC AAA CAT CTT CGG AAA TGG GTC  329
Ile     His Ile         Arg                      40↑          Glu                     50                  Asp
Val Thr Arg Phe Phe Gly His Ser Arg Gln Arg Ala Arg Leu Val Ser Lys Asp Gly Arg Cys Asn Ile Glu Phe Gly Asn Val Glu Ala
GTC ACT CGC TTT TTT GGG CAT TCT CGG CAA AGA GCA AGG CTA GTC TCC AAA GAT GGA AGG TGC AAC ATA GAA TTT GGC AAT GTG GAG GCA  419
            60                                  70                                  80 Val             M 1
Gln Ser Arg Phe Ile Phe Phe Val Asp Ile Trp Thr Thr Val Leu Asp Leu Lys Trp Arg Tyr Lys Met Thr Ile Phe Ile Thr Ala Phe
CAG TCA AGG TTT ATA TTC TTT GTG GAC ATC TGG ACA ACG GTA CTT GAC CTC AAG TGG AGA TAC AAA ATG ACC ATT TTC ATC ACA GCC TTC  509
            90     Leu                         Val 100         Val                         110 Tyr     Pro Glu     Arg
Leu Gly Ser Trp Phe Phe Phe Gly Leu Leu Trp Tyr Ala Val Ala Tyr Ile His Lys Asp Leu Pro Glu Phe His Pro Ser Ala Asn His
TTG GGG AGT TGG TTT TTC TTT GGT CTC CTG TGG TAT GCA GTA GCG TAC ATT CAC AAA GAC CTC CCG GAA TTC CAT CCT TCT GCC AAT CAC  599
            120                 Met             130                         H 5     140                 Phe
Thr Pro Cys Val Glu Asn Ile Asn Gly Leu Thr Ser Ala Phe Leu Phe Ser Leu Glu Thr Gln Val Thr Ile Gly Tyr Gly Phe Arg Cys
ACT CCC TGT GTG GAG AAT ATT AAT GGC TTG ACC TCA GCT TTT CTG TTT TCT CTG GAG ACT CAA GTG ACC ATT GGA TAT GGA TTC AGG TGT  689
            150                             160             M 2             170
Val Thr Glu Gln Cys Ala Thr Ala Ile Phe Leu Leu Ile Phe Gln Ser Ile Leu Gly Val Ile Ile Asn Ser Phe Met Cys Gly Ala Ile
GTG ACA GAA CAG TGT GCC ACT GCC ATT TTT CTG CTT ATC TTT CAG TCT ATA CTT GGA GTT ATA ATC AAT TCT TTC ATG TGT GGG GCC ATC  779
            180                             190                             200
Leu Ala Lys Ile Ser Arg Pro Lys Lys Arg Ala Lys Thr Ile Thr Phe Ser Lys Asn Ala Val Ile Ser Lys Arg Gly Gly Lys Leu Cys
TTA GCC AAG ATC TCC AGG CCC AAA AAA CGT GCC AAG ACC ATT ACG TTC AGC AAG AAC GCA GTG ATC AGC AAA CGG GGA GGG AAG CTT TGC  869
            210                             220                             230         Ile
Leu Leu Ile Arg Val Ala Asn Leu Arg Lys Ser Leu Leu Ile Gly Ser His Ile Tyr Gly Lys Leu Leu Lys Thr Thr Val Thr Pro Glu
CTC CTA ATC CGA GTG GCT AAT CTC AGG AAG AGC CTT CTT ATT GGC AGT CAC ATT TAT GGA AAG CTT CTG AAG ACC ACA GTC ACT CCT GAA  959
            240                 Thr             250                             260
Gly Glu Thr Ile Ile Leu Asp Gln Ile Asn Ile Asn Phe Val Val Asp Ala Gly Asn Glu Asn Leu Phe Phe Ile Ser Pro Leu Thr Ile
GGA GAG ACC ATT ATT TTG GAC CAG ATC AAT ATC AAC TTT GTA GTT GAC GCT GGG AAT GAA AAT TTA TTC TTC ATC TCC CCA TTG ACA ATT 1049
