CLONING OF THE ANION EXCHANGER OF SKATE (*RAJA ERINACEA*) ERYTHROCYTE

Hélène Guizouarn[1], Leon Goldstein[2]
[1]Laboratoire Jean Maetz, Physiologie des membranes cellulaires,
CNRS- Université de Nice, 284 chem. du Lazaret, 06230 Villefranche-sur-Mer, France
[2]Division of Biology and Medicine, Brown University, Providence, RI 02912

Studies on fish red cell volume regulation have shown that the anion exchanger AE1 is involved in swelling-induced permeabilities (Garcia-Romeu, F. et al., Cellular and Molecular Biology, 42:985-994, 1996; Perlman, D.F. et al., Cellular and Molecular Biology, 42:975-984, 1996.). AE1 has been cloned from trout erythrocytes, and when expressed in *Xenopus* oocytes, this anion exchanger (tAE1) induces a large Cl^- conductance associated with an increase in taurine permeability (Fiévet et al., EMBO J. 14:5158-5169, 1995) as well as an increase in permeability of uncharged solutes such as urea or sorbitol (Fiévet et al., Proc. Natl. Acad. Sci. 95(18):10996-11001, 1998). In contrast, AE1 of mammalian erythrocytes does not induce a Cl^- conductance or taurine permeability when expressed in *Xenopus* oocytes. This work suggests that some AE1 isoforms are able to mediate an anion channel associated, volume-sensitive taurine loss. To confirm this feature of fish red cell AE, cloning of skate red cell anion exchanger (sAE) was initiated. Comparison of trout and skate erythrocyte volume regulation reveals both similarities and differences. The hyposmotic swelling of both cells increases taurine permeability and, in both cases, volume regulation is blocked by AE1 inhibitors. However, hyposmotic swelling of the trout erythrocyte induces a large increase in Na^+, K^+ and choline permeabilities not observed in the skate erythrocyte. Two different hypotheses are proposed to explain the induction of osmolyte permeability by AE1: either band 3 functions as a channel of broad specificity allowing structurally and chemically different solutes to cross the membrane, or it regulates different transporters in the membrane. The similarities and differences between the action of skate and trout AE1 in *Xenopus* oocytes will help to understand the role of AE1 in swelling induced permeabilities.

Total RNA from skate red cells was extracted in guanidinium thiocyanate-phenol-chloroform (Chomczynski and Sacchi, Anal. Biochem. 162:156-159, 1987) and polyA RNA purified by oligodT chromatography (Boehringer). cDNA was prepared by reverse transcription with AMV-Reverse transcriptase and oligodT primers (Clontech). Four different PCR were run with two pairs of degenerate primers designed from 4 highly conserved regions within the transmembrane domain of 13 already cloned anion exchangers from different tissues and species. Two other PCRs were run with an upstream primer designed from a partial amino acid sequence of the N terminal part of the anion exchanger of skate red cells (Musch et al., J. Biol. Chem. 274:7923-7928, 1999). The PCR products were cloned in pGEM T-easy vector (Promega). Recombinants were screened using white/blue selection and 14 positive clones were sequenced at the DNA sequencing facility at MDIBL.

Analyses of these sequences yielded 3 different nucleotide sequences of 1004, 1034 and more than 2000 bp (among which 1148 bp are already sequenced) with an open reading frame of 334, 344 and 382 amino-acids respectively. It appears that three different AEs, named sAEa,

sAEb and sAEc, are expressed in skate erythrocytes. There is 65% identity between sAEa and sAEb, 60% between sAEa and sAEc and 68% between sAEb and sAEc within cloned sequences. These three skate AEs are closer to human erythroid anion exchanger (hAE1) than to trout AE1 with, for example, 70% identical amino acids between sAEc and hAE1 and 64% identity between sAEc and tAE1. Until now, three different families of genes were known to code for multiple forms of AEs expressed either in erythroid cells (AE1) or in epithelial cells (AE2 and AE1) or in brain and heart (AE3). A major difference in the transmembrane domain of the 3 isoforms is the size of the extracellular loop between span 5 and 6; it is short in AE1 and much longer in AE2 with a noteworthy exception that AE1 of trout erythrocyte has a large extracellular loop between span 5 and 6 which is called the Z-loop, as in AE2. Among the three cloned sAEs, the size of this connecting loop is variable. Due to the great homology between the transmembrane domain of the different anion exchangers, it is difficult to determine whether these three AEs are three AE1 variants or three different isoforms. To answer this question we need to know the sequence of the N-terminal, cytoplasmic domain of these proteins, which shows considerable variability among AE1, AE2 and AE3.

In conclusion, three different anion exchangers are expressed in skate erythrocytes; their function remains to be characterized. Anion exchangers in red cells are involved in CO_2 transport and, as described in trout and skate red cells, they are also involved in swelling-induced taurine permeability. Expression studies in *Xenopus* oocytes will help to further characterize the function of these three sAEs.

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