A full-length farnesoid X receptor (FXR) from *Leucoraja erinacea*, the little skate

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FXR (NR1H4) is a member of group I of the nuclear receptor superfamily, which consists of 48 members in humans. It was originally identified as a binding protein of retinoid X receptor (RXR) from yeast two-hybrid screening, and classified as an "orphan receptor" [1]. FXR was subsequently found to be specifically activated by bile acids[2,3]. Chenodeoxycholic acid (CDCA) binds with the highest affinity, whereas hydrophilic bile acids are less active. Several key genes are activated by FXR, including genes for enzymes which are involved in bile acid synthesis in the liver (*Cyp7A1*, the rate limiting enzyme for converting cholesterol to bile acids), as well as those related to the transport of bile acids into and out of the liver (*Ntcp*/Slc10a1, the sodium-dependent taurocholate co-transporting polypeptide, and *Bsep*/Abcb11, the bile salt export pump), and uptake in the ileum (*Abst*/Slc10a2, the apical bile salt transporter, and *Babp*, the ileal bile acid binding protein).

FXR orthologs have been cloned and characterized from several other species (Table 1). These orthologues share significant sequence identity, especially in the DNA binding domain (more than 85%). However, ligand specificity is quite diverse. CDCA is the most potent endogenous ligand for human, rat, and mouse FXR alpha form but does not activate *Xenopus laevis* FXR. Lanosterol, a precursor of cholesterol, but not CDCA, binds to the beta form of FXR. Recently, the crystal structure of the rat FXR alpha ligand-binding domain has been solved, and binding residues to 6-ethyl-CDCA have been identified[4]. However, the structure/function relationship of the ligand binding domain and its promiscuous ligand specificity are still unknown. To further elucidate the receptor/ligand structure relationship of FXR, we took an evolutionary approach by identifying an FXR from the evolutionarily primitive little skate, whose predominate bile salt is scymnol sulfate, a sulfated bile alcohol.

Table 1. Sequence comparison of FXR from skate and other species with mouse beta and human alpha.

% Identity	Human alpha	Rat alpha	Mouse alpha	Chicken alpha	*Tetraodon* alpha	Zebrafish alpha	Skate	*Xenopus*	Chicken beta	Mouse beta
Mouse beta	43	44	40	46	46	45	52	54	58	100
Human alpha	100	90	93	79	63	64	57	47	51	43

Degenerate primers were designed to the most conserved DNA binding domain and a DNA fragment was amplified by PCR. DNA sequencing confirmed that this fragment was a part of skate FXR. A full-length 2365 bp sequence was obtained by 5' and 3' RACE from skate liver. The full-length skate FXR cDNA contains 186 bp at the 5' UTR, 1557 bp for the coding region, 722 bp at the 3' UTR, and an AATAAA sequence at 11 bp upstream of the polyA sequence. A Genbank BLAST search indicates that this skate FXR shares homology with all known FXRs. Protein alignment analysis showed that sFXR has 132AA for the AF1 (A/B) domain, 66AA for the DNA binding (C) domain as for other FXRs, 52AA for the hinge (D) domain, and 268AA for the ligand binding (E) domain. The phylogenetic tree is shown in figure 1. Table 1 illustrates sequence comparison of FXR from skate and other species with the mouse beta form and the human alpha form. Of note, skate FXR shares 57% amino acid identity with human FXR, which is lower than all alpha forms, but higher than all beta forms. On the other hand, skate FXR is 52% identical to mouse FXR beta, which is higher than all

alpha forms but lower than all beta forms. Skate FXR contains two insertions in its ligand-binding domain when compared to rat FXR (Fig. 2). These insertions also appear in FXR beta. Further protein hydrophilicity analysis and secondary structure prediction have shown that the inserted portions are highly hydrophilic and likely form coil-coil structures attached to the outer surface of the core helices structure of the ligand binding domain. Because the alpha and beta forms of FXR have different ligand affinities, and because skate FXR may be an intermediate between the alpha and beta forms, we speculate that skate FXR may have an affinity for both CDCA and lanosterol. This hypothesis remains to be tested. We are currently in the process of functionally characterizing skate FXR ligand binding activity. These studies were supported by National Institutes of Health Grants ES03828, ES01247, DK34989, and DK25636.

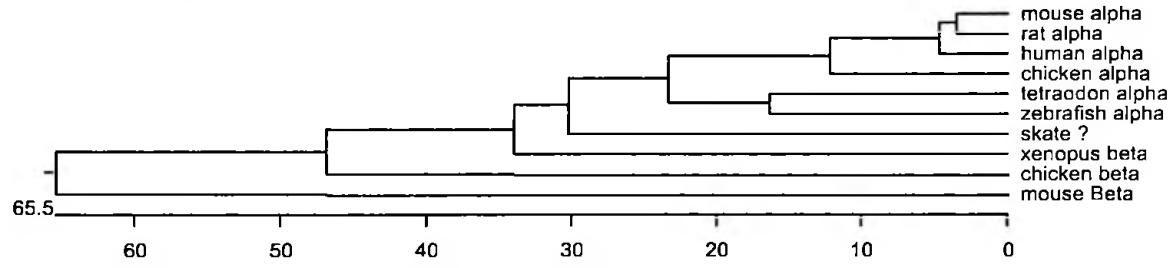

Figure 1. The phylogenetic tree of FXRs from different species.

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RatLBD     EKTELTVDQQTLLDYIMDSYSKQRMPQEITNKILKEEFSAEENFLILTEMATSHVQILVE
SkateLBD   QRVEFTAEQQQLLDYIVEAHHKYRIPQEAARKYLFEPANPVEDFLRLSENATLQVEVLVE
           ::.*:*.:** ********::: * *:*** :.* * *  .. *:** *:* ** :*::***
                      H1                 H2                 H3
RatLBD     FTKRLPGFQTLDHEDQIALLKGSAVEAMFLRSAEIFNKKLPAG-----------------
SkateLBD   FAKRLPGFQTLDNEDQIALLKGSTVEVMFLHSAQLYNQSFTQSSHQYIQDTEHYSTLPGC
           *:**********:**********:**.***:**:::*:.:. .
                           H4       H5
RatLBD     -HADLLEERI-------------------RKSGISDEYITPMFSFYKSVGELKMTQEEYA
SkateLBD   CTNQCFEEHIPAVMMEQSNLDEISIVVPNRTLGIAEEFITPMFDFYRSMGELNVTDTEYA
              : .**:*                    *. **:*:*****.**:*:***::*: ***
              H6                                  H7
RatLBD     LLTAIVILSPDRQYIKDREAVEKLQEPLLDVLQKLCKIYQPENPQHFACLLGRLTELRTF
SkateLBD   LLSATTILFSDRPYLKNKTHVEKLQEPLLEILHKYSKIHHPESPQRFARLLGRLTQLRTL
           **:* .** .** *:*::  **********::*:* .**::**.**:** ******:***:
              H8                      H9                        H10/11
RatLBD     NHHHAEMLMSWRVNDHKFTPLLCEIWDVQ
SkateLBD   NHNHSEVLMSWKMKDQKLTPLLCEIWDVQ
           **:*:*:****::*:*:***********
            H10/11          H12(AF2)
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Figure 2. Rat and skate FXR ligand binding domains (LBD) sequence alignment and comparison. Identical amino acids are labeled by * underneath. The helices which bind to ligand are H3, H5, H7, H10/11, and H12.

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