        Ile 270                             280             Ser             290
Tyr His Val Ile Asp His Asn Ser Pro Phe Phe His Met Ala Ala Glu Thr Leu Leu Gln Gln Asp Phe Glu Leu Val Val Phe Leu Asp
TAC CAT GTC ATT GAT CAC AAC AGC CCT TTC TTC CAC ATG GCA GCG GAG ACC CTT CTC CAG CAG GAC TTT GAA TTA GTG GTG TTT TTA GAT 1139
            300                             310                             320             Val
Gly Thr Val Glu Ser Thr Ser Ala Thr Cys Gln Val Arg Thr Ser Tyr Val Pro Glu Glu Val Leu Trp Gly Tyr Arg Phe Ala Pro Ile
GGC ACA GTG GAG TCC ACC AGT GCT ACC TGC CAA GTC CGG ACA TCC TAT GTC CCA GAG GAG GTG CTT TGG GGC TAC CGT TTT GCT CCC ATA 1229
            330                             340         Gly             350
Val Ser Lys Thr Lys Glu Gly Lys Tyr Arg Val Asp Phe His Asn Phe Ser Lys Thr Val Glu Val Glu Thr Pro His Cys Ala Met Cys
GTA TCC AAG ACA AAG GAA GGG AAA TAC CGA GTG GAT TTC CAT AAC TTT AGC AAG ACA GTG GAA GTG GAG ACC CCT CAC TGT GCC ATG TGC 1319
            360         Ala                 370                             Val         380     Glu
Leu Tyr Asn Glu Lys Asp Val Arg Ala Arg Met Lys Arg Gly Tyr Asp Asn Pro Asn Phe Ile Leu Ser Glu Val Asn Glu Thr Asp Asp
CTT TAT AAT GAG AAA GAT GTT AGA GCC AGG ATG AAG AGA GGC TAT GAC AAC CCC AAC TTC ATC TTG TCA GAA GTC AAT GAA ACA GAT GAC 1409
    Gln 389
Thr Lys Met OC
ACC AAA ATG TAA CAGTGGCTTTTCAACGGGAGTAAAGCAAAGTCTCTAAAGCTCCTAGTACCTAGAAGCATTATGAAGCAGTCAACAATTTAGGGGTACGAAAGTAGGATGAGAG 1524
CCTTCAAAGTCTACCAGCACAAAGACCCCTGAGCCCCGCAATTGTGATCCCACAAGACATGCATCTCCACAAGGCTACTGTATTAGAACGTGCAATGCATTTATATGAAACTGGTGTAT 1643
GGAAGACATAGGTGCTCTCTTGAAATCTTAAATATGATTATTTGAGCTCATATAAGGTGGATTGGAGCAGATAAAATTATCAAAAGTTTCATGAACAGGCCAAACAAAATATTTTTTAA 1762
AGTTTCCTTAAAGAAGTTATGAACTTTAGAAAGGATCAGGGGACAATAATAATCTCATTTTGATTCTACTGATAAGAATGACTCCACTTTTAATGTGGACTTTTACTCATGGAAAAATT 1881
GTCTCCTAATTTGGGGAGATGAACCAACCAATCAATGACAAGAAAACGCTTACACAAAGAACAATTTGAGGCTCTAAGCTTCTCATGTGGTACGTTTAGACAGAGGCTAAATCTGCACA 2000
CTAGAATCTTGATGATACCTTCCTGCAAGACAGAATGCTTTAGTTAAAAGTGGTGATGATATTTCTTTCAATCTGTATTGGATGGCTTAAAGGGCTATAAATCTGTTTATAAAGAGCAT 2119
TTCCTGCTCTTCGAAGACAGCAATGAGGAGTTGGAAGGTGCAAAGTCAGTAGAGAAGGGAATGTATCATTAATGCACCTGAGAAGAAACAGTTTCATGTGTTCCTCCACCTAGAGTTTG 2238
TACTGGAATGCTATTTCTAAAGAAGAAGTGGGAAAGAGAGAGGAATGGGATGGAGCCCCACAGTCAGAATGTTACTATGTCTTTCTTTCCCTGACAGCCCATCTTCCTAAAAGGGACCA 2357
GCTTATGGAAGGCTCGACCTTGAGGGGAAAGTTTTACTGTGAAAGTCTTCTTCAGATCCCCACCTGCATCATTCCGAATGTGTCCTGGAAAAAAACTGGTACTCAAAGCTGCTTAGGAA 2476
TCAAAATGTTTTC                                                                                                          2489
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B

