Published in final edited form as:

Zebrafish. 2007 ; 4(4): 261–275. doi:10.1089/zeb.2007.0520.

Multidrug Resistance-Associated Protein 3 (Mrp3/Abcc3/Moat-D) Is Expressed in the SAE *Squalus acanthias* Shark Embryo– Derived Cell Line

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Abstract

The multidrug resistance-associated protein 3 (MRP3/Mrp3) is a member of the ATP-binding cassette (ABC) protein family of membrane transporters and related proteins that act on a variety of xenobiotic and anionic molecules to transfer these substrates in an ATP-dependent manner. In recent years, useful comparative information regarding evolutionarily conserved structure and transport functions of these proteins has accrued through the use of primitive marine animals such as cartilaginous fish. Until recently, one missing tool in comparative studies with cartilaginous fish was cell culture. We have derived from the embryo of *Squalus acanthias*, the spiny dogfish shark, the *S. acanthias* embryo (SAE) mesenchymal stem cell line. This is the first continuously proliferating cell line from a cartilaginous fish. We identified expression of Mrp3 in this cell line, cloned the molecule, and examined molecular and cellular physiological aspects of the protein. Shark Mrp3 is characterized by three membrane-spanning domains and two nucleotide-binding domains. Multiple alignments with other species showed that the shark Mrp3 amino acid sequence was well conserved. The shark sequence was overall 64% identical to human MRP3, 72% identical to chicken Mrp3, and 71% identical to frog and stickleback Mrp3. Highest identity between shark and human amino acid sequence (82%) was seen in the carboxyl-terminal nucleotide-binding domain of the proteins. Cell culture experiments showed that mRNA for the protein was induced as much as 25-fold by peptide growth factors, fetal bovine serum, and lipid nutritional components, with the largest effect mediated by a combination of lipids including unsaturated and saturated fatty acids, cholesterol, and vitamin E.

Introduction

THE MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 3 (MRP3/Mrp3, or ABCC3, also referred to as multiorganic anion transporter-D, or Moat-D) is a member of the subfamily of transmembrane ATP-binding cassette (ABC) proteins that also includes multidrug resistance-associated proteins 1 and 2 (MRP1/Mrp1 and MRP2/Mrp2, or ABCC1 and ABCC2) and the cystic fibrosis transmembrane conductance regulator (CFTR or ABCC7).¹ MRP3 is capable of supporting a multidrug resistance phenotype and mediating ATP-dependent efflux of a variety of xenobiotic and anionic organic molecules.² Mrp3 and several others of this family are expressed in liver, kidney, and pancreas, as well as other vertebrate tissues.^{2–9}

MRP3/Mrp3 localizes to the basolateral portion of the hepatocyte plasma membrane, where it transports glucuronide conjugates and bile salts from the cell. Mrp3 is inducible upon bile duct

ligation, and may be of particular importance as an alternative system for extracellular removal of bile salts and toxic bile salt metabolites under conditions in which the canalicular export system is compromised.^{3,10} MRP3/Mrp3 is also found on the basolateral membrane of intestinal epithelium,^{3,10} where it has been proposed as a candidate bile salt pump responsible for transport of bile acids into the basolaterally exposed circulation associated with the intestine. However, another transporter, the organic solute and steroid transporter (OST) complex, has been recently found to be the major mediator of this process.¹⁰

Substantial comparative information regarding critical ABC proteins and transport functions has accumulated through the use of primitive marine animals, notably *Squalus acanthias*, the spiny dogfish shark, and *Leucoraja erinacea*, the little skate.^{4,10–15} These animals are marine elasmobranchs, derived from ancestral cartilaginous fish first appeared approximately 400 million years ago. ABC proteins identified and studied in elasmobranchs include CFTR, the bile salt export pump (Bsep), and Mrp2, as well as non-ABC anion/xenobiotic transport proteins (Ost and an ancient member of the organic anion transporting polypeptide [Oatp] family).^{4,12,13} Structure/function studies comparing elasmobranch Bsep and humans exhibiting BSEP mutations have served to confirm and extend observations concerning the critical areas of primary structure for activity of the molecule.¹¹

