I_{Cln}, a chloride channel cloned from kidney cells, is activated during regulatory volume decrease

ANDREAS LAICH, MARTIN GSCHWENTNER, WOLFGANG KRICK, ULRICH O. NAGL, JOHANNES FÜRST, SABINE HOFER, ALEX SUSANNA, ANDREAS SCHMARDA, PETER DEETJEN, GERHARD BURCKHARDT, and MARKUS PAULMICHL

Department of Physiology, University of Innsbruck, Innsbruck, Austria and Center of Physiology, University of Göttingen, Göttingen, Germany

Cell swelling is associated with the activation of chloride as well as potassium channels to allow the cells to expel water by the downhill movement of these ions from the cytosol to the extracellular space. The result of this channel activation is a shrinkage of the cells despite the volume challenging condition. Several proteins have been cloned thus far whose function has been related to chloride permeation associated with this ion-driven volume loss, commonly termed regulatory volume decrease (RVD). The ability for RVD is believed to be crucial for dividing cells, and accordingly no cell line has been found so far that lacks this regulatory mechanism. As generally as RVD is found in various cell types, the underlying channel proteins have to be distributed ubiquitously, and such a general distribution has been shown for most of the proteins expected to act as chloride channels during RVD [1, 2]. However, in mammals, despite the fact that mitosis is taking place in most tissues, which is evidenced by the fact that RVD-related chloride channels can be found in all organs, some of them are subjected to greater volume stress than others; this leads to the finding that the quantitative distribution is quite different. One organ notoriously stressed by changing osmolarities and, therefore, changing water content in the cells, is the kidney.

Both ClC2, a member of the voltage-dependent chloride channel family originally cloned from *Torpedo marmorata* [3], and I_{Cln} [2], a chloride channel cloned from MDCK cells, are expressed in the kidneys of different species. As well, both chloride channels have been shown to be involved in volume regulation upon cell swelling [1, 4].

As shown in Figure 1, I_{Cln} in the rat kidney is expressed in the cortex, in the outer and the inner medullas, as well as in the papilla. All regions are subjected to substantial volume stress. In the cortical region the combined transport of solutes together with sodium leads to a constant water flux into proximal tubular cells [5]. This volume stress has to be constantly counterbalanced by an effective outward movement of ions. An effective mechanism for volume loss after swelling in proximal tubular cells is the activation of potassium and chloride channels [6], as it has been defined for a large number of different cells [6, 7]. The localization of the

I_{Cln} protein is primarily on the apical side of the proximal tubular cells. As shown in Figure 2, we were only able to demonstrate a substantial amount of I_{Cln} protein in apical vesicle preparations of the proximal tubular cells. In the same cells no I_{Cln} signal could be seen in basolateral vesicle preparations. However, small amounts of I_{Cln} also seem to be located in this membrane fraction after substantially (5×) increasing the amount of total protein isolated from these cells used for Western blotting (not shown). In conclusion, we show that I_{Cln} is expressed at high levels in the cortex, outer and inner medullas, and papilla of rat kidney. The major distribution of the swelling-dependent chloride channel I_{Cln} in proximal tubular cells is in the apical membrane, whereas only a small amount can be identified in the basolateral fraction. In the outer and inner medullas as well as in the papilla under antidiuretic conditions, I_{Cln} can mainly be identified in the cytosol. The high level of expression and distribution of the swelling-dependent chloride channel I_{Cln} in the kidney points to the possibility that this protein plays a central role for volume regulation in this organ.

Reprint requests to Markus Paulmichl, M.D., Department of Physiology, University of Innsbruck, Fritz-Pregl-Straße 3, A-6020 Innsbruck, Austria.