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TCACCTAGCATATCCAAACTCTTGCATCAAAGGTGCAGGGACTTGCTCACATCGAGAATCTGGTTGCTTTCTTGGAGACCAAGAAAATGAGTTTTTGTTTCTACATTTACTCCAGCAAT  119
                          1
                          Met Phe Lys His Leu Arg Lys Trp Val Val Thr Arg
CCATGAG ATCAGGGTGTTGACAGAAAGT ATG TTC AAA CAT CTT CGG AAA TGG GTC GTC ACT CGC .........
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C

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TGGATACATTTGGATTTCTCAACATTTGTTCAGCTTCCTAATGACTGTTGTGACAATTGCTCTATACCAGTGAATGCCAACTGTTTATCTCTGCTCTGAACAG GTTTCTTCTCTACTG  118
                                                                                                        ↑
TGCCTTCATCCCCATCCTGGGCCCTGACAAAGACAGTGTGACAAGTTCTGAGCCCCACAGGTGGAATCCCAAACCAAGCAGCTCTTGCTGGTCACCCCAAGATCTACTTATACATGAAG  237
                                                  1
                                                  Met Phe Lys His Leu Arg Lys Trp Val Val Thr Arg
TTTTGAAAATCATCTCCTGAACCTCTTCTGACAGAG ATCAGGGTGTTGACAGAAAGT ATG TTC AAA CAT CTT CGG AAA TGG GTC GTC ACT CGC .........
                                     ↑
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Fig. 1. Nucleotide and predicted amino acid sequences of the inwardly rectifying K^+ channel hROMK1 cDNA. A, Sequence of ROMK1A cDNA. Amino acid residues that differ in rROMK1 are shown above the human sequence. *Arrows*, positions of introns that interrupt the hROMK1 gene in the 5′ untranslated region and between the codons for amino acids Gln-10 and Ile-11. The membrane-spanning domains M1 (residues 81–103) and M2 (residues 154–178) and the pore-forming H5-like domain (residues 128–152) are also noted. The cDNA inserts in λhROMK-5 and λhROMK-1 included nucleotides 1–581 and 576–2489, respectively. The PCR product hROMK-0 included nucleotides 454–764. B, Sequence of the 5′ untranslated region of hROMK1B cDNA including the putative initiating methionine, corresponding to the codon for Met-18 of the hROMK1A cDNA sequence. *Arrow*, location of the internal deletion of 133 bp, corresponding to exon 2. C, Sequence of the 5′ untranslated region of hROMK1C cDNA. The fragment of hROMK1C mRNA was obtained by PCR with primers D and E. *Arrows*, boundaries of the region corresponding to exon 3. The initiating methionine for hROMK1C transcripts corresponds to Met-18 of hROMK1A. Translation initiation and termination codons identified in the 5′ untranslated region are *underlined* and *overlined*, respectively.

1A, *underlined*), all of which are followed closely by a termination codon (Fig. 1A, *overlined*).

The sequence of the cDNA insert in λhROMK-8 had an internal 133-bp deletion spanning nucleotides 149–281, the boundaries of which are at the positions of introns in the hROMK1 gene. Translation of this ROMK1 transcript, designated as hROMK1B mRNA, is predicted to begin at the codon corresponding to Met-18 of hROMK1A mRNA. There is an in-frame translational termination codon upstream of this putative initiating methionine codon. Thus, hROMK1B mRNA encodes a protein of 372 amino acids (M_r 42,664) that is structurally related to hROMK1A but that lacks the 17 amino-terminal residues (Fig. 1B). The other five λ clones encoded a region that is common to both hROMK1A and hROMK1B sequences.

There is 93% amino acid sequence identity between the sequences of hROMK1A and rROMK1. However, there is no homology between the sequences of the amino termini of these two proteins (Fig. 1A). In fact, the sequences diverge at the site of an intron in the human gene, suggesting that there may be a form of hROMK1 having an amino terminus homologous to that of the rat protein, although we have not identified such a sequence.

Analysis of the sequences of hROMK1A and -B indicates that both are likely to have a structure similar to that proposed for members of the inwardly rectifying superfamily of K^+ channels, with intracellularly disposed amino and carboxyl termini and two membrane-spanning segments, M1 and M2, between which is located an H5-like domain that is homologous to the pore-forming region of voltage-gated K^+ channels (4, 5). rROMK1 is a glycoprotein (4). The putative *N*-linked glycosylation site located in the putative extracellular loop connecting the M1 and H5 segments is conserved in the human and rat proteins, suggesting that hROMK1 may also be glycosylated.

Tissue distribution. The tissue distribution of hROMK1 mRNA was examined by RNA blotting and RT-PCR analyses. RNA blotting studies using poly(A)$^+$ RNA from various human tissues showed a single transcript of ~3.0 kb in human kidney (Fig. 2). This result contrasts with similar studies of rROMK1 expression, which showed the presence of multiple kidney transcripts varying in size from 2.2 to 12 kb (4). There was no detectable hybridization of the hROMK1 probe to poly(A)$^+$ RNA from heart, brain, placenta, lung, liver, skeletal muscle, or pancreas.