Although *in vivo* studies of a molecular and physiological nature have been critical in elucidating the nature of elasmobranch anion/xenobiotic transport, a tool lacking until recently was cell culture. At the same time, fish cell lines are finding increased use as substitutes or initial tools in the study of various areas of marine toxicology.¹⁶ Cell culture approaches allow reliable control of the extracellular environment and provide an opportunity to study a number of transport phenomena at the cellular and subcellular levels.¹⁴ These include approaches to the study of mechanisms by which environmental effectors, xenobiotics, drugs, and their metabolites influence substrate specificity and kinetics of transport or cellular expression of transport proteins at the transcriptional, posttranscriptional, translational, and posttranslational stages. Recently, we have derived from the embryo of the spiny dogfish shark the SAE cell line, the first continuously proliferating cell line from a cartilaginous fish.^{15–19} The line is of mesenchymal stem cell origin. In this report, we describe the identification of Mrp3 in the SAE cell line, cloning of the molecule, and characterization of molecular aspects, and the ability of this ABC transport protein to be induced by lipids, peptide growth factors, and fetal bovine serum (FBS) *in vitro*.

Results

S. acanthias Mrp3 primary structure

The full-length shark Mrp3 cDNA contains 5048 bp and encodes for 1544 amino acids, with a 37-bp 5' untranslated region (UTR) and a 380-bp 3' UTR (Fig. 1). A polyadenylation AATAAA sequence appears 14 nucleotides 5' to the polyA sequence. The protein is predicted to have 15 transmembrane regions organized into three membrane-spanning domains and two intracellular nucleotide-binding domains, with one additional, significantly long intracellular domain between membrane-spanning domains 1 and 2 (Figs. 1 and 2). The membrane-spanning domains contain, respectively (amino to carboxy-terminal), 5, 4, and 6 transmembrane regions. Each nucleotide-binding domain contains the ABC-characteristic Walker A and B nucleotide sequences and ABC protein signature sequence (Fig. 1). PCR assays using a portion of the 3' UTR of the mRNA as primer target indicated that the protein was expressed ubiquitously in 11 shark tissues tested, including gill, spleen, spiral gut, and rectal gland (not shown). Mrp3 in mammals also is expressed in most tissues.

There are 2 predicted extracellular asparagine-linked glycosylation sites (Fig. 1), 14 possible serine phosphorylation sites, 1 predicted potential tyrosine phosphorylation site, and 1

threonine phosphorylation site. There are five potential protein kinase C phosphorylation sites. Particularly interesting are positions 1154–1165 (Fig. 3), containing three serines (KRLESVSRSP^Y) all of which are predicted to function as potential phosphorylation sites. The middle serine is strongly predicted as a protein kinase C site. The sequence is between transmembrane regions 13 and 14 and is identical in sharks, humans, and chickens (Fig. 3).

Multidrug resistance-associated protein-3 sequence comparisons among vertebrate families

The primary sequence of *S. acanthias* Mrp3 was compared to the deduced primary sequence for Mrp3 from species representing four other families of vertebrates: mammals, birds, amphibians, and bony fishes (teleosts) (Fig. 3 and Table 1). Areas of strong conservation were intermittent throughout the protein, and particularly high in the intracellular carboxy-terminal portion. The overall protein lengths and percent identities among 12 representatives of five families of vertebrates and the protochordate *Ciona intestinalis* are given in Table 1. Among the sequences for which full representation was available, the shark sequence showed highest identity to chicken (*Gallus gallus*, 72%), frog (*Xenopus tropicalis*, 71%), stickleback (*Gasterosteus aculeatus*, 71%), and medaka (*Oryzias latipes*, 69%). We also identified from a previously sequenced expressed sequence tag derived in the laboratory from little skate a partial sequence of an Mrp3-like protein. This sequence showed 87% amino acid identity with shark, and over 215 amino acids represented an amino acid region from transmembrane region 15 through the nucleotide-binding site. This strong homology with the shark amino acid sequence is representative of the relatively close evolutionary relationship between the two species. The little skate and the dogfish shark diverged 125–150 million years ago.

