# Regulation of NKCC2 expression in the gut of *Fundulus heteroclitus* on change in salinity

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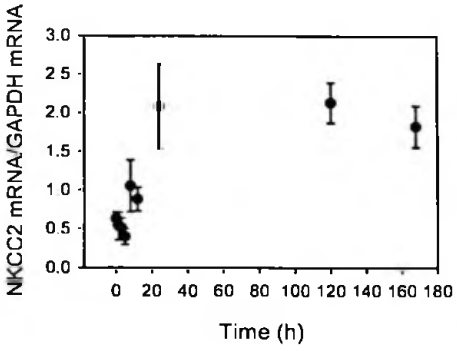


Fig.1. Levels of NKCC2 mRNA in the gut of the killifish increase during the fresh- to salt-water adaptation.

Killifish, *Fundulus heteroclitus*, is an estuarine fish that regulates its ionic and osmotic balance in the face of constantly changing water salinity. NKCC1 in the chloride cells of the gill plays an essential role in salt secretion and the expression level of gill NKCC1 is dramatically upregulated in salt-water adapted killifish, permitting increased NaCl efflux.[1] In addition to NKCC1a in gill, we have also reported NKCC1b (also called NKCC3) in brain and kidney, and NKCC2 in the gut and the kidney[2]. Here we present evidence that NKCC2, localized at high concentrations in the apical membrane of the intestinal epithelial cells, markedly changes its expression during changes in water salinity.

Fresh-water adapted killifish were exposed to sea water for different times, fish were sacrificed, and gut tissue was harvested to determine levels of NKCC2 mRNA using quantitative PCR. As illustrated in figure 1, the level of NKCC2 mRNA in the gut epithelium rapidly increases about 4-fold during the fresh- to salt-water adaptation; the steady-state was reached within 24-48h. In other experiments, a rapid decrease in NKCC2 expression level was seen on the transition from salt water to fresh water, also complete within 48 hours (not shown). We have previously reported that early in the adaptation to salt water, before the animal has had a chance to synthesize NKCCs, there is a pronounced increase in the regulatory phosphorylation state of NKCC1 consistent with increased utilization of available transporters[3]. Interestingly, we have been unable to find significant changes in the phosphorylation level of NKCC2 during the same time period (Harmel, Djurisic, Weiss, and Forbush, unpublished).

The increase in the expression of NKCC2 in the killifish gut during adaptation to salt water permits increased salt uptake though the intestinal epithelium. This result is consistent with a homeostatic mechanism whereby teleost fish in salt water counteract whole body osmotic water loss by continuous absorption of water through the gut accompanying bumetanide-sensitive NaCl absorption[4]; excess NaCl is then secreted through the gills, by the well-known mechanism involving NKCC1.

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1. **Scott, G.R., J.G. Richards, B. Forbush, P. Isenring, and P.M. Schulte.** Changes in gene expression in gills of the euryhaline killifish *Fundulus heteroclitus* after abrupt salinity transfer. *Am J. Physiol.* 287:C300-309, 2004.
2. **Djurisic, M., P. Isenring, and B. Forbush**. Cloning, tissue distribution and changes during salt adaptation of three Na-K-Cl cotransporter isoforms from killifish, *Fundulus Heteroclitus*. *Bull. Mt Desert Isl. Biol. Lab.* 42: 89-90, 2003.
3. **Flemmer, A.W., R. Behnke, and B. Forbush**. Changes in phosphorylation state of the Na-K-Cl cotransporter in chloride cells of the gill of *fundulus heteroclitus* during salt adaptation. *Bull. Mt Desert Isl. Biol. Lab.*38: 80-82, 1999.
4. **Halm, D.R., E.J. Krasny Jr., and R.A. Frizzell.** Electrophysiology of flounder intestinal mucosa. II. Relation of the electrical potential profile to coupled NaCl absorption. *J Gen Physiol.* 85:865-83, 1985.