RT-PCR was also used to study the tissue distribution of hROMK1 mRNA. Using primers A and B from the large exon that includes most of coding region of the hROMK1 gene (exon 4 in our nomenclature) and that is common among hROMK1A and -B mRNAs, a 150-bp RT-PCR product was observed in all of the tissues examined, with the highest levels of this product being in kidney and pancreatic islets (Fig. 3). Similar RT-PCR analysis of GAPDH mRNA expression showed similar levels of this mRNA in these tissues (Fig. 3). The primers used to amplify the 195-bp GAPDH-specific PCR product span two small introns and would be expected to generate a fragment of 480 bp on amplification of chromosomal DNA. A DNA fragment of this size was not observed, indicating that the PCR products observed in Fig. 3 were cDNA derived and not generated by amplification of small amounts of chromosomal DNA present in the RNA preparations.

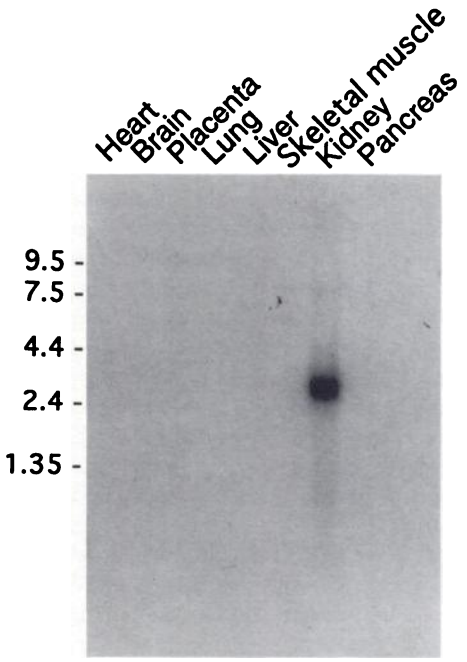

Fig. 2. Tissue distribution of hROMK1 mRNA determined by RNA blotting. Each lane contains 2 μg of poly(A)$^+$ RNA from various tissues. The single transcript of 3.0 kb seen in kidney was evident after an overnight exposure, and there was no detectable hybridization to transcripts in the other tissues even after a 7-day exposure. The sizes of internal RNA standards (kb) are shown on the *left*.

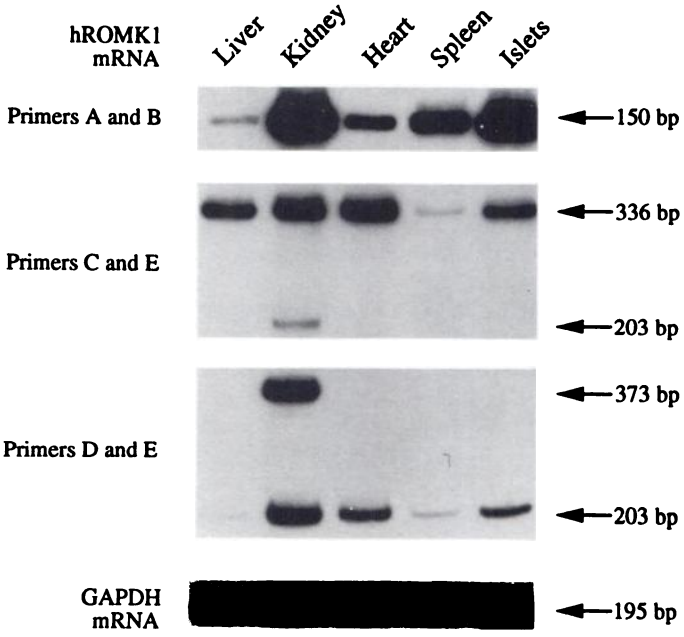

Fig. 3. Tissue distribution of hROMK1 mRNA determined by RT-PCR. The various PCR fragments derived from hROMK1A, hROMK1B, hROMK1C, and GAPDH mRNAs are shown. The sizes of PCR products are shown on the *right*. *Top*, RT-PCR products obtained using primers A and B; *second panel*, products obtained using primers C and E; *third panel*, products obtained using primers D and E; *bottom*, RT-PCR of GAPDH mRNA.

