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Two highly homologous members of the ClC chloride channel family in both rat and human kidney

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ABSTRACT **We have cloned two closely related putative Cl^- channels from both rat kidney (designated rClC-K1 and rClC-K2) and human kidney (hClC-Ka and hClC-Kb) by sequence homology to the ClC family of voltage-gated Cl^- channels. While rClC-K1 is nearly identical to ClC-K1, a channel recently isolated by a similar strategy, rClC-K2 is 80% identical to rClC-K1 and is encoded by a different gene. hClC-Ka and hClC-Kb show ≈90% identity, while being ≈80% identical to the rat proteins. All ClC-K gene products are expressed predominantly in the kidney. While rClC-K1 is expressed strongly in the cortical thick ascending limb and the distal convoluted tubule, with minor expression in the S3 segment of the proximal tubule and the cortical collecting tubule, rClC-K2 is expressed in all segments of the nephron examined, including the glomerulus. Since they are related more closely to each other than to the rat proteins, hClC-Ka and hClC-Kb cannot be regarded as strict homologs of rClC-K1 or rClC-K2. After injection of ClC-K cRNAs into oocytes, corresponding proteins were made and glycosylated, though no additional Cl^- currents were detectable. Glycosylation occurs between domains D8 and D9, leading to a revision of the transmembrane topology model for ClC channels.**

The bulk of Cl^- filtered into the proximal tubule across the glomerular membrane must be reabsorbed subsequently along the nephron. Mechanisms such as $Na^+/K^+/2$ Cl^- cotransport, K^+/Cl^- cotransport, various anion exchangers, and Cl^- channels (1) are important in this transepithelial transport as well as in cellular homeostatis.

Recently, our knowledge of Cl^- channels has been extended dramatically by molecular biological tools (2–5). At least two different structural classes of anion channels are found in the kidney: the cystic fibrosis transmembrane conductance regulator (CFTR; refs. 4 and 6) and several members of the ClC family of voltage-gated Cl^--channels (7–9).

We have used a combined reverse transcriptase–polymerase chain reaction (RT-PCR) and hybridization approach to clone members of the ClC Cl^--channel family from rat and human kidney (rClC-K and hClC-K, respectively).§ rClC-K1 is nearly identical to the previously cloned ClC-K1 from rat kidney (9) and has limited intrarenal expression, while the closely related rClC-K2 is expressed in every nephron segment examined.

In humans there are two closely related ClC proteins that have 90% identity to each other (hClC-Ka and hClC-Kb). The high intraspecies identity precludes a conclusive correlation of rat channels with human counterparts. In our hands, these putative channels could not be expressed functionally, in contrast to reports for ClC-K1 (9). ClC-K proteins can be synthesized both in oocytes and *in vitro* and are glycosylated in the presence of pancreatic microsomes. Identification of the glycosylation sites leads to a revision of the working model for transmembrane topology of these channels (5, 10).

MATERIALS AND METHODS

Cloning of Renal Members of the ClC Cl^- Channel Family. Degenerate oligonucleotides against segments conserved in ClC Cl^- channels were used as primers for PCR. Rat kidney poly(A)$^+$ RNA was reverse-transcribed and used as a template for PCR using 60 cycles consisting of 92°C for 1 min, 45°C for 1.5 min, and 72°C for 2 min. The primers amplifying the rClC-K1 fragment had the following sequences: AAGAATTCTCTGGAATCCCIGARMTGAA, where R = G or A and M = C or A, and AATCTAGASGTGASCTCIATGCTGAA, where S = G or C. The cloned and sequenced PCR fragment then was used to screen cDNA libraries of rat kidney (in λgt11, provided by Mike M. Mueckler, St. Louis, and in λUni-Zap, Stratagene no. 937503) and human kidney (in λgt10, Clontech HL 1123a) under high stringency. Complete cDNAs were assembled from the resultant partial clones (rClC-K2, hClC-Ka, and hClC-Kb). Since no rClC-K1 clone could be obtained from two different cDNA libraries, we used RT-PCR with primers directed against the published sequence (9) to clone rClC-K1. Amino acids in rClC-K1 differing from the published ClC-K1 sequence (except for a stretch containing frameshift mistakes in ref. 9; see the legend to Fig. 1) were changed to construct a cDNA equivalent to ClC-K1.

