A NOVEL RENAL CHLORIDE CHANNEL, OmClC-K,

PLAYS AN IMPORTANT ROLE FOR FRESHWATER

ADAPTATION IN MOZAMBIQUE TILAPIA

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EXTENDED ABSTRACT ONLY - DO NOT CITE

The kidney plays an important role in osmoregulation in freshwater teleosts, which are exposed to the danger of osmotic loss of Na^+ and Cl^-. Since the primary function of the kidney in fresh water is to excrete excess water, it generates hypotonic urine by reabsorbing most of the filtered solutes. Nishimura et al. (1983) isolated and perfused nephron tubules from freshwater rainbow trout, *Oncorhynchus mykiss*, and showed that the net water movement across the distal tubule was very low, but the net Cl^- reabsorption exists. These results suggest that in freshwater trout, which lack the loop of Henle, the distal tubule acts as a diluting segment such as the early distal segment of the frog kidney and the mammalian thick ascending limb of Henle's loop (TAL) (Burg and Green, 1973). The TAL of the mammalian kidney is involved in the urinary dilution and concentration processes by actively reabsorbing NaCl through the complex mechanism. Previous electrophysiological studies have demonstrated that two Cl^- are taken up with one Na^+ and one K^+ by the TAL cells via the bumetanide-sensitive Na-K-2Cl cotransporter and then transported across the basolateral membrane. Major advances have been made over the past few years in our understanding of NaCl transport mechanisms in this nephron segment. This has been fueled by the molecular identification of the Na-K-2Cl cotransporter (NKCC2) (Gamba et al., 1994), K^+ secretory channels (ROMK) (Ho et al., 1993) and basolateral Cl^- channel (ClC-K2) (Adachi et al., 1994; Kobayashi et al., 2001) expressed in the TAL. Despite abundant data in mammals, however, the identification of the ion transporters in the teleostean nephron was not done at all.

In the present study, to identify the molecular mechanisms of the teleost kidney, we have cloned OmClC-K chloride channel from Mozambique

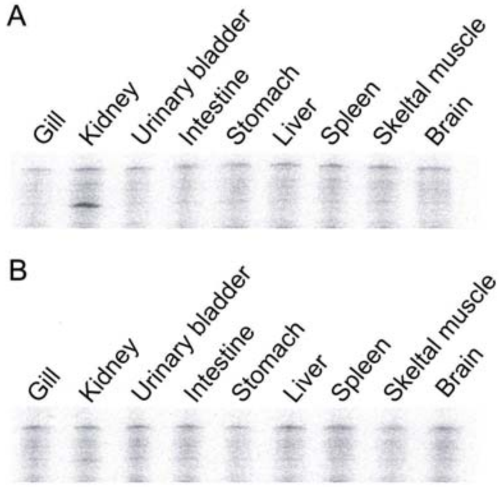

Figure 1. Tissue distribution of OmClC-K in freshwater- (A) and seawater-adapted (B) tilapia by RNase protection assay.

tilapia, *Oreochromis mossambicus*, which is a homologue of the mammalian kidney-specific chloride channel, ClC-K. The cDNA of OmClC-K encodes a protein, whose amino acid sequence has high homology to *Xenopus* and mammalian ClC-K (*Xenopus* ClC-K, 41.8%; rat ClC-K2, 40.9%; rat ClC-K1, 40.1%). In order to assess the role of OmClC-K in the osmoregulation of tilapia, changes in expression of its mRNA was examined in various tissues after altering environmental salinities using the ribonuclease protection assay. The mRNA of OmClC-K was expressed exclusively in the kidney and the expression level of mRNA was significantly increased in freshwater-adapted fish (Figure 1).

To investigate the physiological role of the OmClC-K, we sought to determine its exact localization in the kidney of freshwater tilapia by immunohistochemistry. The immunoreactive cell was located in specific nephron tubules. In adjacent sections, immunoreaction of Na^+, K^+-ATPase was observed on the same tubules that were immunoreactive to OmClC-K (Figure 2).

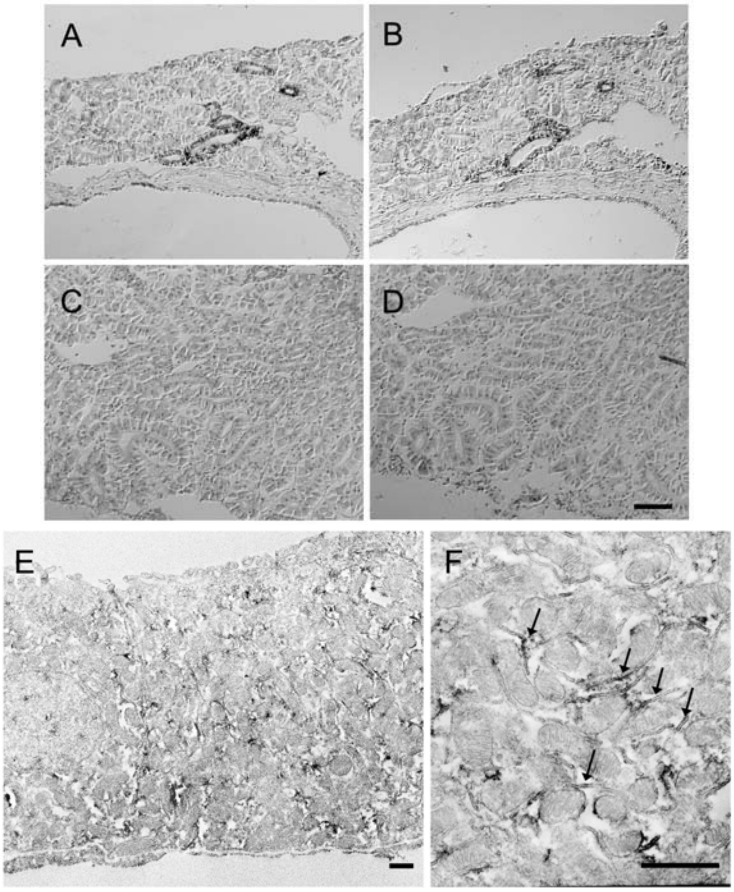

Figure 2. The serial sections of the kidney, stained with Na^+, K^+-ATPase antibody (A, C) and OmClC-K antibody (B, D), in freshwater- (A, B) and seawater-adapted tilapia (C, D). Scale bar = 100 μm.
E. Immunoelectron micrographs of the distal tubule stained with anti-OmClC-K in tilapia kidney adapted to freshwater. F. a high magnification of E. Immunostaining (arrows) is mainly located on the infolding basolateral membrane. Scale bar = 500 nm.

To identify the OmClC-K immunoreactive nephron, we performed the immunoelectron microscopy. Ultrastructural observations revealed that OmClC-K-immunoreactive cells of freshwater-adapted fish had a few microvilli, a rich population of mitochondria and an extensive labyrinth of basolateral membrane infolding (Figure 2). Immunoreaction of OmClC-K was recognizable on the structure of basolateral membrane infolding.

Therefore, the OmClC-K-positive tubules may correspond to the distal tubules of freshwater tilapia. Judging from the results obtained from mammalian ClC-K, OmClC-K is involved in the transepithelial Cl^- reabsorption by the kidney of hyperosmoregulating, freshwater fish.

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