Comparisons of shark Mrp3 to human MRP3, polymorphisms, and site-directed mutations

The shark sequence showed 999 amino acids identical to the human sequence, representing 64% identity. The amino acid identity between shark and human among the various protein domains is shown in Table 2. The highest shark/human identity (81%) was seen in nucleotide-binding domain 2, and least identity (49%) was seen in the intracellular domain of approximately 100 amino acids between membrane-spanning domains 1 and 2. The membrane-spanning domains showed 60–65% shark/human identity. The highest identity between shark and human among the transmembrane regions was in the second and third region of membrane-spanning domain 1, the third region of membrane-spanning domain 2, and the fifth and sixth regions of membrane-spanning domain 3. Identity in these last two regions was 78% between shark and human. The final transmembrane region of membrane-spanning domain 3 in particular is important in conferring substrate specificity and transport rate for the human protein.²⁰

Lang *et al.*⁵ and Lee *et al.*²¹ identified seven individual amino acid polymorphisms in the coding region of human MRP3, and Zhang *et al.*²⁰ introduced site-directed mutations into the substrate-binding site of the MRP3 transporter and examined alterations in specificity and efficiency of transport. A comparison of the natural polymorphic changes in MRP3 with the corresponding amino acids of shark Mrp3 shows conservative or semiconservative changes in all cases except one (human position 68), which showed no specific pattern (histidine and tyrosine in human, and valine in shark) (Table 3). In one case (human position 513), the corresponding amino acid in the shark was the same as the human polymorphism (lysine). Alterations by site-directed mutagenesis of polar amino acid residues within the final transmembrane region²⁰ have identified seven critical residues. Six of these were identical in the analogous shark positions. However, an interesting anomaly is an alanine in the shark position analogous to MRP3–1233-ser. Alteration of this site to alanine in the human protein causes a reduction in transport rate.²⁰

Regulation of shark Mrp3 mRNA in vitro

In an attempt to determine if Mrp3 expression might be regulated by components present in the SAE cell culture environment, candidate signaling and nutritional medium supplements were tested for the ability to influence Mrp3 expression *in vitro*. Medium supplements chosen for further scrutiny were the peptide growth factors insulin, fibroblast growth factor (FGF), and epidermal growth factor (EGF), as well as FBS, and the lipid mixture chemically defined lipid (CDL). CDL is composed of fatty acids arachidonic, linoleic, linolenic, myristic, oleic, palmitic, palmitoleic, and stearic, as well as DL- α -tocopherol acetate, cholesterol, and emulsifiers. The cells were incubated for 24 h in basal nutrient medium with all supplements present or in medium in which each supplement was individually added. A control was also included in which none of the medium supplements were added. RNA was isolated, and expression of shark Mrp3 was compared under each condition by quantitative real-time PCR (qPCR) with normalization to shark elongation factor-1 α (EF-1 α) (Fig. 4). A 25-fold induction in the presence of the full combination of supplements was seen when compared to conditions in which no supplements were added. The addition of CDL alone led to a 13-fold induction, and FBS alone caused a 7-fold induction. Insulin caused a fivefold induction, and EGF and FGF each caused inductions of approximately threefold.

Discussion

We have characterized an ABC protein from the spiny dogfish shark, an elasmobranch descended from cartilaginous fish of ancient lineage. The gene is a member of the ABCC transmembrane protein family and appears to be the ortholog of MRP3/Mrp3 of other species, showing over 70% amino acid identity in some cases. The full-length cDNA encodes for a protein of 1544 amino acids, 17 amino acids longer than its human counterpart. Sites of potential phosphorylation and glycosylation were identified, and several sites for phosphorylation are predicted to be of particular likelihood. The most likely tyrosine protein kinase substrate is tyr-739. The sequence at positions 1154–1165 (KRLESVSRSPY) is near a highly conserved substrate specificity and rate-critical areas present in the last transmembrane region, and may be a site of protein kinase C-mediated regulation. Effects of protein kinase C activation on Mrp3 expression and mRNA induction have been reported,²¹ but phosphorylation sites within the protein are unknown. Overall amino acid identity was well conserved through a variety of species, including five families of vertebrates and the protovertebrate (tunicate) *C. intestinalis*.