Site-Directed Mutagenesis. Point mutations, as well as the *Torpedo* 5′ untranslated sequence, were introduced to the ClC-K cDNAs by recombinant PCR (10) with *Pfu* DNA polymerase (Stratagene). Stretches amplified by PCR were sequenced fully.

RNA (Northern) Blots. A nylon membrane [described previously (7, 8)] blotted with 10 μg of poly(A)$^+$ RNA from different tissues was reprobed under high stringency with cDNA probes labeled with ^{32}P by random priming.

RT/PCR for ClC-K Distribution and Analysis. Total RNA was prepared (11, 12) from different tissues and microdissected renal segments. Five glomeruli, two to five segments (0.5–1.5 mm) from the mid-convoluted (S2) and straight (S3) portions of the proximal tubule, the cortical thick ascending limb (cTAL), the distal convoluted tubule (DCT), and the cortical collecting tubule (CCT) were isolated from adult rat kidney as described (13). The RNAs were reverse-transcribed (14) into cDNAs that were used as templates in PCR. PCR was performed in a final volume of 100 μl of buffer (50 mM KCl/4.5 mM $MgCl_2$/0.1 mg of gelatin per ml/10 mM

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Abbreviations: rClC-K and hClC-K, Cl^- channels from rat and human kidney, respectively; RT-PCR, reverse transcriptase–PCR; DCT, distal convoluted tubule; CCT, cortical collecting tubule; cTAL, cortical thick ascending limb.

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§The sequences reported in this paper have been deposited in the GenBank data base (accession nos. Z30663, Z30643, and Z30644 for rClC-K2, hClC-Ka, and hClC-Kb, respectively).

Tris·HCl, pH 8.3) containing 10% (vol/vol) dimethyl sulfoxide, 40 μM dNTP, 1 μCi (37 kBq) of [α-^{32}P]dCTP, 1 unit of *Taq* polymerase, 100–200 ng of each oligonucleotide primer, and 50 ng of β-actin primers (internal control). We used the following primers: rClC-K1, sense AGACCATGCTGTCCGGTGTGG and antisense GAAGGGGGCTGCGAAAACT; rClC-K2, AAACCATCTTGACAGGTGTGA and GATTGGGGCGGCGAAGACC; and β-actin, CGTGGGCCGCCCTAGGCACCA and TTGGCCTTAGGGTTCAGGGGGG. Amplification reactions were subjected to 20–35 cycles of the following thermal cycling program: 94°C for 30 sec, 54°C for 30 sec, and 72°C for 1 min. Amplification products were run on a 4% polyacrylamide gel and autoradiographed.

Expression of kidney ClC Gene Products in Oocytes and *in Vitro*. Full-length cDNAs of all four ClC-Ks were assembled and cloned into pBluescript plasmid. To boost expression, we replaced their 5′ untranslated sequence with that of ClC-0 (5), a previously successful strategy (7, 8). We also inserted rClC-K2, hClC-Ka, and hClC-Kb cDNAs into the pSP64T vector containing the 5′ β-globin untranslated sequence (15). Capped cRNA was transcribed *in vitro* and injected into *Xenopus* oocytes (5–50 ng per oocyte). After 1–5 days oocytes were analyzed using the two-electrode voltage clamp. cRNAs having the ClC-0 5′ end were also translated *in vitro* by using rabbit reticulocyte lysate with or without dog pancreatic microsomes (Promega) in the presence of [^{35}S]methionine. Analysis was by SDS/PAGE and autoradiography, in some experiments preceded by immunoprecipitation.

Antibodies and Immunoprecipitation. A peptide representing the C terminus of rClC-K2 (Cys-Lys-Lys-Ala-Ile-Ser-Thr-Leu-Thr-Asn-Pro-Pro-Ala-Pro-Lys) was synthesized, coupled via its cysteine to keyhole limpet hemocyanin, and used to raise polyclonal antibodies in rabbits. rClC-K proteins were solubilized in buffer containing 0.5% Nonidet P-40 and immunoprecipitated by using this antiserum and protein A-sepharose and were analyzed by SDS/PAGE and autoradiography.