E-mail: Markus.Paulmichl@uibk.ac.at

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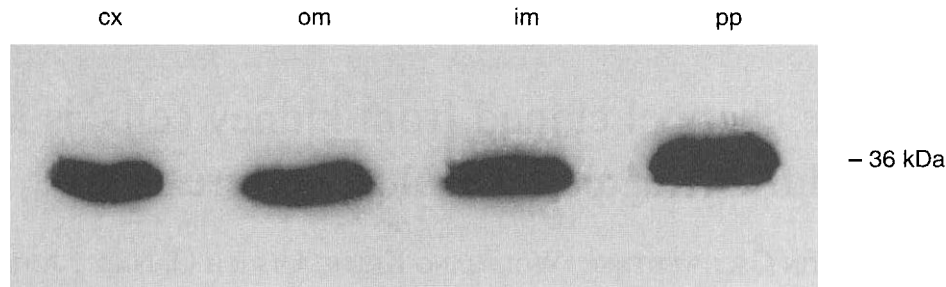
Fig. 1. *Identification of the I_{Cln} protein in different regions of the rat kidney.* The I_{Cln} protein with an apparent molecular weight of 36 kDa can be located in the cortex (cx), inner and outer medulla (im and om, respectively) and papilla (pp). The whole cell protein (cytosol as well as membrane fraction) was used. For the Western blot 100 μg of total protein was sized by SDS gel electrophoresis and the different size proteins blotted on nitrocellulose membrane (Schleicher & Schüll, Germany). Standard Western blotting techniques [8] were used to identify the I_{Cln} protein. As the first selective antibody, we used polyclonal antipeptide antibodies raised against a synthetic peptide comprising the C-terminal end of the I_{Cln} protein. After affinity purification the antibodies were used in a 1:1000 dilution and then recognized by chemoluminescence (peroxidase-labeled second antibody; Boehringer Mannheim).
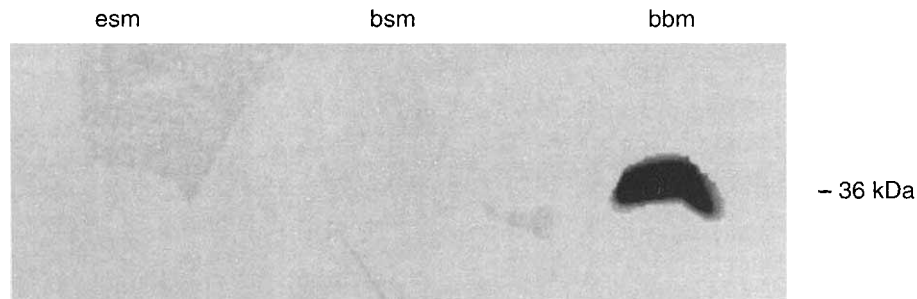

Fig. 2. *Identification of the I_{Cln} protein in different vesicle preparations of proximal tubular cells.* The I_{Cln} protein can be identified in vesicles from the brush-border (apical) membrane (bbm). No signal was detected in vesicles from the basolateral membrane (bsm), nor in the endocytotic vesicles (esm). Pig kidneys were obtained from the local slaughterhouse and immediately after their removal transferred to the laboratory. The renal cortex tissue was isolated and homogenized. Brush-border membrane vesicles were purified from the homogenate using the Mg^{2+}/EGTA precipitation technique originally described by Biber et al [9]. Basolateral membrane vesicles were prepared from the outer cortex by a Percoll density centrifugation technique using a 12% (wt/vol) Percoll gradient according to Werner and Roch-Ramel [10]. Endocytotic vesicles were enriched from cortex homogenate by differential centrifugation and separated from other membranes on a 16% (wt/wt) Percoll gradient. The details of isolation have been described previously [11]. The different isolation procedures were monitored by the measurement of respective enzyme activities to ensure the purity of the preparations. As the marker enzyme for the brush border membrane, the leucine aminopeptidase (EC 3.4.11.1) was determined using L-leucine-4-nitro-anilide as a substrate. The specific acitivity of the Na^+/K^+-ATPase (EC 3.6.1.3), a marker enzyme of basolateral cell membranes, was measured with a coupled optical test. In the preparations of brush border membrane vesicles the specific activity of leucine aminopeptidase was enriched by a factor of 11 ± 1 (SEM, $N = 3$) over the activity of the homogenate. The specific activity of Na^+/K^+-ATPase in the preparations of basolateral membrane vesicles was 18 ± 1 ($N = 3$) times that of the homogenate, proving sufficient purification of the vesicles. As a marker for endocytotic vesicles, ATP-driven proton pump activity was measured in the presence of chloride with acridine orange [11].

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