To examine the tissue distribution of alternatively spliced ROMK1 transcripts described above, RT-PCR was also carried out using primers specific for ROMK1A and -B mRNAs. The forward primers were selected from the region of the 5′ untranslated region upstream of the deleted segment present in

ROMK1A mRNA (primer C) and from the deleted region itself (primer D), and the reverse primer was from the large common coding exon (primer E). Using primers C and E, the 336-bp product derived from hROMK1A mRNA was observed in all of the tissues examined, whereas an additional fragment derived from hROMK1B mRNA was seen only in kidney (Fig. 3, *second panel*). Using primers D and E, a 203-bp fragment derived from hROMK1A mRNA was seen in all tissues (note that this 203-bp segment has a different sequence, compared with the fragment of identical size generated by RT-PCR of kidney RNA with primers C and E). In addition to this common band, there was an extra larger fragment of 373 bp evident in RNA from kidney, suggesting additional, alternatively spliced, ROMK1 transcripts in this tissue. This 373-bp PCR product was cloned and sequenced (Fig. 1C). The sequence showed the presence of a 170-bp insert between codons 10 and 11 of the hROMK1A cDNA sequence (Fig. 1C). As a consequence of this insertion, hROMK1C mRNA would be expected to encode a protein identical to hROMK1B. Thus, alternative splicing in kidney appears to generate a family of related mRNAs that encode structurally related proteins, of 389 and 372 amino acids, that differ only in the sequence at their amino termini.

Expression of hROMK1 in *Xenopus* oocytes. The functional properties of hROMK1A were determined after expression of this protein in *Xenopus* oocytes. Currents were recorded in OR-2 medium containing 2.5 mM KCl, 1–3 days after injection of hROMK1A sRNA. To enhance detection of K^+ channel currents, recordings were also made in high-external KCl solutions. The activity of rROMK1 is blocked in the presence of external Ba^{2+} (3). This property was used in this study to separate hROMK1A currents from endogenous Ba^{2+}-insensitive inwardly rectifying K^+ currents and Ca^{2+}-activated Cl^- currents. Fig. 4 shows that oocytes injected with hROMK1A sRNA and studied in 50 mM KCl solution expressed an inwardly rectifying current of 8.0 ± 0.9 μA (n = 6, V_m = −160 mV). The membrane potential of the voltage-clamped oocytes was held at 0 or −60 mV and then stepped to potentials between −160 and 50 mV. In noninjected oocytes, voltage steps to −160 mV infrequently elicited a small inward current that was not reduced in amplitude in the presence of 5 mM extracellular $BaCl_2$. Currents in most uninjected oocytes were negligible, similar to those shown in Fig. 4A. Oocytes injected with hROMK1A sRNA displayed a large increase in conductance in an inward direction (Fig. 4B), which was blocked by superfusion of the oocyte with $BaCl_2$ (Fig. 4C). The peak amplitude of the Ba^{2+}-sensitive current at −160 mV in 50 mM K^+ solution was 6.5 ± 0.6 μA for three oocytes. As seen in Fig. 4C, some injected oocytes expressed a significant amount of outward current that was Ba^{2+} insensitive and may be attributable to an endogenous Ca^{2+}-activated Cl^- current. The current-voltage relationships for currents in the presence and absence of $BaCl_2$ for an uninjected oocyte and an oocyte expressing hROMK1A are shown in Fig. 5.

The selectivity of hROMK1A was determined in experiments in which shifts in current reversal potential were determined as a function of changes in external K^+ concentration, for the Ba^{2+}-sensitive current component. Experiments were carried out in solutions in which the extracellular Na^+ was isosmotically replaced with K^+. The reversal potential of the Ba^{2+}-selective current varied linearly with changes in the logarithm of the external K^+ concentration (−78 mV with 2.5 mM K^+,