RESULTS

Cloning of Putative Rat and Human Kidney Chloride Channels. We used degenerate primers directed against conserved regions within the ClC family of voltage-gated Cl^- channels to amplify sequences from rat kidney mRNA by RT-PCR. A novel, homologous fragment was cloned and used to screen rat kidney cDNA libraries under high stringency. All cDNAs isolated were only 80% homologous to the PCR fragment used for screening, indicating that they originated from a different ClC gene. While this work was in progress, Uchida *et al.* (9) published the sequence of a rat kidney channel that they named ClC-K1. Our original PCR fragment is identical in sequence to a portion of this cDNA. We named the putative channel corresponding to our PCR fragment rClC-K1, and the clones isolated by library screening rClC-K2. Within the coding region, sequence identity is 85% at the nucleic acid level and 83% at the protein level. A full-length rClC-K1 cDNA was cloned by RT-PCR. After accounting for obvious mistakes in the published sequence (9) (Fig. 1), its sequence differs at nine amino acid positions from the published ClC-K1 sequence (see Fig. 1). At most, three of these represent PCR artifacts in our clone, since in the remaining cases they conform to other ClC-K sequences. Eleven independent clones isolated from a human kidney cDNA library also fell into two closely related classes. They are related more to each other (94% and 91% identity at the RNA and protein level, respectively) than to the rat genes. Since no unambiguous cross-species correlation is possible, we named them hClC-Ka and hClC-Kb.

Structural Features of ClC-K Proteins. Like other members of this gene family, ClC-K proteins also have 13 conserved hydrophobic domains (D1 through D13). ClC-K proteins are the smallest known members of the ClC family (687 amino acids, calculated molecular mass = 75 kDa). One distinctive feature of ClC-K channels is the insertion of two additional amino acids in putative transmembrane domain D3. There also are six more amino acids in the stretch linking D8 and D9, introducing another consensus site for N-linked glycosylation nine residues downstream of a highly conserved one. Further potential glycosylation sites are found between D4 and D5 for rClC-K2, hClC-Ka, hClC-Kb, and ClC-2. In all ClC-K proteins, further sites are present after D12. However, the current model for ClC channels (5, 10) localizes all these sites to the cytoplasm, suggesting that they may not be used.

Tissue Distribution of ClC-K Messages. Northern analysis of rat tissues demonstrates that ClC-K expression is highly specific for the kidney (Fig. 2*A*). Rabbit kidney showed expression both in the medulla and in cortex, while no signal was detected in the MDCK, LLC-PK1, BSC-1, COS, and HEK293 kidney cell lines (data not shown).

Because of their high degree of identity, Northern analysis is unsuitable for differentiation between ClC-K isoforms. We synthesized primers designed to amplify specifically rClC-K1 and rClC-K2 fragments. Both primer pairs amplified correctly sized products from rat kidney RNA (Fig. 2*B*). Analytical digests confirmed their identities. Amplification of β-actin was used as an internal control. Slight amounts of correctly sized amplification products also were obtained in brain (ClC-K1 and ClC-K2) and muscle (ClC-K2).

To assess intranephronal ClC-K distribution, we used RT-PCR on microdissected tubules (Fig. 3). ClC-K1 was found predominantly in cTAL and DCT, and to a lesser degree in the S3 segment of the proximal tubule and the CCT. ClC-K2 was expressed in all nephron segments investigated, which includes the S2 segment and the glomerulus.

Expression and Glycosylation of ClC-K Gene Products. Since we could not observe the expression of additional currents in oocytes injected with any of the native ClC-K cRNAs, we replaced their 5′ untranslated region with that of ClC-0, a previously successful strategy (7, 8). Additionally, we inserted the cDNAs into the pSP64T vector, which uses β-globin 5′ and 3′ untranslated regions to stimulate expression in *Xenopus* oocytes (15). We also analyzed rClC-K1 after mutating it to conform to the published sequence (9).

While we easily could detect Cl^- currents in oocytes expressing ClC-0 or ClC-1 cRNAs, we were unable to detect new Cl^- currents in the same batches of oocytes injected with ClC-K cRNAs. Since ClC channels are multimers (16) and rClC-2, rClC-K1, and rClC-K2 are coexpressed in some segments of the nephron, they may form functional heterooligomers. However, ClC-K cRNAs remained nonfunctional even when coexpressed in several different combinations.

