FUNDULUS HETEROCLITUS 14-3-3.a INHIBITS AN ENDOGENOUS CHLORIDE CHANNEL IN *XENOPUS LAEVIS* OOCYTES

Devulapalli Chakravarty, Andrea Kohn, Robert Greenberg, and Dietmar Kültz
The Whitney Laboratory
University of Florida, St. Augustine, FL 32080

We have previously cloned a phospho-adapter protein called 14-3-3.a from *Fundulus heteroclitus* gill epithelium. Furthermore, we have shown that this protein and its corresponding gene are induced during transfer of fish from seawater to fresh water. 14-3-3 proteins bind to other proteins when these are phosphorylated on Serine or Threonine and are important modulators of such phospho-proteins and signaling complexes. Thus, we hypothesize that 14-3-3.a plays a key role in controlling the adaptive remodeling of fish gill epithelium that takes place during salinity change. Here we report that 14-3-3.a inhibits an endogenous Ca^{2+}-activated chloride channel (CaCl) in *Xenopus* oocytes. 14-3-3.a cRNA was transcribed from pGEMT vector using T7 RNA polymerase. Fifty nanoliter of 14-3-3.a cRNA were injected into oocytes and the current generated by a calcium-activated chloride channel that is endogenously expressed in *Xenopus* oocytes was monitored by whole cell recording after a 48 h incubation period. A control group, in which *Xenopus* oocytes were injected with 50 nL water was analyzed in parallel to the 14-3-3.a-injected oocytes. The results of this experiment demonstrate that fish 14-3-3.a inhibits a chloride channel (Fig. 1).

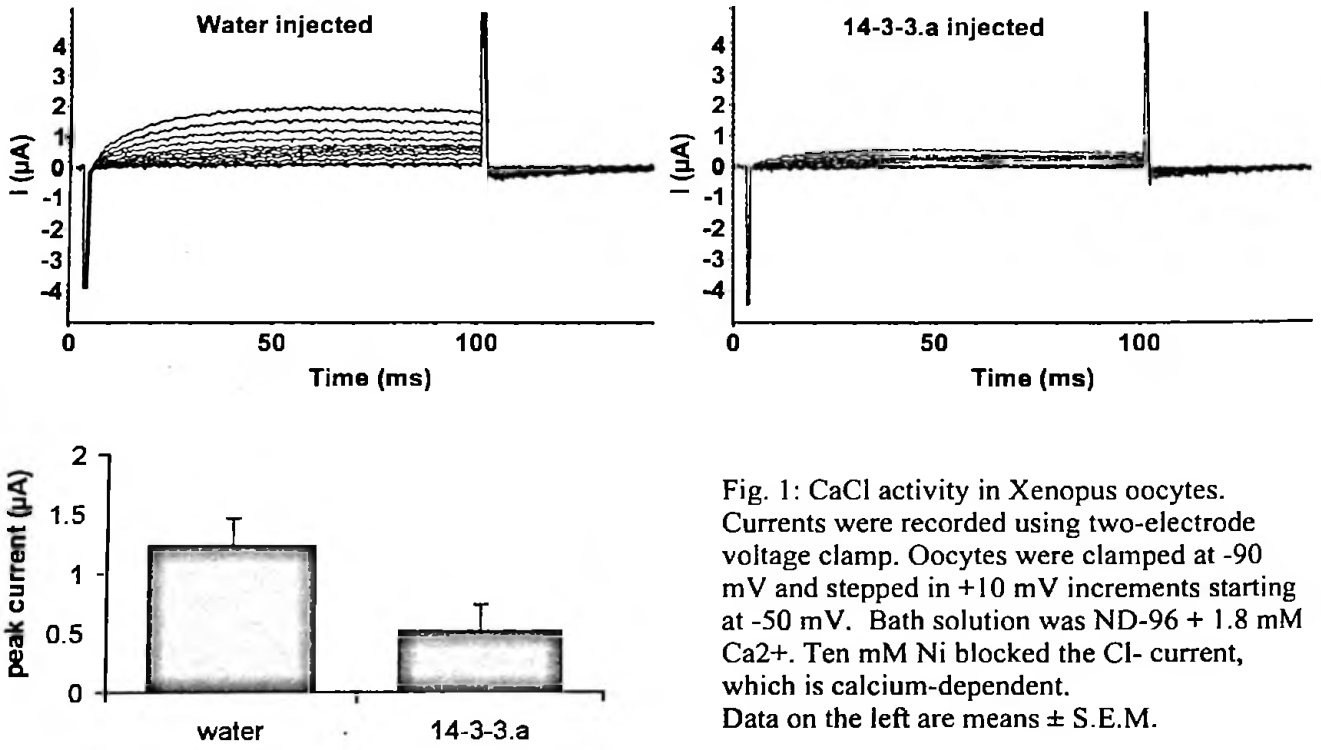

Fig. 1: CaCl activity in Xenopus oocytes. Currents were recorded using two-electrode voltage clamp. Oocytes were clamped at -90 mV and stepped in +10 mV increments starting at -50 mV. Bath solution was ND-96 + 1.8 mM Ca2+. Ten mM Ni blocked the Cl- current, which is calcium-dependent.
Data on the left are means ± S.E.M.

Since a CFTR-type chloride channel mediates salt extrusion across gill epithelium in seawater fish and 14-3-3.a is down-regulated under such conditions we interpret these data as evidence for a potential role of 14-3-3.a in the regulation of salt transport across teleost gills. This work was supported by the Salisbury Cove Research Fund (DK) and NSF MCB-0114485 (DK).