EXPRESSION OF THE SECRETORY Na-K-Cl COTRANSPORTER IS INCREASED DURING LONG TERM SALT ADAPTATION IN THE KILLIFISH, *FUNDULUS HETEROCLITUS*

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The killifish, *Fundulus heteroclitus,* is an excellent model of a euryhaline fish that has been used extensively to study the process of salt adaptation. In the short term, isolated gill epithelium responds to increased osmolarity with an increase in bumetanide-sensitive transpepithelial Na^+ and Cl^- transport of (Zadunaisky *et al, J. Memb. Biol.* 143:207, 1995), implicating the Na-K-Cl cotransporter (NKCC1) as part of the secretory apparatus involved in adaptation. Over the long term, proliferation and enlargement of the secretory "chloride cells" of the gill epithelium accompanies adaptation to changes in salinity. In the present study, we make use of a monoclonal antibody specific to the Na-K-Cl cotransporter to demonstrate that long-term changes in expression of the transporter occur in response to changes in salinity.

Killifish were acquired from estuaries feeding into Frenchman Bay, Maine. They were kept in fresh water or slowly adapted to either seawater or "2X seawater", containing twice the amount of NaCl. At the end of the 5-week adaptation period, the fish were pithed, their gills removed, and the tissue flash frozen in liquid nitrogen. Frozen gills were crushed in homogenization buffer containing protease inhibitors and membranes prepared from these homogenates by differential centrifugation. Samples of these membranes were separated by SDS-polyacrylamide gel electrophoresis, transferred to Immobilon, and probed with the primary antibody, T4, raised to a fusion protein of the T84-cell Na-K-Cl cotransporter (Lytle *et al. Am J. Physiol.* 269:C1496, 1995).

Figure 1 shows a T4 Western blot of three independent samples at each of the adaptation conditions. The cotransporter runs as a band at ~180 kDa. At higher levels of expression, proteolytic fragments appear between ~100 and 150 kDa The fresh water fish expressed detectable amounts of Na-K-Cl cotransporter in their gills, showing that a secretory machinery exists even in the non-adapted fish, enabling it to compensate for short term changes in osmolarity. The seawater-adapted fish showed a dramatic increase in cotransporter protein, 2-fold above the freshwater fish, consistent with an increased expression of the secretory transporter in a saline environment to handle salt elimination in the steady state. The 2X seawater fish showed the greatest increase in signal, which is more than 5-fold above the freshwater samples. This suggests that the fish are able to adapt to extreme salinities, such as that which they might encounter in a partially evaporated tidepool. Overall, these data show that expression of the NaKCl cotransporter increases dramatically with an increase in environmental salinity, consistent with a critical role for NKCC in the gill during saltwater adaptation. A similar pattern

of upregulation has been observed in the trout, *Salmo gairdneri* (Behnke et al. *Bull. Mt. Desert. Is. Biol. Lab.* 35:24, 1996).

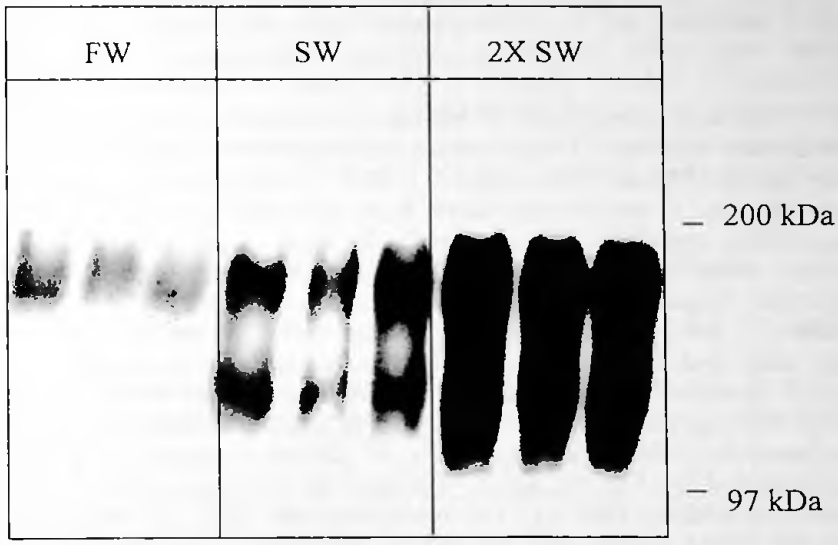

Figure 1. Gills were excised from killifish after the adaptation period and frozen in liquid nitrogen. Enriched cell membrane preparations were made from pools of frozen gills (5 fish per sample). Aliquots of equal amounts of total protein were separated on 7.5% acrylamide gels. The fully glycosylated cotransporter runs as the upper band, a proteolytic fragment constitutes the lower band.

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