Immunoprecipitation experiments of injected oocytes demonstrated the synthesis of correctly sized ClC-K proteins (Fig. 4*A*). When translated *in vitro*, all ClC-K proteins, as well as ClC-0, ClC-1, and ClC-2, were shifted to higher molecular weights when core glycosylation was made available by adding microsomes (Fig. 4 *B* and *C*). With rClC-K1, this shift appears larger than with rClC-K2. This challenges the working model of ClC channels (5, 10), which places all consensus sites for N-linked glycosylation into the cytoplasm. The first site between D8 and D9 is the only one conserved in all ClC members; ClC-K proteins have a second site nearby. Using site-directed mutagenesis, we eliminated both sites in rClC-K1 and rClC-K2 and reexamined glycosylation (Fig. 4*C*). In each case, no shift was observed upon the addition of microsomes, suggesting that this segment is extracellular.

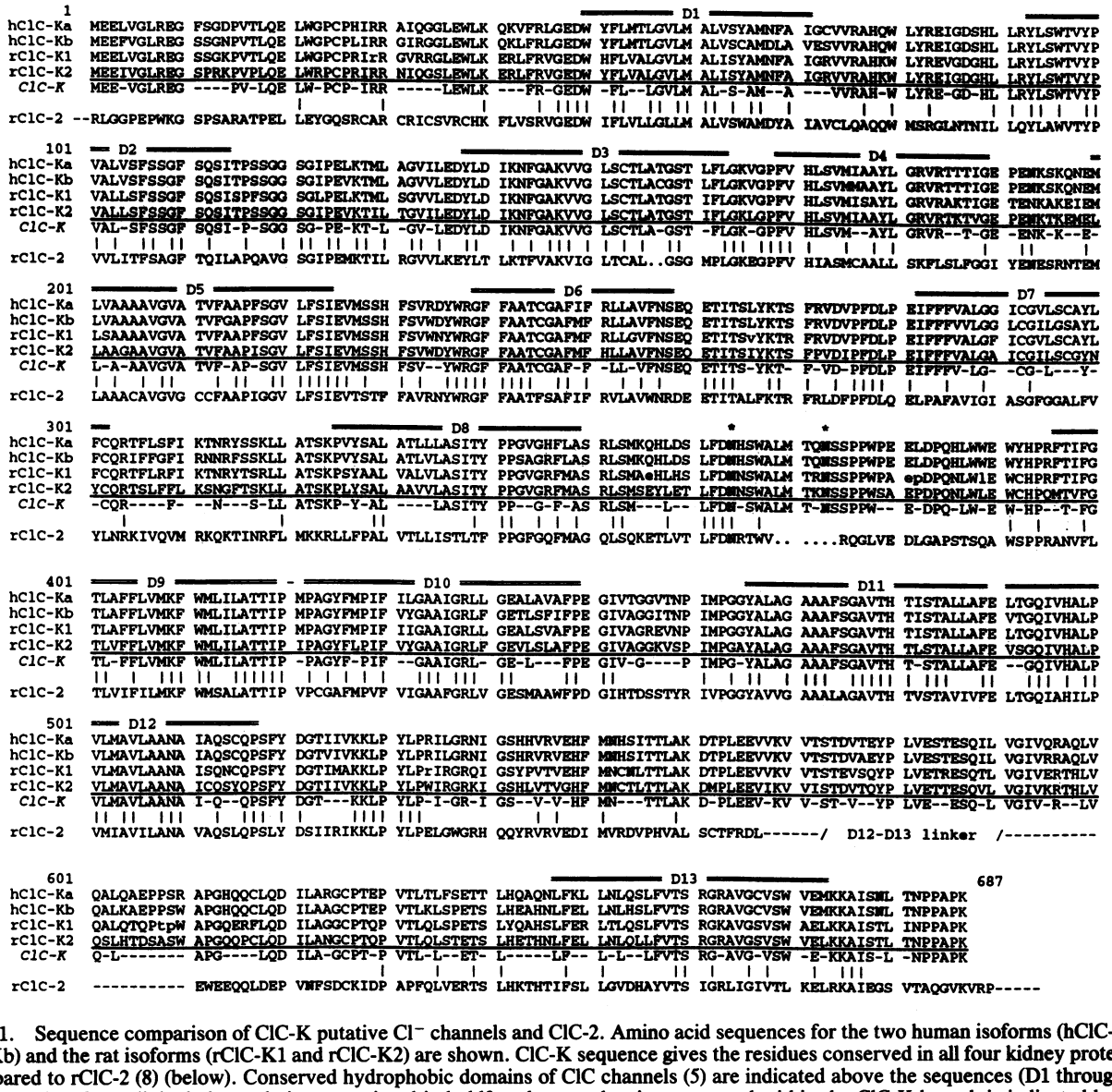

FIG. 1. Sequence comparison of ClC-K putative Cl^- channels and ClC-2. Amino acid sequences for the two human isoforms (hClC-Ka and hClC-Kb) and the rat isoforms (rClC-K1 and rClC-K2) are shown. ClC-K sequence gives the residues conserved in all four kidney proteins and is compared to rClC-2 (8) (below). Conserved hydrophobic domains of ClC channels (5) are indicated above the sequences (D1 through D13). Consensus sites for N-linked glycosylation are printed in boldface letters; the site conserved within the ClC-K branch is indicated by a star, and the site conserved in all ClC channels is indicated by a boldface star. Lowercase letters in the rClC-K1 sequence indicate positions where differences to ClC-K1 (9) were found: R27 → P, V266 → I, E356 → Q, E381 → D, P382 → A, L389 → P, R534 → W, T608 → A, P609 → S (last amino acid as in ref. 9). The ClC-K1 sequence in ref. 9 lacks three cytosines at positions 1311, 1344, and 1403 (initiator A=1), leading to three frameshifts in the D10 region.

DISCUSSION

