Bile duct obstruction and drainage modulates *Bsep* and *Ostα* gene expression in *Leucoraja erinacea*, the little skate

Shi-Ying Cai[1], Shuhua Xu[1], Ned Ballatori[2,3], and James L. Boyer[1,3]
[1] Liver Center, Yale University School of Medicine, New Haven, CT 06520
[2] Dept. of Environmental Medicine, Univ. of Rochester School of Medicine, Rochester, NY 14642
[3] Mount Desert Island Biological Laboratory, Salisbury Cove, Maine 04672

Bile salts play an important role in maintaining normal body functions not only because they facilitate food digestion and nutrient absorption, but also because they serve as key signaling molecules in the enterohepatic circulation. Bile salts activate nuclear receptors, specifically FXR, and regulate the expression of a number of genes in mammals[1]. Several key genes which are involved in bile salt synthesis in the liver and their enterohepatic circulation are regulated by FXR, including Cyp7A1 (the rate limiting enzyme converting cholesterol to bile salts) via regulation of Shp, and Bsep (Abcb11), the bile salt export pump. In addition, a bile acid response element in the human BSEP promoter region has been identified[2]. We have previously cloned and functionally characterized a Bsep orthologue from the liver of the small skate, *Leucoraja erinacea*. However it is not known how this gene is regulated [3]. More recently, we have identified Ostα (organic solute transporter alpha) as a heteromeric partner for the basolateral bile acid efflux transporter in ileum[4,5]. Ostα is highly expressed in skate liver, but whether it undergoes adaptive regulation is not known. To address these questions, we performed bile duct ligation and biliary drainage in the small skate, procedures that increase and decrease bile retention in the liver and circulation.

Sham, common bile duct ligation (CBDL), and bile drainage (BD) were carried out for 7 days in four skates for each group. Skates were caught by trawl from the coast of Maine. Total RNA was extracted with Trizol from liver, kidney, and intestine and further treated with Qiagen RNeasy kit. A set of primers and TaqMan probe were designed with Primer Express Software (Applied Biosystem Inc.) and synthesized by IDT (Integrated DNA Technology) for skate *Bsep*, *Ostα*, and *β-actin* genes, respectively. Quantitative real-time RT-PCR was performed on an ABI 7700 DNA Sequence Detection System. To minimize variations in gene expression between individual animals, equal amount of total RNA from each sample were pooled together for each group. β-Actin was used as a reference and data from CBDL and BD groups were compared with the sham animals. The results suggest that Bsep is up-regulated in CBDL liver by 41% and downregulated in BD liver by 53% (Fig. 1A). These findings are consistent with observations obtained from mammalian liver and HepG2 cell lines[2,3], and suggest that skate Bsep may also be regulated by FXR in response to the retention or depletion of the bile salt (bile alcohol) pool. Similar to Bsep in liver, Ostα expression in kidney was up 48% in the CBDL group and down 74% in the BD group (Fig. 1C). But Ostα was down-regulated in both CBDL and BD intestines by 51% and 39% respectively (Fig. 1D). These results suggest that the expression of skate Ostα gene in kidney and intestine is also regulated by the systemic retention of bile. However no significant difference was observed for Ostα gene among sham, CBDL, and BD treatment in liver (Fig.1B). We also measured the relative abundance of skate Ostα gene expression in liver, kidney and intestine by using β-actin as a reference. These results show that the Ostα is most abundant in liver, since the mRNA ratio was 800:150:1 liver>kidney> intestine, consistent with our previous report[4]. Taken together, our findings indicate that bile salts (e.g. skate symnol sulfate), modulate gene expression in the small skate, presumably via FXR, which we have also recently characterized from skate liver. These studies were supported by National Institutes of Health Grants ES03828, ES01247, DK34989, and DK25636.

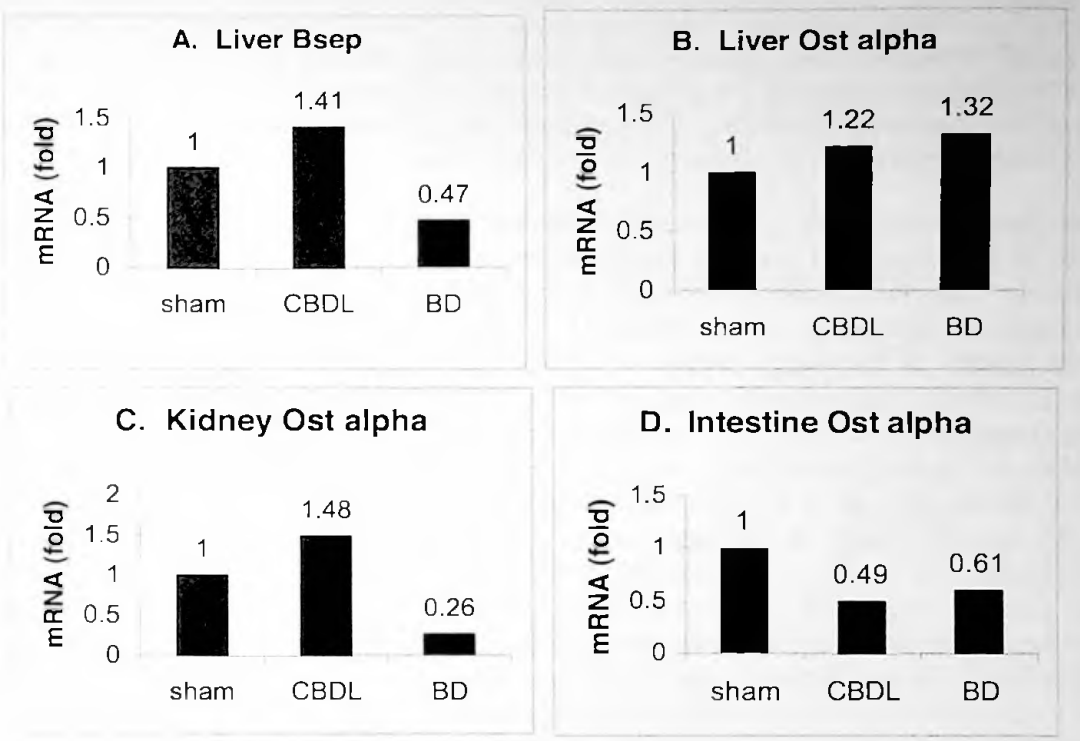

Figure 1. Skate Bsep and Ostα expression in sham, CBDL, and BD treated liver, kidney and intestine by Q-RT-PCR. A, skate Bsep expression in liver from pooled samples; B, skate Ostα expression in liver from pooled samples; C, skate Ostα expression in kidney from pooled samples; D, skate Ostα expression in intestine from pooled samples.

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