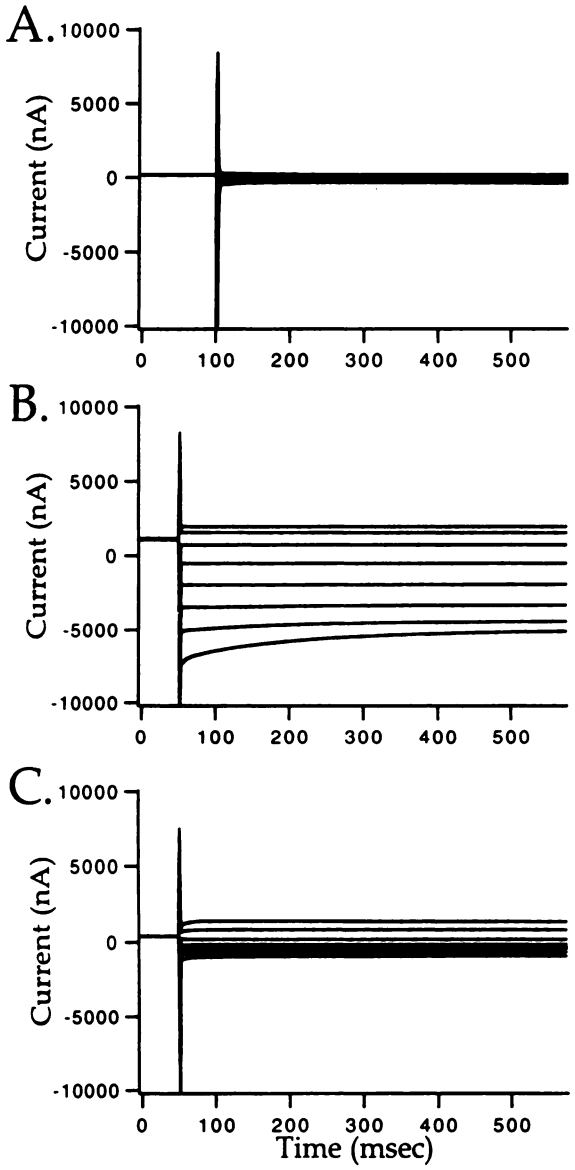

Fig. 4. Expression of hROMK1A in *Xenopus* oocytes. A, Current recording elicited from an uninjected control oocyte. B, Currents recorded from an oocyte injected with hROMK1A sRNA. C, Currents recorded from the same oocyte as in B, in 5 mM $BaCl_2$. All currents were recorded in OR-2 solution containing 50 mM KCl, 48 mM NaCl, 0.3 mM $CaCl_2$, and 0.5 mM $MgCl_2$; 5 mM $BaCl_2$ was added to this solution without regard for osmolarity. Currents were elicited in response to voltage steps to −160, −130, −100, −70, −40, −10, 20, and 50 mV; V_h was 0 mV.

−63 mV with 10 mM K^+, −35 mV with 20 mM K^+, and −13 mV with 50 mM K^+, for a representative oocyte), as would be predicted from the Nernst potential for a perfectly K^+-selective channel. Data from studies of oocytes expressing hROMK1A (n = 13) gave a 51-mV change in reversal potential/10-fold change in external K^+ concentration. Oocytes injected with RNA prepared from the hROMK1B cDNA were also found to give Ba^{2+}-sensitive currents that were indistinguishable from those described for hROMK1A.[2]

Exon-intron organization of the hROMK1 gene (*KCNJ1*). The sequences of human kidney hROMK1 cDNAs suggested that hROMK1 mRNA was alternatively spliced. The

[2] R. Martin, D. Hanck, and L. H. Philipson, unpublished observations.