Both the rat and the human kidney express two highly homologous members of the ClC Cl^--channel family, respectively named rClC-K1 and -K2 and hClC-Ka and -Kb. They stem from different genes judging from their differences in sequence and their differential intrarenal distribution. Their surprisingly high degree of homology (83% in rat and 91% in human) suggests comparatively recent evolutionary divergence. The degree of intraspecies homology makes it impossible to deduce from sequences alone which rat protein would be the direct functional homologue of a given human protein.

A functional correspondence between the rat and the human putative channels may only be established after either the transcriptional regulation (promoter structure), intrarenal distribution, or channel properties of all four proteins are known. Dehydration increases ClC-K1 transcription in the rat kidney (9), but such studies are unfeasible in the human. Difficulties in working with human kidney precluded investigation of the intrarenal distribution of hClC-K messages. rClC-K mRNAs show a different distribution along the rat nephron. Among the segments examined, rClC-K1 is predominantly expressed in the cTAL and DCT, while rClC-K2 is expressed in every nephron segment we examined. For ClC-K1, Uchida *et al.* (9) reported high expression in the medullary thin ascending limb, a segment that we did not investigate. ClC-K message was not detected in any kidney cell line we studied, suggesting highly differentiation dependent expression. Identification of low levels of message in muscle, brain, and intestine (Fig. 3*A*) by RT-PCR may represent illegitimate transcription without functional significance.

The intranephronal distribution suggests different functions for both isoforms. These may result from differences in biophysical properties, polarized expression, or control of gene expression. ClC channels are probably multimers with more than three identical subunits (16). The high homology within the ClC-K subfamily may allow the formation of heterooligomers with novel functional properties. The ubiquitous ClC-2 channel is also coexpressed with ClC-K gene products, but functional heterooligomers between these more divergent proteins (≈45% homology) seem less likely.