The most conserved regions in alignments with the human primary structure are the second nucleotide-binding domain and certain transmembrane regions within each of the membrane-spanning domains. These observations support the expectation that these regions serve critical functions for the protein related to catalytic activity, substrate recognition, and transport function.^{1,13} Polymorphisms found in human MRP3 were examined relative to the analogous amino acids in alignments with the shark. In all cases but one, the polymorphisms in human and the corresponding amino acids in shark were of a conservative nature, and in some cases were identical. In one case (human position 513), the corresponding amino acid in the shark was the same as the human polymorphism. A single polymorphism (human position 68) did not show conservation with the shark, but also did not show similarity within the human, suggesting that it is not in a critical portion of the protein. None of the polymorphic changes shown in Table 3 have been found to affect MRP3 function in the human.^{5,21}

In human–shark alignments, an alanine is found in the shark amino acid position analogous to serine 1233 in the human MRP3. When this site is altered to alanine in the human protein by site-specific mutagenesis, overall reduction in transport rate of multiple substrates (taurocholate, etoposide, and 12- β -estradiol-17- β -D-glucuronide) is observed.²⁰ That the deduced shark primary structure indicates alanine at this position may mean that the transport

capability of the shark protein is reduced compared to the human protein. Alternatively, the appearance of alanine at this position in the shark protein may represent an adaptation leading to a conformational change more amenable to the lower temperature at which the shark protein functions, and the different membrane lipid composition of the cold water-adapted plasma membrane of the shark compared to human.

Mrp3 expression in the SAE cell line *in vitro* was increased approximately 25-fold in the presence of a combination of medium supplements including FBS, three peptide hormones, and a lipid mixture containing saturated and unsaturated acids, vitamin E, and cholesterol. When tested individually, the strongest inducer of Mrp3 was the lipid mixture, with effects also observed from each of the other supplements. FBS caused a sevenfold induction of Mrp3 mRNA within 24 h, possibly the result of the combination of lipids and peptide growth factors present in this supplement. Lipid effects may indicate induction by substrate-related compounds.³ The peptide growth factors insulin, EGF, and FGF are presumed to act through initial protein kinase activity. A variety of nuclear receptors and transcription factors have been reported to enhance or inhibit expression of MRP3 under various conditions. These include Sp1, Sp3, PXR, CAR, Ahr, PPAR α , Nrf2, and RXRa:RXRa.^{6–8,22–27} Kubo *et al.*²⁸ have shown that docosahexanoic acid induces Mrp3, further supporting our observation that Mrp3 is induced with lipid mixtures as well as FBS. The mechanisms for the effects we report here may well involve the interaction of multiple signaling modes and remain to be determined.

Materials and Methods

Cell line and culture conditions

The SAE cell line was maintained in LDF medium (50% Dulbecco's modified medium [Gibco BRL, Carlsbad, CA], 35% L-15 [Sigma Chemical, St. Louis, MO], 15% Ham's F-12 [Gibco BRL], 2 mM sodium bicarbonate, and 15 mM HEPES buffer [pH 7.2])^{15,19} supplemented with 10 μ g/mL insulin (Sigma Chemical), 10 μ g/ μ L transferrin (Sigma Chemical), 10 nM selenous acid (Sigma Chemical), 50 ng/mL human recombinant EGF (R&D Systems, Minneapolis, MN), 50 ng/mL basic FGF (R&D Systems), 2 mM GlutaMAX (Gibco BRL), 0.1 $\times$ minimum essential medium (MEM) nonessential amino acids (Gibco BRL), 0.05 $\times$ essential amino acids (Sigma Chemical), 1:1000 CDL (Gibco BRL), and 2% heat-inactivated FBS (Hyclone, Logan, UT). Cells were grown at 18°C in T-25 flasks (Nalgene Nunc, Rochester, NY) and passaged using 2 mg/mL collagenase (Sigma Chemical) in LDF medium and 0.2% trypsin (Sigma Chemical) with 1 mM ethylenediaminetetraacetic acid.

Cloning of shark Mrp3

Total RNA was extracted from SAE cells (12th passage, 3×10^6 cells) with TRIzol Reagent (Invitrogen, Carlsbad, CA). Initial PCR using primers designed for detection of Mrp2⁴ identified a product that was further examined by sequencing and determined to be a cDNA that was not MRP2, but was related in sequence. For the synthesis of cDNA, 3 μ g of total RNA was reverse transcribed using the SuperScriptTM III First-Strand Synthesis System for RT-PCR (Invitrogen) according to the manufacturer's instructions. The reaction mixture contained 0.2 μ L of cDNA, 200 nM 5' and 3' primers, 0.625 U of AmpliTaq[®] DNA polymerase (Applied Biosystems, Foster, CA), 1.5 μ L of 10 $\times$ Buffer for AmpliTaq (Applied Biosystems), and 200 μ M of each deoxynucleotide triphosphate (Promega, Madison, WI). Amplification was carried out with a PTC-200 DNA Engine[®] Cyclor (MJ Research, Waltham, MA) with 40 cycles of template DNA denaturation at 95°C for 15 s, primer-template annealing at 60°C for 20 s, and primer extension at 72°C for 30 s. An aliquot (10 μ L) of the PCR reaction mixture was analyzed by gel electrophoresis in a 1.2% E-Gel[®] Pre-cast Agarose Electrophoresis System (Invitrogen). A 100-bp ladder marker (Promega) was used for the estimation of the size of the PCR product. DNA fragments were visualized with a UV transilluminator.

