MOLECULAR IDENTIFICATION AND TISSUE DISTRIBUTION OF A MULTIDRUG RESISTANCE ASSOCIATED PROTEIN (Mrp2) ISOLATED FROM THE LIVER OF THE LITTLE SKATE, *RAJA ERINACEA*

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Members of the MRP subfamily of ABC transporters function as multidrug export pumps for a variety of organic conjugates and are widely expressed. One member of this family, human MRP2 and rodent Mrp2 is localized to the apical membrane of hepatocytes where it functions to extrude a diverse group of compounds including bilirubin-glucuronide, glutathione and glutathione conjugates. We have previously described an ATP-dependent GSH and glutathione S-conjugate transporter in isolated plasma membrane vesicles from skate liver (Rebbeor et al. AJP 279:G417-G425, 2000). These studies support in-vivo and in-vitro studies from our laboratory in the free swimming skate (*Raja erinacea*) and isolated skate liver and hepatocyte cultures that demonstrate biliary excretion of Mrp2 substrates.

To identify this transporter at the molecular level, we developed degenerate oligonucleotide primers that hybridized with two highly conserved regions in Mrp2s from human, rabbit, mouse, *C.elegans* and yeast. Using RT-PCR, a 410 band fragment was amplified from both skate liver and kidney total RNA. DNA sequencing confirmed that this fragment coded for a portion of the Mrp2 protein. A ^{32}P labeled fragment was prepared and used to screen a skate liver cDNA library. Colonies were isolated that contained up to 4 kb of the *Mrp2* gene. After 5'RACE, a full-length skate *Mrp2* gene was identified, which contains 5870 bp and encodes 1564 AA, with 197 bp 5'UTR, and 1033 bp 3'UTR. Genbank BLASTing indicates that this cDNA is an ABC transporter with the highest identities corresponding to human MRP2 and rabbit Mrp2 (56% each), mouse and rat Mrp2s (54%), and *C.elegans* Mrp2 (44%). Figure 1 illustrates a phylogenetic tree for the Mrp2 family. Computer modeling predicts 17 transmembrane domains, 2 ATP binding cassettes, 2 N-glycosylation sites, 6 PKC phosphorylation sites, and one tyrosine phosphorylation site in skate Mrp2. An apical targeting sequence, TAL has also been found at the C-terminal of sMrp2. Northern-blot analysis in multiple skate tissues showed that sMrp2 is a 6 kb transcript and is expressed in liver, kidney, and intestine. A rabbit polyclonal antibody was raised to a C-terminal peptide of sMrp2 and detects a 180 kD band in skate liver vesicle membrane in a Western-blot. Peptide competition experiments completely eliminate the staining of this 180 kD band. Immunofluorescence studies localize sMrp2 to the canalicular membrane of skate liver, the apical membrane of proximal convoluted tubes of the skate kidney, and the apical membrane of enterocytes in the skate small intestine. Mutation sites in the hyperbilirubinemic Dubin-Johnson Syndrome in man and the TR$^-$ /Groningen/EHBR rats are all conserved from this evolutionary sMrp2 precursor.

These findings indicate that a member of the Mrp2 subfamily is highly expressed in skate liver at the canalicular apical excretory domain. It is likely that this transporter is responsible for the canalicular excretion of a variety of organic anions (BSP, bilirubin, biliverdin, carboxyfluorescein diacetate, lucifer yellow) that we have demonstrated previously are excreted

in high concentrations in bile of this marine vertebrate. Supported by DK 25636(to JLB), DK48823 (to NB) and ES 03828 from the USPHS

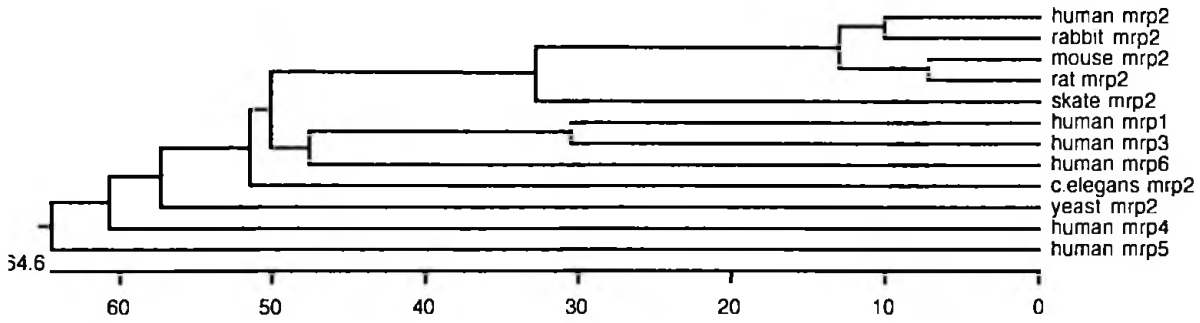

Figure 1. Phylogenetic tree of MRP2/Mrp2s and other human MRPs.