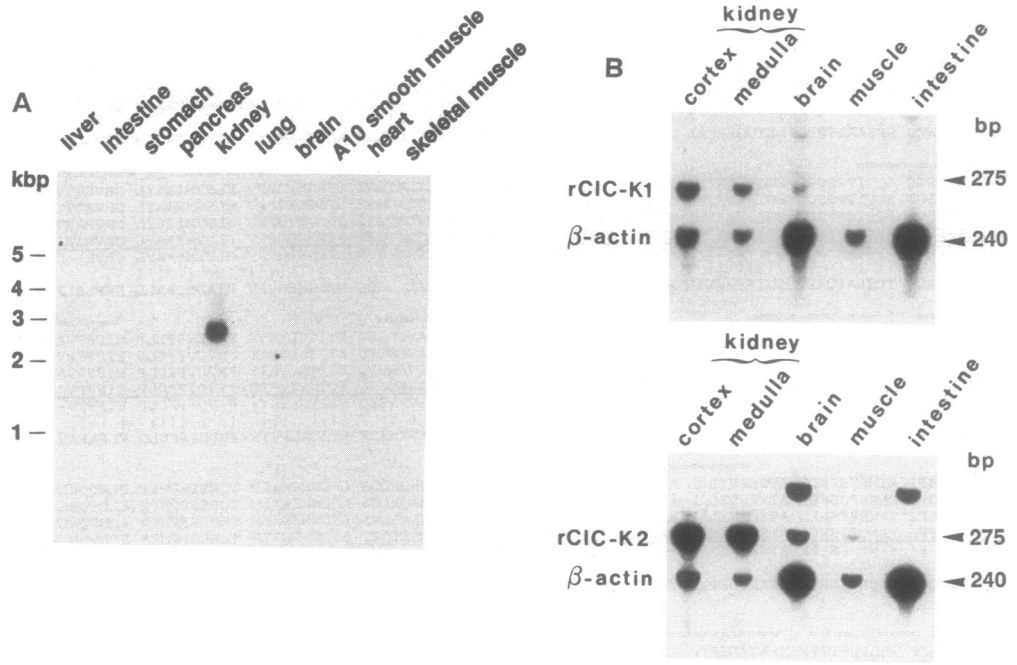

FIG. 2. Tissue distribution of rClC-K messages. (*A*) Northern analysis with rClC-K2 as a probe, which also detects the highly homologous rClC-K1. (*B*) RT-PCR analysis of rClC-K1 and rClC-K2 expression in different rat tissues. RNAs from several tissues were submitted to RT-PCR [35 cycles for rClC-K1 (*B Upper*) and 30 cycles for rClC-K2 (*B Lower*)]. A β-actin fragment was coamplified as internal standard. With ClC-K2 primers we observed products of aberrant size in rat brain and intestine. Sequencing of the large brain product revealed that it represented unspliced rClC-K2 message, possibly because of contaminating genomic DNA. Identities of the correctly sized bands were verified by restriction digests with *Bss*HII and *Fsp* I, which cut only the rClC-K1 or the rClC-K2 fragment, respectively (data not shown).

In contrast to Uchida *et al.* (9), we were unable to functionally express any of the putative ClC-K channels, while other ClC channels were easily expressible. Occasionally we observed time-independent, slightly outwardly rectifying Cl^- currents. Since they could also be observed in negative controls, we attributed these to endogenous oocyte Cl^- channels. Oocytes contain several different Cl^- channels that can be activated by second messengers, during maturation, or by injection of RNA coding for nonrelated proteins (16–20), making analysis of newly expressed Cl^- currents difficult. We also exchanged the untranslated regions of our cDNAs, mutated rClC-K1, to conform to the published sequence of ClC-K1 (9), and coinjected ClC-K complementary RNAs with other ClC cDNAs. Nonetheless, since by sequence ClC-K proteins are members of an established Cl^--channel family, we favor the view that they are anion channels or are subunits in such channels. ClC-K channels may serve roles in transepithelial transport or be involved in housekeeping functions such as cell-volume regulation. Indeed, ClC-2 is activated by hypotonicity (10), and volume regulation may be of special importance for epithelial cells transporting large amounts of solute and water (21).

