Bile salts are weak modulators of *Fxr*, *Bsep*, *Ostα, and Ostβ* gene expression in isolated hepatocytes from *Leucoraja erinacea*, the little skate

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Bile salts modulate gene expression by activating nuclear receptors in mammals[1]. Chenodeoxycholic acid (CDCA), the major bile acid in human, is the most potent ligand of the nuclear receptor FXRα/Fxrαs (NR1H4), although it activates mouse Fxrα to a less extent than human FXRα. FXRα/Fxrα plays a pivotal role in maintaining bile salt homeostasis by regulating the expression of several key genes involved in bile salt synthesis, metabolism and transport[1]. Upon bile salt stimulation, FXRα/Fxrα directly up-regulates BSEP/Bsep (ABCB11/Abcb11), the bile salt export pump and the organic solute transporter, OSTα-OSTβ/Ostα-Ostβ, the basolateral bile salt efflux transporter both in vivo and in vitro, including isolated hepatocytes and hepatoma cell lines[2,3]. The bile salt response elements in these genes have been identified[4]. FXRα/Fxrα also down-regulates the expression of Cyp7A1, the rate limiting enzyme converting cholesterol to bile salts, and NTCP/Ntcp (SLC10A1/slc10a1), the sodium-dependent taurocholate cotransporting polypeptide, by controlling the expression of Shp (Nr0b2), the small heterodimer partner. Interestingly, bile salts have been shown to stimulate the expression of FXRα/Fxrα itself[3]. In addition, FXRα/Fxrα has also emerged as a key player in lipid and glucose metabolism. Furthermore, Fxrβ, a homolog of Fxrα in rodents does not respond to bile salts but to lanosterol, a precursor of cholesterol, but its functional role is unknown. We have previously identified and functionally characterized several genes involved in bile salt transport from the liver of the small skate, *Leucoraja erinacea*, including Bsep, Ostα-Ostβ, and an Oatp[5,6]. However it is not known if the expression of these genes is modulated by bile salts as are their mammalian orthologues. In a previous study we indirectly assessed whether skate Fxr was able to regulate these genes in the small skate following bile duct ligation (BDL) and found that skate Bsep and Ostα mRNA expression were slightly but significantly up-regulated. To more directly investigate if bile salts and Fxr play a role in this regulation, mRNA expression was assessed in isolated skate hepatocytes following treatment with bile salts and other nuclear receptors ligands.

Skates were caught by trawl from the coast of Maine and hepatocytes were isolated by collagenase perfusion. Hepatocytes were cultured in 6-well plates by maintaining in serum-free Elasmobranch Ringer solution at 12°C. Bile salts and chemicals were freshly prepared as 10X solution and directly added to the culture medium to treat hepatocytes for up to 6 days. Total RNA was extracted from cultured hepatocytes using Trizol and purified with Qiagen RNeasy MinElute Cleanup kit. Five microgram of total RNA from each sample was reverse-transcribed to cDNA as template for quantitative PCR. A set of primers and TaqMan probe were designed with Primer Express Software (Applied Biosystem Inc.) and synthesized by Integrated DNA Technologies Inc. (Coralville, IA) for skate *Bsep*, *Ostα*, *Ostβ*, *Fxr*, and *β-actin* genes, respectively. Quantitative real-time RT-PCR was performed on an ABI 7500 DNA Sequence Detection System. β-Actin was used as a reference to normalize the amount of target genes. Data from treated cells were normalized to controls values set as 1. Surprisingly, unlike their mammalian orthologues, mRNA expression of skate *Bsep* and *Ostα* was not significantly altered in isolated hepatocytes when treated with bile salts, scymnol sulfate, the major skate bile salt, CDCA, or skate bile for 48 hours (Fig. 1A). The expression of these genes was also not enhanced by ligands of other mammalian nuclear receptors, including lanosterol for mouse Fxrβ, rifampicin for PXR, bilirubin for CAR, all-trans retinoic acid and TTNPB for RAR (Fig. 1A). The

