cDNA LIBRARIES PRODUCED FROM THE LITTLE SKATE RAJA ERINACEA AND WINTER FLOUNDER PSEUDOPLEURONECTES AMERICANUS EPITHELIUM

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Contemporary molecular biology techniques allow cDNA libraries to be made from tissues with relative ease. cDNA libraries are useful in cloning cellular proteins in that they are based on the mRNA molecules which exist in the cells of a tissue at the time those cells are collected. Thus cDNA libraries are specific to a given tissue or cell and represent the proteins being produced within those cells, unlike libraries based on genomic DNA. They also offer the advantage that the nucleotide information they contain can be directly translated into the protein peptide sequence. Using such cDNA libraries a number of ion transport proteins and channels have now been cloned. Through cloning of homologous transport proteins from different species, one can gain insight into the structure-function relation of the protein by comparison of the amino acid sequence with the biophysical properties. Such studies take advantage of the evolutionary process and can quickly reveal areas of a protein that are important for function. Some proteins show a high degree of homology across species, such as the cystic fibrosis transmembrane conductance regulator (CFTR) which is 72% homologous between human and the dogfish shark Squalus acanthias (Marshall et. al., J. Biol. Chem. 266:22749-22754, 1991). The biophysical characteristics of CFTR between human and shark are also similar with only a small difference in the single channel conductance (Hanrahan et. al. Ped. Pulmon. abs., 1992 and in this bulletin). Comparison of species differences in similarly functioning proteins can also lend information as to the evolutionary divergence of structure and function. Such information can be used to complement the physiological studies of ion transport in marine species which have been so important in our understanding of mammalian ion transport.

cDNA libraries were constructed from the gill and kidney of a teleost fish, the winter flounder Pseudopleuronectes americanus and from the alkaline and rectal gland of an elasmobranch, the little skate Raja erinacea. Gill arches from two flounder were excised and the epithelial cells scraped from the cartilage with a glass slide. A single flounder kidney was exposed , removed intact, and rapidly cut into pieces on ice before homogenization with a tissue homogenizer. Three pairs of alkaline glands were carefully dissected away from the surrounding tissue and removed one at a time. The glands were then opened and the epithelial cell layer scraped using a glass slide. Three skate rectal glands were removed and cut into pieces on ice and homogenized. All tissues were immediately homogenized into a buffer containing mRNase inhibitors. PolyA mRNA was isolated using the FastTrack mRNA Kit (InVitrogen). A sample of mRNA was quality analyzed from each preparation by formaldehyde gel electrophoresis prior to cDNA synthesis. cDNA was synthesized from 5μg mRNA and uni-directionally inserted into the Uni-ZAP vector using the ZAP-cDNA synthesis Kit (Stratagene). The cDNA was size fractionated on a Sephacryl S-400 column and only the larger sized cDNA fractions (>500 bP, by gel analysis) ligated into vector. ZAP-cDNA was then packaged using Gigapack II Gold Packaging Extract (Stratagene) to produce the 1° library. 1° library was then titered and 1×10^{6} plaques amplified, to produce ~200 mL of high titer ($>10^{8}$ pfu/mL) library. The amplified library was subsequently plated at low density so that isolated clones could be obtained. 20 individual plaques were randomly picked from each library and subjected to PCR (32 cycles, 5 min elongation) with T3 and T7 primers to estimate the range of insert sizes.

Table 1. Initial Library Titer and the largest insert size detected by PCR in 20 random picks from each library constructed.

Tissue	1° Titer pfu/library	Largest insert detected (kb)
Flounder Gill	7.0×10^5	4.5
Flounder Kidney	7.4×10^5	2.5
Skate Alkaline Gland	4.2×10^5	3.4
Skate Rectal Gland	4.4×10^5	3.5

The titer of the 1° library is used to indicate how well the library represents the proteins being synthesized in the given tissue, and is shown in Table 1. Membrane proteins can be rare, only representing a small fraction of the total cellular protein, likewise the mRNA that codes for those proteins can be rare with representation as low as 1 copy in 50-100K mRNA molecules. The low mRNA copy number thus requires high titer libraries for membrane protein cloning. Ion channels and transporters can be large requiring > 2kb of message to code for the full length protein. We chose to use PCR as a means of quickly estimating the insert sizes within each library. Since the success rate of PCR is decreased with greater lengths of DNA, this technique is only useful in determining that inserts at least as large as the one detected occurs in the library. The largest insert size detected within the 20 samples from each library are listed in Table 1, however some samples did not successfully PCR which could indicate insert sizes beyond the capability of our PCR conditions. The titer and insert size range of these libraries should make them useful for cloning ion channel and transporter proteins or other cellular proteins > 20 kD (> 500 bp of mRNA) in size. Since cDNA was incorporated into the ZAP vector system these libraries can be both nucleotide and immunoscreened, and inserts directly rescued into the pBluescript plasmid.

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