STRATEGIES FOR A MOLECULAR CHARACTERIZATION OF RENAL Na/P_i COTRANSPORT SYSTEMS IN FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

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In the past three years a variety of renal Na/P_i cotransport systems have been cloned. They can be divided into two different groups: The first type, characterized in rabbit kidney (rabbit NaPi-I), plays a yet unknown role in renal P_i handling. Type II Na/P_i cotransporters have been cloned from rat and human kidney cortex. They contribute significantly to the reabsorption of P_i from the primary urine and are responsive to changes of blood P_i levels (Magagnin et al., Proc. Natl. Acad. Sci. USA 90: 5979-5983, 1993). Functional studies using brush border membrane vesicles and cell culture models lead to the hypothesis (Renfro et al., Comp. Physiol. Basel, Karger, vol 7: 216-240, 1990) that flounder kidney expresses a corresponding system. Since we had the rat Na/P_i cotransporter cDNA (rat NaPi-II) available we wanted to search for a Na-dependent P_i transporter in flounder renal tubules. Two different strategies were chosen: In a first set of experiments mRNA from different flounder tissues were analyzed for mRNA species related to NaPi-II by Northern blot. The second approach involved the functional expression of Na/P_i cotransport activity in Xenopus laevis oocytes.

Total RNA from different flounder tissues (renal tubules, erythrpoetic tissue, intestine) was extracted following the guanidinium thiocyanate-phenol-chloroform method described by Chomczynski and Sacchi (Anal. Biochem. 162, 156-159, 1987). After oligo(dT) affinity purification mRNA was length separated on a 1% denaturing agarose gel. Nucleic acids were blotted to a nylon membrane and hybridized to ^{32}P labelled rabbit NaPi-I and rat NaPi-II cDNA. The autoradiographs showed no crossreactivity between flounder mRNA and rabbit NaPi-I. Very weak signals were observed with mRNA from renal tubules and intestine hybridized to rat NaPi-II cDNA. In order to confirm these preliminary observations total mRNA from flounder renal tubules was length separated over a 6-20% sucrose gradient. The mRNA fractions were analyzed by Northern blot using a coding region fragment of rat NaPi-II as a probe. A single band was found in two neighbouring fractions (#6 and #7) representing mRNA species of 2-3 kb in length. This result provided us with the basic information needed to clone this system related to mammalian Na/P_i cotransporters.

We tested the relevance of this observation on a functional level using the Xenopus laevis oocyte expression system. All the methods dealing with oocytes as well as the flux measurement assay for Pi and sulfate have been described (Werner et al. J. Biol. Chem. 265: 12331-12336, 1990). The above mentioned mRNA fractions (5-20 ng/ oocyte) were injected, and three days later the oocytes were assayed for P_i and sulfate transport activity. Water and total mRNA injected oocytes were used as controls. The

results are presented in Figure 1A and 1B. The expression of Na/P_i transport activity in oocytes with mRNA derived from flounder tubules turned out to be difficult. The effects (fraction #7 was supposed to give the highest signal according to the results from the Northern blot) were hardly reproducible and often influenced by side effects. The extent to which these irregularities can be attributed to different Pi-transport-inducing mRNA species in flounder kidney may be answered by having the flounder type-II Na/Pi cotransporter clone available. Even though different P_i transporting systems may exist in renal epithelium the increased flux rates in #9 are likely to reflect systemic problems.

Figure 1A

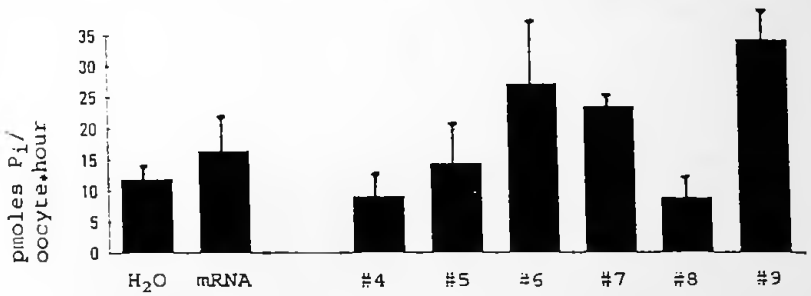

Figure 1B

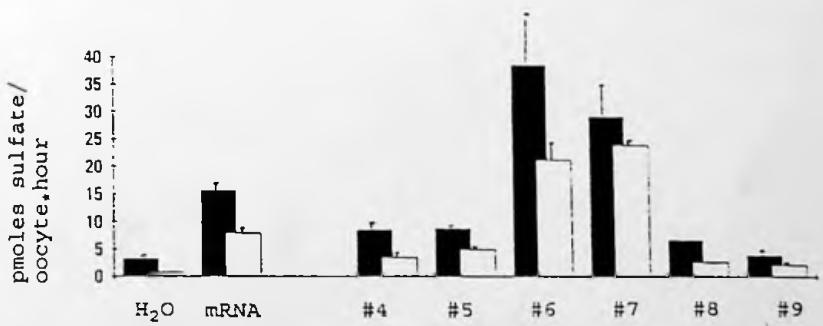

Figure 1A: Na-dependent P_i transport activity in water (50 nl/oocyte), total mRNA (20 ng/50nl), and fractionated mRNA (5-15ng/50nl) injected oocytes. After injection the oocytes were incubated for 3 days and then assayed for transport. The bars represent the mean $\pm$ SD.

Figure 1B: Sulfate transport activity was determined in Xenopus laevis oocytes to demonstrate the integrity of the injected mRNA. The oocytes were injected and incubated as above. The filled bars represent the total sulfate transport activity, the open bars the sodium-independent component.

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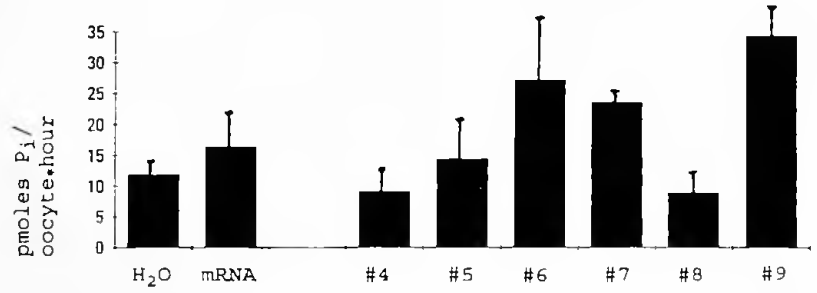
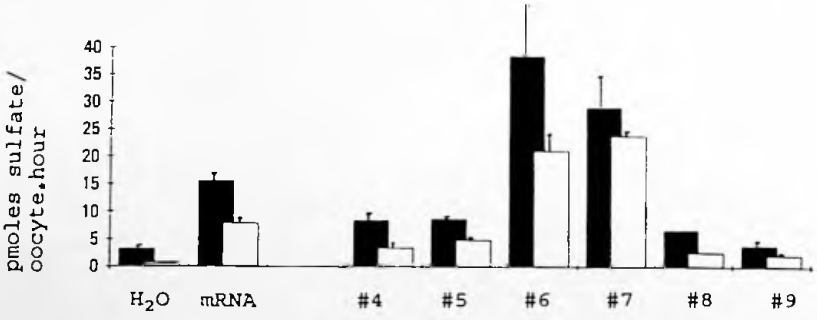


Figure 1B



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