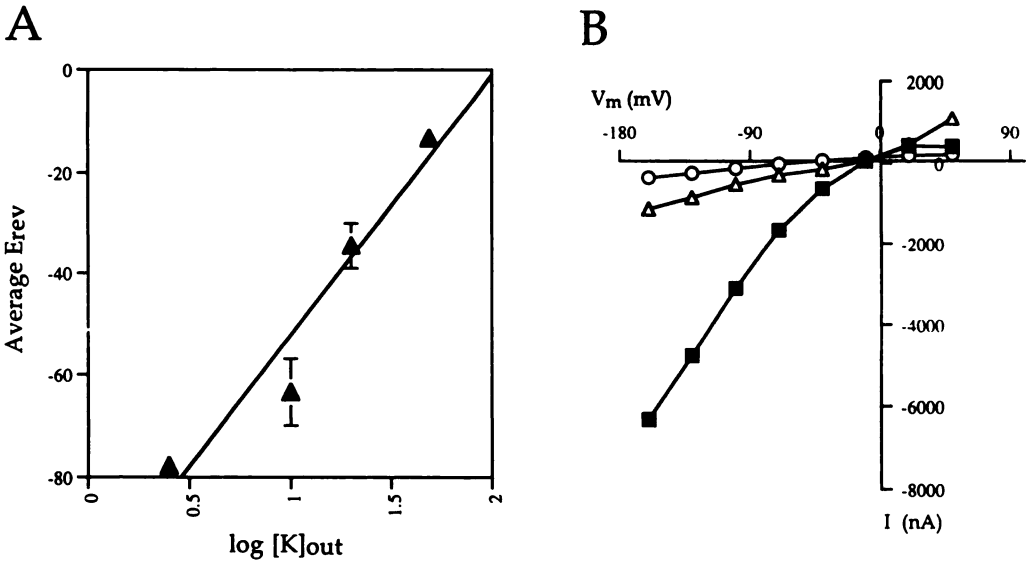

Fig. 5. Current-voltage relationship and K^+ selectivity. A, Dependence on external potassium concentrations of reversal potentials of Ba^{2+}-sensitive currents. Potassium was replaced by equimolar sodium, such that $[K]_{out} + [Na]_{out} = 98$ mM ($n = 13$). The data were fitted with a line with slope of >51 mV, demonstrating high ionic selectivity for K^+ over Na^+. B, Current-voltage curve for Ba^{2+}-sensitive currents (■), Ba^{2+}-insensitive currents in the same oocyte (Δ), and an uninjected control oocyte (○). All currents were recorded in 50 mM K^+.

hROMK1 gene (gene symbol, *KCNJ1*) was isolated from a λ phage library by hybridization with a ^{32}P-labeled hROMK1 cDNA probe. Ten positive clones were isolated and two of these clones, λhgROMK-1 and λhgROMK-7, spanned a region of ~30 kb (Fig. 6A). The partial sequence of the hROMK1 gene revealed a small exon encoding a part of the 5′ untranslated region of hROMK1C mRNA (exon 3) and a large exon beginning at the codon for Ile-11 and including the remainder of the protein-coding and 3′ untranslated regions of hROMK1 mRNA (exon 4). Exons corresponding to nucleotides 1–281 of the hROMK1A cDNA sequence were not present in λhgROMK-1, λhgROMK-7, or the other genomic clones and were not identified on rescreening of the library. Therefore, the technique of inverse PCR (15) was used to clone these exons. As shown in Fig. 6 and Table 1, the results suggest that *KCNJ1* consists of four exons and three introns. hROMK1A mRNA is generated from exons 1, 2, and 4, ROMK1B mRNA is from exons 1 and 4, and ROMK1C mRNA is from exons 2, 3, and 4 (Fig. 6B).

Chromosomal localization of *KCNJ1*. The chromosomal location of *KCNJ1* was determined by examination of its segregation in a panel of reduced human/rodent somatic cell hybrids and by fluorescence *in situ* hybridization. The hybrid cell studies indicated that the 420-bp *KCNJ1*-specific PCR product was observed only in cell line NA1092A, which contains human chromosome 11 (data not shown).

The assignment of *KCNJ1* to chromosome 11 was confirmed and its regional localization was determined using the technique of fluorescence *in situ* hybridization. Hybridization of biotin-labeled λhgROMK-1 DNA to human metaphase chromosome resulted in specific labeling only of chromosome 11 (Fig. 7). Specific labeling of 11q23-q25 was observed on four (17 cells) or three (8 cells) chromatids of the chromosome 11 homologues in 25 cells examined. Of 92 signals observed (92 of 100 11q chromatids from 25 metaphase cells were labeled), 67 (73%) were located at 11q24, two (2%) were located at the 11q23-q24 junction, and 13 (14%) were located at the 11q24-q25 junction. The remaining 10 signals were located at 11q23 (2.2%) or 11q25 (8.9%). Specific labeling of 11q24 was obtained in an additional hybridization experiment using this probe. These results localized *KCNJ1* to chromosome band 11q24.

Concluding remarks. The present study indicates that tissue-specific alternative splicing of hROMK1, one member of the superfamily of inwardly rectifying K^+ channels, may generate a family of structurally related proteins that differ in sequence at their amino termini. The consequences of sequence differences at the amino terminus of hROMK1 for its func-

