POSITIVE IDENTIFICATION AND AMPLIFICATION OF Na^+/H^+ ANTIPORTER cDNA PREPARED FROM CRAB (CARCINUS MAENAS) GILL mRNA

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The Na^+/H^+ antiporter in crab and lobster is electrogenic, apparently exchanging 2 Na^+ for 1 H^+ (Shetlar and Towle, Am. J. Physiol. 257:R924-R931, 1989; Ahearn and Franco, Am. J. Physiol. 259:F758-F767, 1990). Despite its functional distinction from the electroneutral Na^+/H^+ antiporter of vertebrate tissues, we have hypothesized that the crustacean antiporter may share a structural relationship with the vertebrate antiporters. Sequence information is now available for four different isoforms of the antiporter from five mammalian species and one fish species (Counillon and Pouysségur, Soc. Gen. Physiol. Series 48:169-186, 1993).

Downloading many of the known antiporter sequences from the GENBANK database and aligning them using DNASIS and MULTALIN software has revealed a number of regions of homology among the vertebrate antiporters. On the basis of these conserved regions, we designed several pairs of degenerate oligonucleotide primers directed toward amplification of the crab antiporter by the polymerase chain reaction (PCR). The amino acid sequences which provided the basis for these primers are indicated by the designations 5A, 3F, and 4R in Figure 1.

We isolated Carcinus gill mRNA under RNAse-free conditions and submitted the mRNA to reverse transcription using oligo-dT as the primer. Employing the resulting cDNA as a template, we were able to amplify a 700-base-pair fragment using one pair (3F and 4R) of the newly designed primers. Following ligation into the TA cloning vector, transformation of E. coli and overnight culture, the cloned plasmid was isolated and purified. Upon sequencing the 3F-4R fragment using the dideoxynucleotide method modified to reduce premature chain terminations (Sanger et al., Proc. Natl. Acad. Sci. USA 74:5463-5467, 1977; Kho and Zarbl, Biotechniques 12:228-230, 1992), we discovered a nucleotide sequence possessing an open reading frame for protein synthesis.

A second PCR amplification employing a different forward primer (5A) but the same reverse primer (4R) produced an expectedly larger (800-base-pair) fragment whose sequence completely overlapped that of the first 700-bp fragment. Moreover, translation of the 5A-4R nucleotide sequence to the corresponding amino acid sequence again revealed an open reading frame. Analysis with MULTALIN demonstrated substantial homology of the crab fragment with the human, pig, and trout NHE-1 isoforms (Fig. 1), particularly in the putative membrane-spanning regions of the antiporter protein.

A search of the complete 146,000-sequence GenBank database using the BLAST algorithm (Altschul et al., J. Mol. Biol. 215: 403-410, 1990) revealed ten high-scoring matches with our 800-bp crab cDNA sequence, all of them Na^+/H^+ antiporter sequences from a variety of tissues and species. We are thus very confident that we have isolated and sequenced a portion of the actual crab Na^+/H^+ antiporter. The fact that the Carcinus sequence is not identical but similar to other Na^+/H^+ antiporter sequences suggests that the crustacean electrogenic antiporter belongs to the larger family of Na^+/H^+ antiporters and is not totally unique.

We have employed the 800-bp Carcinus antiporter fragment as a DNA probe in Northern blot analysis of Carcinus gill messenger RNA. Following electrophoresis of the mRNA and transfer to a nylon filter, hybridization with the ^{32}P-labelled probe disclosed a corresponding mRNA of approximately 4,000 nucleotides. We have therefore sequenced approximately 20% of the total cDNA sequence coding for the crustacean antiporter. Current work is devoted to completing the sequencing of the antiporter, permitting studies of its physiological function with respect to salinity acclimation.

