PARTIAL CLONING OF THE cDNA ENCODING THE RENAL ISOFORM OF THE Na-K-Cl COTRANSPORTER (sNKCC2) FROM THE KIDNEY OF DOGFISH SHARK, *SQUALUS ACANTHIAS*

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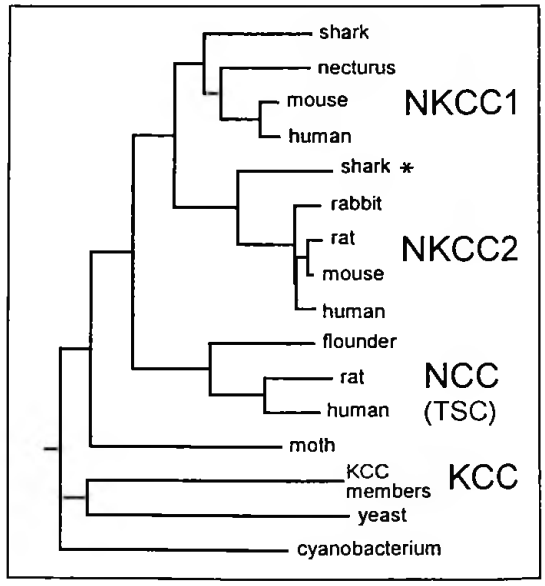
Two distinct isoforms of the Na-K-Cl cotransporter have been identified: the basolateral Na-K-Cl cotransporter (NKCC1), particularly prominent in secretory epithelia and first cloned from shark rectal gland (Xu, J-C, et. al., Proc. Natl. Acad. Sci. USA, 91, 2201-2205, 1994); and the apical Na-K-Cl cotransporter (NKCC2) previously reported only in thick ascending limb of the loop of Henle of mammalian kidney. Phylogenetic analysis of available cDNAs has suggested that the two cotransporter isoforms arose from a gene duplication event that occurred early in animal evolution (cf. Gillen, C.M. et al., J. Biol. Chem., 271, 16237-16244, 1996). We previously addressed the question as to whether the point of divergence occurred before the evolution of elasmobranchs using immunolocalization studies in the dogfish shark kidney. NKCC was identified on both apical and basolateral membranes of epithelial cells (Biemesderfer, D., et. al., Am J Physiol. 270, F927-36, 1996), however the use of monoclonal antibodies did not distinguish whether one or two isoforms were present. The present experiments were undertaken to search for the renal isoform in the shark.

PolyA-RNA was isolated from homogenized shark kidney using polydT-cellulose affinity chromatography; RT-PCR was performed on this polyA RNA using degenerate primers. Sites for upstream and downstream PCR primers were selected for a high degree of sequence conservation among NKCC family members, and oligonucleotides were produced so that where divergence was present at a given base position, nucleotides were used in ratios approximating the ratios of occurrence in the family. The primers flanked the region encoding transmembrane helices 5-7 of the predicted structure of shark NKCC1 (nucleotides 1632-2149). A high-yield RT-PCR product (~520 bp) was obtained and sub-cloned into the PCR^{TMII} vector (Invitrogen). Two clones were isolated and sequenced bi-directionally.



A diagram of the distances between cation-chloride cotransporter family members is presented in the accompanying figure. The sum of horizontal segments connecting two members is proportional to the degree of amino acid sequence divergence, calculated within the region of the PCR product. The diagram includes the Na-K-Cl cotransporters (NKCCs), the Na-Cl cotransporter (NCC, or TSC), and the K-Cl cotransporter (KCC). The newly obtained sequence is more similar to the mammalian NKCC2s than to shark NKCC1, demonstrating that the cDNA is that of the shark renal Na-K-Cl cotransporter (sNKCC2, denoted in the figure by the asterisk). The analysis also predicts that the NKCC1 – NKCC2 gene duplication event occurred quite a bit earlier than the evolution of the elasmobranchs, perhaps at a point near the origin of the vertebrates.

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