expression of skate Fxr was also unaffected by these ligands (Fig. 1A). Similar results were obtained when ligands were incubated for 24 or 72 hours for Fxr, Bsep, and Ostα. However, Ostβ was increased about 2-fold when hepatocytes were treated with scymnol sulfate for 3 and 6 days (Fig. 1B). In summary, these results indicate that bile salts are weak modulators of bile salt transporter gene expression in cultured skate hepatocytes when compared with their mammalian orthologues, suggesting that bile salts evolved later in evolution as ligands for the regulation of genes involved in bile salt transport. These studies were supported by National Institutes of Health Grants ES01247, DK34989, DK25636, and the NIEHS Center for Membrane Toxicity Studies (ES03828).

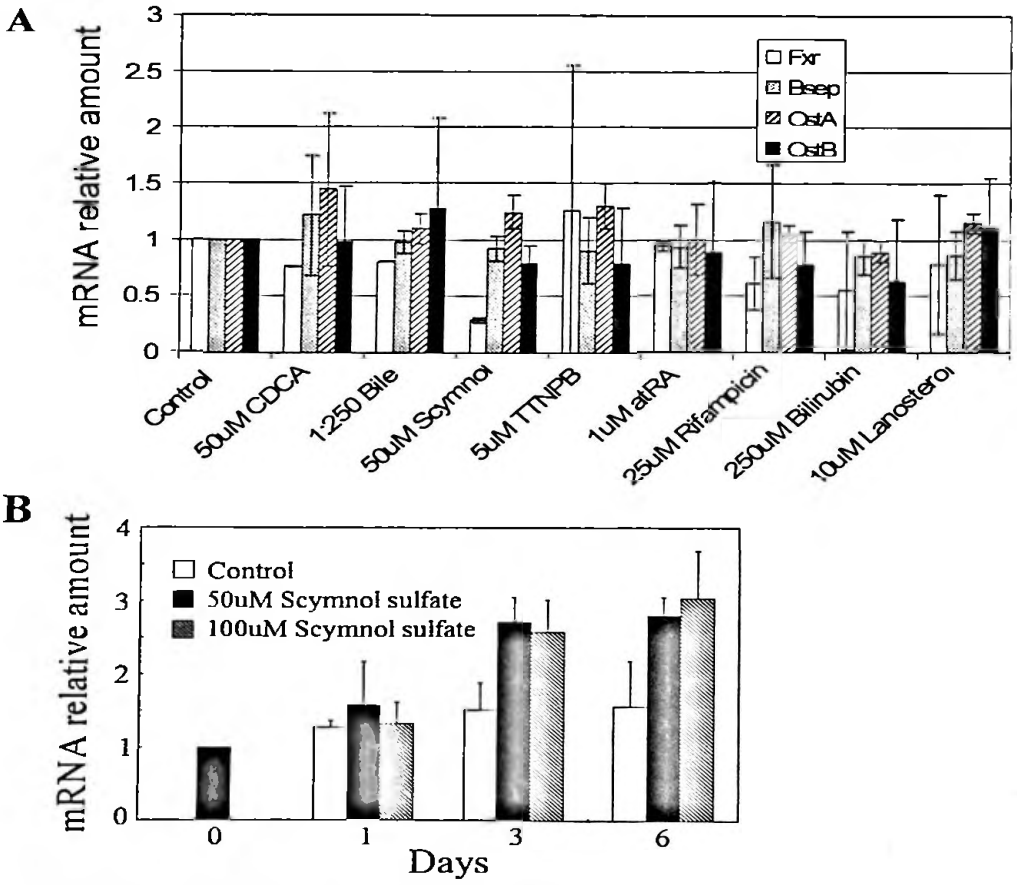

Figure 1. Relative mRNA expression of genes from cultured skate hepatocytes by quantitative real-time PCR. Data normalized to β-actin. A. Bsep, Ostα, Ostβ, and Fxr gene from hepatocytes treated with the indicated chemicals and concentrations for 48 hrs at 12°C, N≥3. B. Time course of Ostβ expression when treated with scymnol sulfate.

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