LOCALIZATION OF ENDOGENOUS XENOBIOTIC TRANSPORTERS IN ISOLATED CLUSTERS OF POLARIZED SKATE (*RAJA ERINACEA*) HEPATOCYTES

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Isolated skate hepatocytes retain their structural and functional integrity as clusters of polarized cells arranged around a central bile canaliculus. This in vitro model has served as an excellent system for the characterization of xenobiotic transport mechanisms given that the secretion of fluorescent transport substrates can be monitored in living cells using confocal microscopy. Recent studies have examined the expression and function of the transport proteins Bsep (bile salt excretory protein) and Mrp2 (multidrug resistance protein 2) in these cells using antibody probes generated against the mammalian homologues of these proteins. More recently, PCR-based methods have allowed for the generation of peptide antibodies against the endogenous skate forms of Bsep and Mrp2 as well as the organic anion transporting polypeptide Oatp (Cai et al., Bull. MDIBL 39:1-2, 2000; Cai et al., Bull. MDIBL 40:83-85, 2001). The objective of the present study was to study the cellular and subcellular distributions of these endogenous transporters and to begin to examine their expression during long term culture. In addition preliminary studies were done to characterize transporter regulation in these cells. Particular attention was paid to the possibility that cAMP may be instrumental in regulating Mrp2 localization as has been shown with mammalian hepatocytes (Roelofsen et al., J. Cell Sci. 111:1137-1145, 1998).

The isolation and culture conditions for skate hepatocytes have been described previously (Ballatori et al., Bull. MDIBL 37:85-86, 1998). The cells were allowed to settle onto poly-L-lysine coated coverslips and then fixed in either ice cold methanol or acetone. The cells were then rehydrated in PBS and stained with the appropriate peptide-specific rabbit polyclonal antibodies against skate Mrp2, Bsep, or Oatp, followed by labeling with goat anti-rabbit secondary antibodies conjugated with fluorescent compounds. Additional characterization of transporter localization was performed by double labeling the skate cells for filamentous actin using fluorescent phalloidin or staining nuclei using the DNA dye propidium iodide. Fluorescently labeled cells were viewed using a 40X (1.0 NA) water-immersion objective lens on an Olympus Fluoview laser scanning confocal microscope. For the Mrp2 regulatory studies cultures of skate hepatocyte clusters were exposed to dibutrylcAMP and the transport of the Mrp2-specific substrate sulforhodamine was analyzed using a Zeiss 510 multiphoton imaging system. These same cultures were then processed for immunofluorescent staining with anti-skate Mrp2.

Figure 1 shows that skate Mrp2 displays an apical membrane concentration typical of that seen with this transporter in mammalian cells. In addition faint punctate fluorescent objects are visible in the cytoplasm of the skate hepatocytes and these may correspond to vesicle-associated skate Mrp2. The distribution of skate Oatp appears opposite to that of skate Mrp2, given that skate Oatp is concentrated on the basolateral margins of the cells and appears excluded from the apical

membranes which form the bile canaliculus (arrow). Bsep distribution appears to combine elements of each of these other transporters since it concentrates in the apical region but clearly also displays some basolateral labeling. This is unexpected and may reflect cross reactivity or the fact that all of the transporter has not been trafficked to the apical membrane. All three of the endogenous transporters appeared to maintain their polarized localization pattern in skate clusters kept in culture for up to seven days. The results of the Mrp2 cAMP regulation studies suggested that cAMP may alter Mrp2 localization patterns and canalicular geometry, however these results are very preliminary and need to be substantiated through additional experimentation.

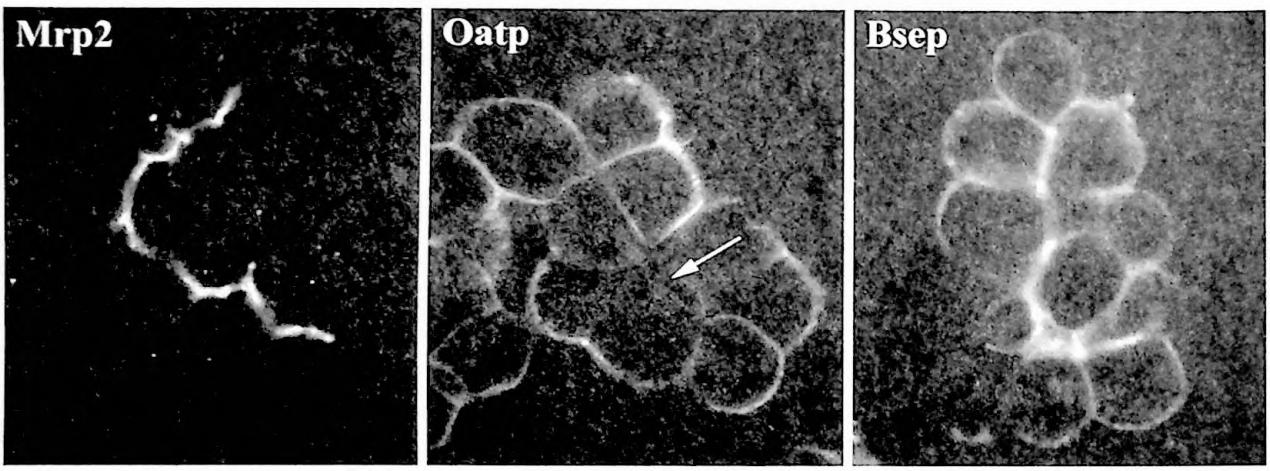

Figure 1: Immunofluorescent labeling of skate Mrp2, Oatp and Bsep in freshly isolated skate hepatocyte clusters.

Analysis of the distribution and function of endogenous xenobiotic tranporters is an important step in the continuing characterization of the novel experimental system represented by isolated skate hepatocyte clusters. These cells have advantages over isolated mammalian hepatocytes, including maintenance of structural and functional polarity and long term viability, and as such provide an exceptional *in vitro* model for determining xenobiotic transport mechanisms. Future experiments will concentrate on determining the possible regulation of xenobiotic transporters in the skate hepatocyte system. Additional cAMP-based studies will be carried out along with experiments aimed at determining if transporter expression and/or localization is impacted by exposure to environmental toxins.

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