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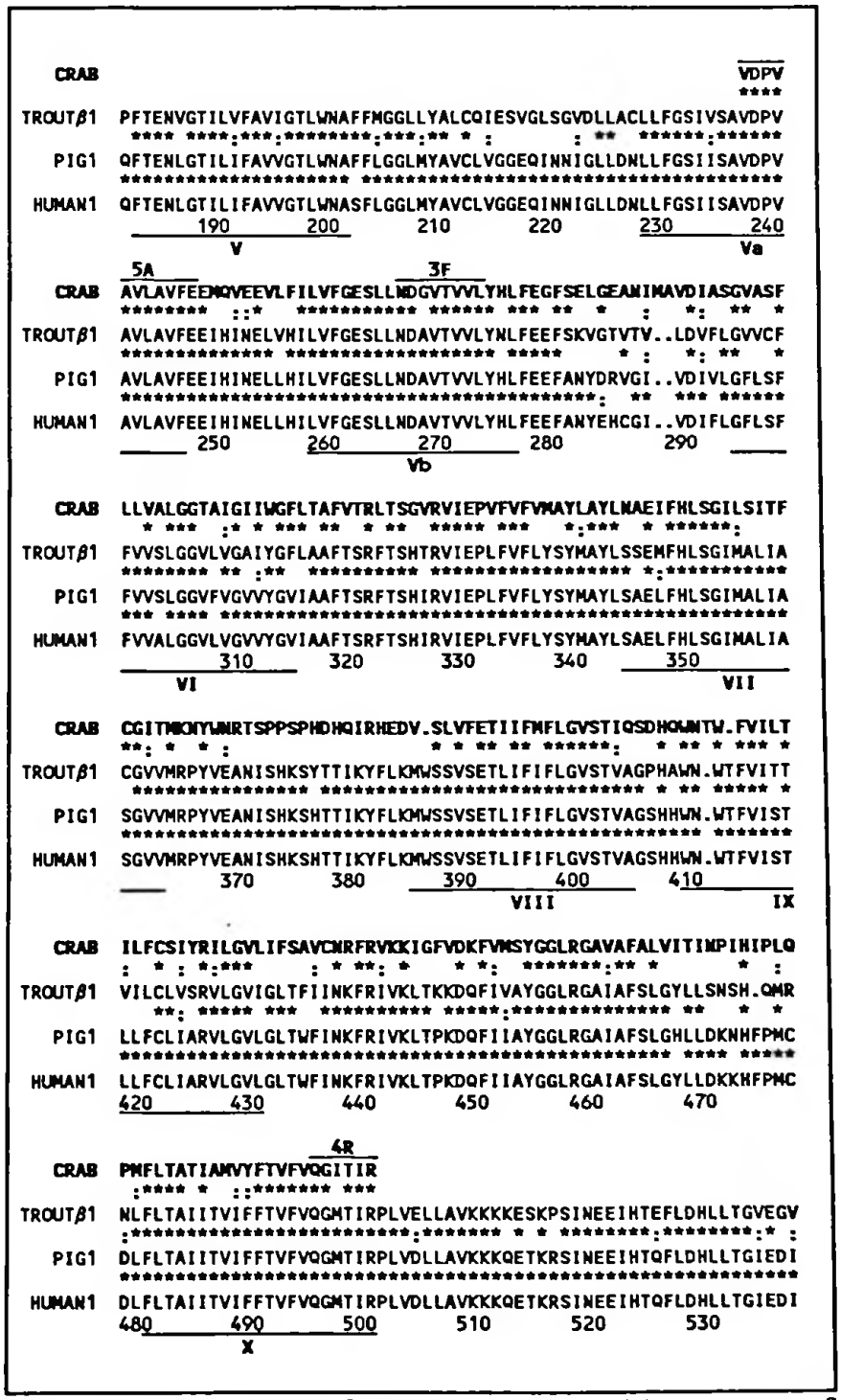

Figure 1. Alignment of deduced amino acid sequence of crab antiporter fragment with selected target sequences. Putative membrane-spanning domains are numbered (V-X) after Counillon and Pouysségur, 1993.