MOLECULAR IDENTIFICATION OF THE BILE SALT EXPORT PUMP (SISTER OF P-GLYCOPROTEIN) CLONED FROM LIVER OF THE SMALL SKATE, *RAJA ERINACEA*.

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Bile secretion is a fundamental function of vertebrate liver. Bile is formed by osmotic filtration following the transport of osmotically active substances from the liver into bile. Several members of the ATP Binding Cassette (ATP) super gene family reside on the apical canalicular membrane of the hepatocyte and function as export pumps to form this secretion. Recently, an ABC transporter known as the bile salt export pump (bsep) or sister of P-glycoprotein (spgp) has been cloned from rat and human liver (Gerloff et al., J. Biol. Chem 273:10046-10050,1998; Strautnieks SS, et al. , Nat. Genet. 20:233-238,1998). When rat bsep is expressed in Sf9 cells, it transports bile salts. Mutations in BSEP in man result in an inability to excrete bile salts into bile and the syndrome of Progressive Familial Intrahepatic Cholestasis type 2. Previous studies from this laboratory (Ballatori et al. Bull. MDIBL 38:46-47,1999) have provided functional and structural evidence for a bsep-like protein in the canalicular membrane of skate liver. We now report the cloning of a full length bsep skate homologue.

A skate liver cDNA library was screened using probes designed from the spgp ATP binding cassette domain of *Fundulus heteroclitus* (~400 bp RT-PCR product, a gift from Peter Cooper) and the spgp carboxy terminal genomic DNA from the winter flounder , *Pseudopleuronectes americanus* (~200bp , a gift from Peter David) with low stringency washes. Positive clones were then sequenced. Genbank data base searches identified several clones that were closely matched to MDR1 (P-glycoprotein) while others were matched to both rat bsep and human BSEP. Following two rounds of library screening, 3 full-length skate bsep clones were identified. The full-length sequence contains an open reading frame which encodes for 1348 amino acids (27 amino acids larger than human and rat bsep) and shares ~ 65% identity to human BSEP. The clone contains 580 bp 3' UTR and 480 bp 5' UTR.

Northern analysis of RNA from multiple tissues from the small skate (Fig 1) demonstrated a 5 kb transcript only in liver, similar to the expression of this gene in rat and human . Using a single exon region as probe, Southern-blot analysis demonstrated that skate bsep is a single copy gene.

Figure 2 illustrates a phylogenetic tree generated by cluster analysis that compares several members of the ABC family of transporters know to be present in liver, in particular, bsep, Mdr1 (P-glycoprotein) and Mrp2 (multidrug resistance associated protein). This analysis reveals that bsep and Mdr1 share a common ancestor and are differentiated from a primitive Mdr gene. Since bsep does not have close homology to invertebrate mdr proteins in the fly, *C.elegans* or yeast and is liver specific, we speculate that bsep may first appear in vertebrates The substrates

for skate liver bsep remain to be determined. However it is anticipated that the ancestrial Mdr protein should have a broad range of substrates as is the case for the yeast Mdr homologue Bat1 which transports the bile salt taurocholate with low affinity.

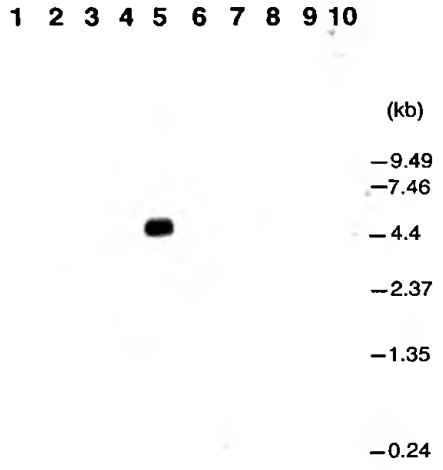

Figure 1. Northern-blot of multiple skate tissues by using P32 labeled skate bsep gene fragment as probe. Lane 1, brain; 2, heart; 3, kidney; 4, intestine; 5, liver; 6, pancreas; 7, rectal gland; 8, spleen; 9, stomach; 10, testes.

Figure 2. Phylogenetic tree of skate bsep and mdrs from different species. The tree was generated by MegAlign module of Lasergene software.