EXPRESSION OF A POTASSIUM CHANNEL HOMOLOGOUS TO THE INWARD RECTIFYING POTASSIUM CHANNEL (IRK1) IN *Pseudopleuronectes americanus* INTESTINE

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The intestine of the winter flounder (*Pseudopleuronectes americanus*) can actively absorb or actively secrete potassium (K^+). Secreted K^+ leaves the intestinal cell by means of K channels in the apical membrane, but the molecular nature of these channels is not known (Dawson, D.C. and R.A. Frizzell, Pflugers Archiv. 414:393-400, 1989). K channels are critical components of epithelial cells, such as those in the kidney and gastrointestinal tract, that regulate and maintain fluid composition. Virtually every known salt absorptive or salt secretory process in these polar cells is either directly or indirectly dependent on K channels. Functional studies suggest that epithelial cells express a range of K channel subtypes that subserve different physiological functions. Despite their importance, progress in analyzing the molecular basis for K channel function has been hampered by the absence of molecular probes for these proteins. Recently expression cloning has yielded cDNAs for K channels that are members of a new family, the inward rectifying potassium channels (IRKs), distinct from that defined by the well-known voltage-dependent channels expressed in excitable cells. Members of the IRK1 family have been identified in a variety of tissues, including heart (Ashen *et al.*, Am. J. Physiol. 268:H506-H511, 1995), brain and renal cells (Ashen *et al.*, Am. J. Physiol. 268:H506-H511, 1995; Ho *et al.*, Nature 362:31-38, 1993). Members of this K channel family exhibit a number of distinguishing characteristics that permit them to be identified in electrophysiological assays in native cells and expression systems, although there is variation in expression of traits from one family member to another. All members of the family exhibit moderate to pronounced inward rectification, conducting K^+ more effectively in the inward direction even when external K^+ is lower than internal K^+. Their rectifying property dictates that at the resting potential of the cell, usually positive to E_K, they will mediate outward currents that are virtually voltage independent. Thus, near E_K, IRK channels will stabilize the resting potential, but during a large depolarization these channels prohibit excess loss of K^+ from the cell. The mechanistic basis for this inward rectification appears to be a fast component mediated by intracellular Mg^{2+} and a slower component mediated by intracellular polyamines (Jan, L. Y. and Y. N. Jan, Nature 371:119-122, 1994; Lopatin *et al.*, Nature 372:366-369, 1994).

Two members of this new family are the rat renal outer medullary K channel (ROMK1) (Ho *et al.*, Nature 362:31-38, 1993) and the mouse macrophage inwardly rectifying K channel (IRK1) (Kubo *et al.*, Nature 362:127-133, 1993). The goal of the study described here was to ascertain if either of these two members of the IRK family could be found in the winter flounder intestinal epithelium or bladder.

Ten micrograms of total RNA isolated from winter flounder intestinal columnar epithelium and bladder (Chirgwin *et al.*, Biochem. 18:5294-5299, 1979) was size fractionated on a 1% agarose-formaldehyde gel, transferred to a nylon membrane (Thomas, P. J., Proc. Natl. Acad. Sci. USA 77:5201-5205, 1980), and probed with a digoxigenin (DIG)-labeled RNA derived from either the ROMK1 or IRK1 cDNAs (Holtke, H.-J. and Kessler, C., Nucleic Acids Res. 18 (19):5843-5851, 1990). We were careful to restrict, as much as possible, the tissue sample from intestine to the columnar epithelial cells by using gentle scraping of stripped intestine to isolate surface epithelial cells. The entire tissue from an intact bladder was minced and homogenized to isolate total RNA. Integrity of the RNA samples was verified by ethidium bromide staining before transfer (Fig. 1A). The filters were washed at high stringency (0.5 x SSC, 0.1% SDS, 65°C).