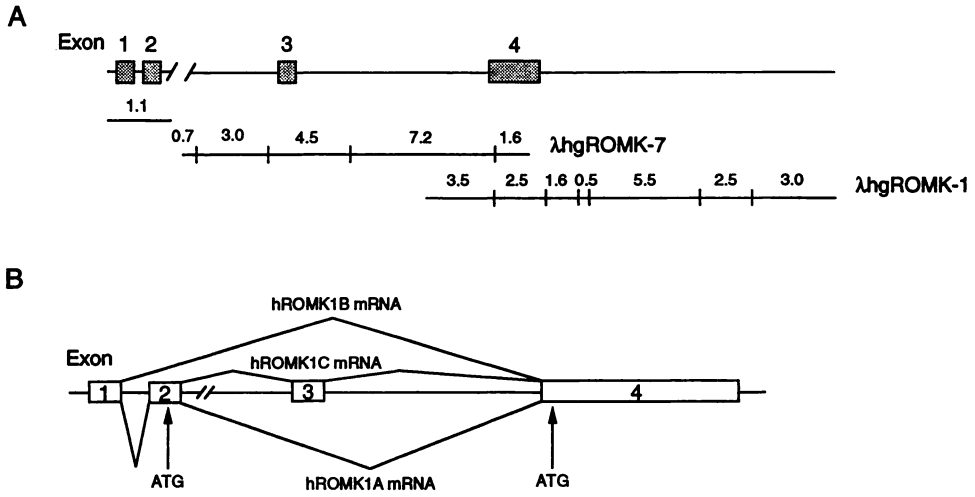

Fig. 6. Map of the hROMK1 gene, *KCNJ1*. A, Positions of exons (shown as *boxes*) and the structures of the two overlapping segments of the gene cloned in λ phage are indicated. *Vertical lines*, natural *Eco*RI sites. Sizes (in kb) of each *Eco*RI fragment are indicated. Exons 1 and 2 were cloned by inverse PCR. B, Schematic representation showing the pattern of alternative splicing of hROMK1 mRNAs. Exons are shown as *boxes* and numbered *1* to *4*. The putative ATG triplets at which translation is initiated are noted.

TABLE 1

Exon-intron organization of the hROMK1 gene (*KCNJ1*)

Exon and intron sequences are in capital and lower-case letters, respectively.

Exon	Exon size	Sequence at exon-intron junction		Intron size
		5′ Splice donor	3′ Splice acceptor	
	bp			*Kb*
1	>148	CATGAG gtaagaggagaaaca	cattttctgatgtag GACTTT	0.5
2	133	GAACAG gtatggagcccccct	gttgtattttaccag GTTTCT	>4
3	170	ACAGAG gtgagtggcttgatt	ttgatgtgttttcag ATCAGG	10.5
4	>2208			

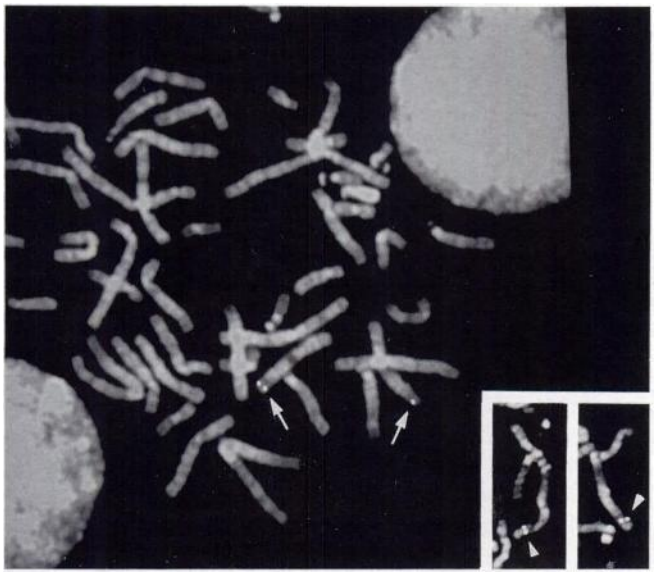

Fig. 7. Fluorescence *in situ* hybridization of biotin-labeled λhgROMK-1 DNA to human metaphase cells obtained from phytohemagglutinin-stimulated peripheral blood lymphocytes. A partial metaphase cell is shown. *Arrows*, chromosome 11 homologues. Specific labeling was observed at 11q24. *Inset*, two chromosome 11 homologues with specific labeling at 11q24 (*arrowheads*). Images were obtained using a Zeiss Axiophot microscope coupled to a cooled charge-coupled device camera. Separate images of DAPI-stained chromosomes and the hybridization signal were merged using image analysis software (Image 1.47 and GeneJoin).

tional properties or regulation are not completely clear. Because the channel properties of hROMK1A and rROMK1 proteins appear very similar despite the differences in sequence of the amino-terminal 10–12 residues, the sequence differences in this region may affect the properties of this inwardly rectifying K^+ channel in other ways, such as its disposition within the cell, its regulation, or its interaction with other proteins. The availability of cDNA clones encoding different forms of ROMK1 will allow these hypotheses to be tested directly.

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Send reprint requests to: Jun Takeda, Howard Hughes Medical Institute, University of Chicago, 5841 S. Maryland Ave., MC1028, Chicago, IL 60637.