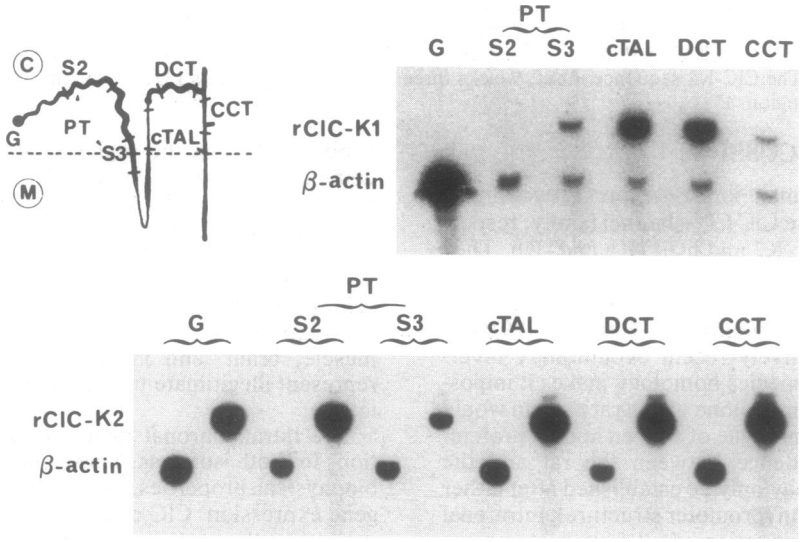

FIG. 3. Intrarenal distribution of rClC-K1 and rClC-K2 measured by RT-PCR on microdissected tubules. (*Upper Left*) Schematic of nephron segments. G, glomerulus; PT, proximal tubule (S2 and S3 segments). Dashes represent the border between kidney cortex (C) and medulla (M). (*Upper Right*) Intrarenal distribution of amplification products for rClC-K1 and β-actin (internal standard). (*Lower*) Intrarenal distribution of amplification products for rClC-K2 and β-actin expression from the same cDNA initially divided in two equal parts. Number of amplification cycles was 25 for ClC-K1 and 35 for ClC-K2.

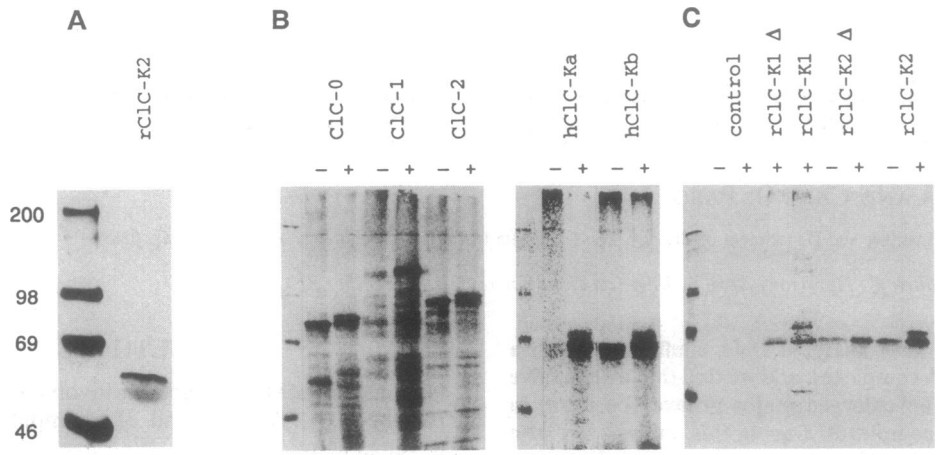

FIG. 4. Expression and glycosylation of different members of the ClC Cl^- channel family. (*A*) Immunoprecipitation of rClC-K2 protein expressed in *Xenopus* oocytes. Oocytes were injected with rClC-K2 message and labeled with [^{35}S]methionine. A solubilized membrane fraction was immunoprecipitated by using an antiserum directed against a C-terminal ClC-K peptide. (*B* and *C*) *in vitro* translation of ClC proteins in the absence (lanes −) or presence (lanes +) of dog pancreatic microsomes. In *C*, the products were immunoprecipitated with the anti-ClC-K antiserum before SDS/PAGE. For rClC-K1 and rClC-K2, we also analyzed mutants in which both consensus glycosylation sites between domains D8 and D9 were eliminated by replacing the asparagine residues by glutamine (N364 → Q, N373 → Q) (indicated by Δ in construct name).

Several possibilities may explain a lack of functional expression. ClC-K proteins may not function as Cl^- channels but perhaps transport other substances. We may miss another essential subunit, or the expression system may be inappropriate. Alternatively, the channel may be regulated by second messengers, but preliminary experiments testing several candidates did not reveal any novel currents. Finally, the channel may serve an intracellular role, even though other ClC proteins can be functionally expressed in the plasma membrane of different cells (5, 7, 8, 22, 23).

Whereas we cannot distinguish between these possibilities, we have shown that proteins of the correct size are synthesized both in oocytes and *in vitro* and that these are inserted into membranes and glycosylated. Though all ClC proteins contain consensus sites for N-linked glycosylation, their use seemed unlikely as they were thought to be cytoplasmic (5, 10). Our present observations indicate that this model is incorrect. Elimination of consensus sites between D8 and D9 abolished glycosylation with ClC-K proteins. This is probably not due to protein misfolding, since the equivalent mutant of ClC-1 is still functional in the oocyte (C. Lorenz, K. Steinmeyer, and M. Pusch, personal communication). Thus, the D8–D9 linker cannot be cytoplasmic, and one of the less likely candidates for transmembrane spans (such as D3, D4, or D8) may not cross the membrane. Alternatively, an additional, less hydrophobic segment on the N-terminal side of D8 may span the lipid bilayer.