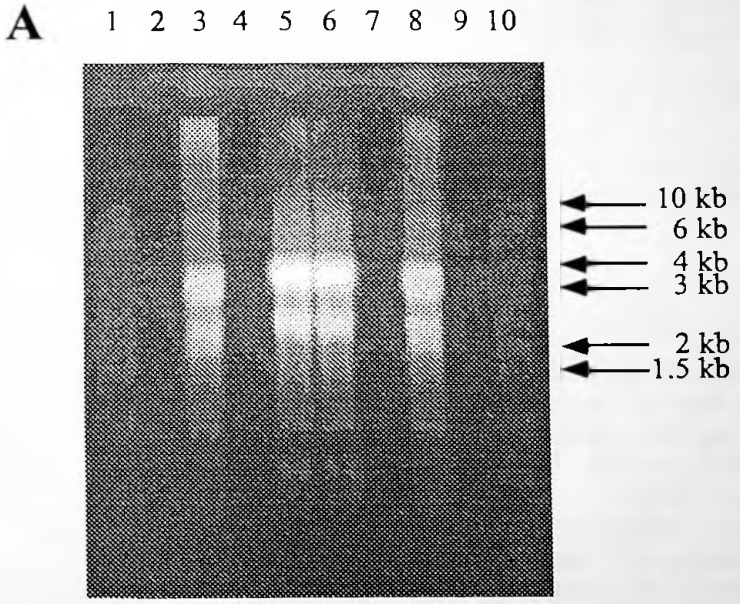

Figure 1A. Gel of total RNA isolated flounder intestinal epithelium and bladder. 10 µg of total RNA isolated from bladder (lanes 3 and 8) and intestinal epithelium (lanes 5 and 6) was run on a 1% agarose-formaldehyde gel along with 2 µg RNA markers (lanes 1 and 10), capillary transferred onto a nylon membrane and probed with either DIG-labeled ROMK1 or IRK1 RNA probes.

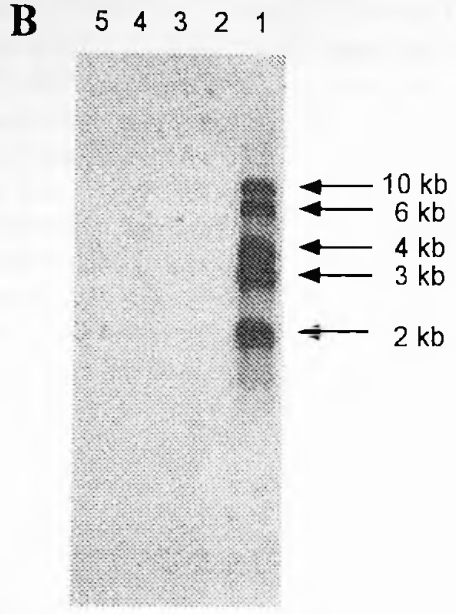

Figure 1B. Northern blot analysis of total RNA isolated from flounder bladder (lane 3) and intestinal epithelium (lane 5) probed with DIG-labeled ROMK1 RNA. No homologous sequences were detected in either bladder or intestinal epithelium. High stringency wash was 0.5 x SSC, 0.1% SDS, 65°C.

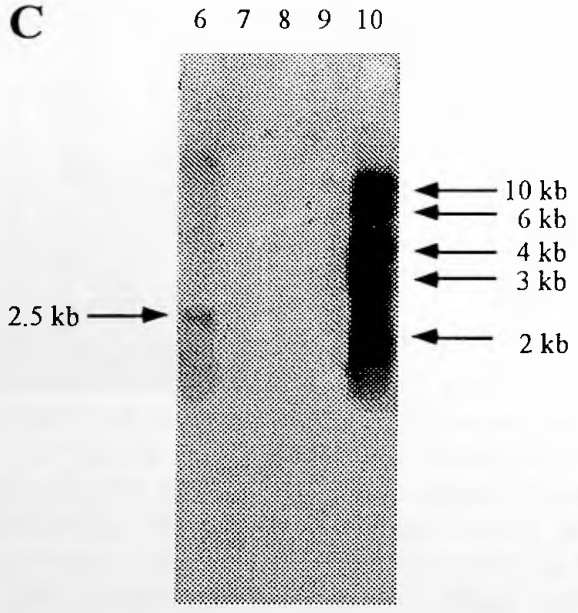

Figure 1C. Northern blot analysis of total RNA isolated from flounder bladder (lane 8) and intestinal epithelium (lane 6) probed with DIG-labeled IRK1 RNA. Flounder intestinal epithelium revealed a transcript of approximately 2.5 kb. High stringency wash was 0.5 x SSC, 0.1% SDS, 65°C.

Northern blot analysis of total RNA from flounder intestinal epithelium (Fig. 1C) revealed a transcript of approximately 2.5 kilobases (kb) using a DIG-labeled RNA IRK1. Hybridization with a ROMK1 probe detected no homologous sequences in either flounder intestinal epithelium or bladder (Fig. 1B). These results provide evidence for the expression in flounder intestinal cells of a K channel homologous to the inward rectifier, IRK1, and raises the possibility that this channel could be responsible for K^+ secretion.

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