ISOLATION AND IN VITRO EXPRESSION OF DOGFISH (SQUALUS ACANTHIAS) mRNA

Alison I. Morrison, Robert E. Shetlar
Max-Planck-Institut fuer Systemphysiologie, D-4600 Dortmund, F.R.G.

The rectal gland, the kidney and the intestine of the dogfish (Squalus acanthias) have been successfully used as models for mammalian transepithelial solute transport. Initial studies of the transport components of these tissues involved the isolation of mRNA and its subsequent characterization in terms of its composition and ability to be expressed in an in vitro translation system. Dogfish were maintained in live cars until needed. The fish were killed by spinal transection and the rectal glands, intestine and/or kidneys were removed, placed on dry ice and stored at -80'C until required. Total RNA was isolated by the method of Ullrich et al. (Science, 196:1313-1319, 1977). mRNA was isolated using an oligo dT affinity column (Aviv and Leder, PNAS. 96:1408-1413, 1972).

The amounts of mRNA recovered from the different tissues per gram wet weight were 19.4 µg from rectal gland, 9.5 µg from kidney and 10.6 µg from intestine. Agarose gel electrophoresis revealed that the mRNA samples contained a broad variety of mRNAs of different size but, in contrast to results for rabbit kidney and intestine, distinct mRNAs in much higher concentration were also present. These bands correspond to 3.30, 2.18, 1.80, 1.14 kilo base pairs (kbp) in the rectal gland, 3.30, 2.18, 1.61, 1.14 kbp in the kidney and 1.69, 1.17 kbp in the intestine. These mRNAs probably represent multiple copies of the mRNA coding for particular proteins of importance for the cells. It is interesting to note that identical organ specific patterns were observed in the skate mRNA isolated according to the same procedure.

To investigate the expression of mRNA obtained from the rectal gland, experiments were carried out using the Amersham Reticulocyte lysate kit. In pilot experiments it was found that a concentration of 180 mM magnesium and 0.70 mM potassium per reaction was required for the maximal expression of shark rectal gland mRNA. After fluoro-autoradiography of the expressed shark rectal gland protein, several bands were visible the most prominent at 87, 75, 62 and 50 kDa. From the mRNA data, expression of proteins of estimated molecular weight 121, 80, 59, 42 kDA would have been expected if the full sequence of the mRNA was translated. The discrepancy may be due to a number of possible factors such as post translational processing or premature termination of translation. Further studies are being carried out to examine these possibilities and determine the functional role of the expressed shark rectal gland proteins.

These studies thus demonstrate that it is possible to obtain mRNA from shark tissue which can be translated into protein. The functional role of this protein remains to be determined.

Supported by a fellowship from the Lucille P. Markey Trust to AIM and a travel grant from the Max-Planck-Institut to AIM and RES.