In summary, we have shown that kidney of both rat and human each express highly homologous members of the ClC channel family. Differential expression along the nephron suggests specialized functions for each of these isoforms. Glycosylation of ClC-K proteins at sites previously thought to be cytoplasmic necessitates a revision of the topology model that is applicable to all members of this gene family.

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1. Reeves, W. B. & Andreoli, T. E. (1992) *Annu. Rev. Physiol.* **54**, 29–50.
2. Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E. D. & Betz, H. (1987) *Nature (London)* **328**, 215–220.
3. Schofield, P. R., Darlison, M. G., Fujita, N., Burt, D. R., Stephenson, F. A., Rodriguez, H., Rhee, L. M., Ramachandran, J., Reale, V., Glencorse, T. A., Seeburg, P. H. & Barnard, E. A. (1987) *Nature (London)* **328**, 221–227.
4. Riordan, J. R., Rommens, J. M., Kerem, B. S., Alon, N., Rozmahel, R., Grzelczak, Z., Zielenski, J., Lok, S., Plavsik, N., Chou, J. L., Drumm, M. L., Ianuzzi, M. C., Collins, F. S. & Tsui, L. C. (1989) *Science* **245**, 1066–1073.
5. Jentsch, T. J., Steinmeyer, K. & Schwarz, G. (1990) *Nature (London)* **348**, 510–514.
6. Crawford, I., Maloney, P. C., Zeitlin, P. L., Guggino, W. B., Hyde, S. C., Turley, H., Gatter, K. C., Harris, A. & Higgins, C. F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9262–9266.
7. Steinmeyer, K., Ortland, C. & Jentsch, T. J. (1991) *Nature (London)* **354**, 301–304.
8. Thiemann, A., Gründer, S., Pusch, M. & Jentsch, T. J. (1992) *Nature (London)* **356**, 57–60.
9. Uchida, S., Sasaki, S., Furukawa, T., Hiraoka, M., Imai, T., Hirata, Y. & Marumo, F. (1993) *J. Biol. Chem.* **268**, 3821–3824.
10. Gründer, S., Thiemann, A., Pusch, M. & Jentsch, T. J. (1992) *Nature (London)* **360**, 759–762.
11. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
12. Cartier, N., Lacave, R., Vallet, V., Hagege, R., Hellio, J., Robine, S., Pringault, E., Cluzeaud, F., Briand, P., Kahn, A. & Vandewalle, A. (1993) *J. Cell Sci.* **104**, 695–704.
13. Vandewalle, A., Wirthensohn, G., Heidrich, H. G. & Guder, W. G. (1981) *Am. J. Physiol.* **240**, F492–F500.
14. Akli, S., Chelly, J., Lacorte, J.-M., Poenaru, L. & Kahn, A. (1991) *Genomics* **11**, 124–134.
15. Krieg, P. A. & Melton, D. A. (1984) *Nucleic Acids Res.* **12**, 7057–7070.
16. Steinmeyer, K., Lorenz, C., Pusch, M., Koch, M. C. & Jentsch, T. J. (1994) *EMBO J.* **13**, 737–743.
17. Peres, A. & Bernardini, G. (1983) *Pflügers Arch. Eur. J. Physiol.* **399**, 157–159.
18. Webb, D. J. & Nuccitelli, R. (1985) *Dev. Biol.* **107**, 395–406.
19. Boton, R., Singer, D. & Dascal, N. (1990) *Pflügers Arch. Eur. J. Physiol.* **416**, 1–6.
20. Attali, B., Guillemare, E., Lesage, F., Honoré, E., Romey, G., Lazdunski, M. & Barhanin, J. (1993) *Nature (London)* **365**, 850–852.
21. Montrose-Rafizadeh, C. & Guggino, W. B. (1990) *Annu. Rev. Physiol.* **52**, 761–772.
22. Bauer, C. K., Steinmeyer, K., Schwarz, J. R. & Jentsch, T. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11052–11056.
23. Pusch, M., Steinmeyer, K. & Jentsch, T. J. (1994) *Biophys. J.* **66**, 149–152.