For full-length cDNA cloning, 3' RACE PCR was performed with newly synthesized cDNA, and reverse transcribed with 5' primer designed from internal nucleotide sequence of the RT-PCR product (5'-ACTGGTTCCGTGGTTAATGC-3') and 3' primer (5'-GGCCACGCGTCGACTAGTAC-3'). The PCR conditions were same as described above. 5' RACE PCR was performed with 1 µg of newly prepared SAE cell line RNA using the SMARTTM RACE cDNA Amplification Kit (Clontech Laboratories, Mountain View, CA) according to the manufacturer's instructions. 5' RACE PCR was further utilized for full cDNA sequencing.

Sequence analysis

The NCBI BLASTX program (<http://ncbi.nlm.nih.gov/BLAST/>) was used to deduce the amino acid sequence from the nucleotide sequence. Ortholog searching was carried out through the Ensemble database (<http://ensembl.org/index.html>). The Sosui server (<http://bp.nuap.nagoya-u.ac.jp/sosui/sosuiframe0.html>) was used to predict transmembrane regions of the protein based on the amino acid sequence. Domain and motif searching was carried through the Prosite database (<http://au.expasy.org/prosite/>). Multiple alignment of the amino acid sequence was carried out using ClustalX (1.83) software.

Induction of Mrp3 in the SAE cell line

To identify the potential cell culture conditions affecting expression of shark Mrp3 mRNA, SAE cells (1.0×10^6 cells/well of six-well plates, Nalgene Nunc) were incubated for 4 days at 18°C in basal LDF nutrient medium containing amino acids, selenous acid, transferrin, and GlutaMax. Following this, other medium supplements (FBS, insulin, FGF, EGF, and CDL) were added to individual wells at the concentrations routinely used in the culture medium. A control was also included, in which none of the medium supplements were added. After incubation for 24 h, total RNA was isolated from each well, and cDNA was synthesized. The expression of shark Mrp3 was compared by qPCR with shark EF-1 α as the normalizer. The qPCR assay was performed in a 200-µL tube with a total volume of 25 µL. The reaction mixture contained 12.5 µL of 2×master mix of Brilliant® SYBR® Green QPCR Reagents (Stratagene, La Jolla, CA), 0.375 µL of 1:500 diluted ROX reference dye (Stratagene), 100 nM of 5' and 3' primer, and 0.5 µL of cDNA. Amplification and detection was carried out with an Mx 3000P® QPCR system (Stratagene). Conditions were 95°C for 10 min, 40 cycles of template DNA denaturation at 95°C for 15 s, primer-template annealing at 55°C for 20 s, and primer extension at 72°C for 20 s. Data were analyzed with MX pro software (Stratagene).

Acknowledgments

Supported by NIH grants R01-RR019732 and P20-RR016463 and by National Institute of Environmental Health Sciences Center Grant ES03828. We thank Dr. S.-Y. Cai for *Ciona* sequence and helpful discussions. D.B. thanks Amber Miller.

References

1. Annilo T, Chen ZQ, Shulenin S, Constantino J, Thomas L, Lou H, et al. Evolution of the vertebrate ABC gene family: analysis of gene birth and death. *Genomics* 2006;88:1–11. [PubMed: 16631343]
2. Haimeur A, Conseil G, Deeley RG, Cole SP. The MRP-related and BCRP/ABCG2 multidrug resistance proteins: biology, substrate specificity and regulation. *Curr Drug Metab* 2004;5:21–53. [PubMed: 14965249]
3. Soroka CJ, Lee JM, Lee F, Azzaroli A, Boyer JL. Cellular localization and up-regulation of multidrug resistance-associated protein 3 in hepatocytes and cholangiocytes during obstructive cholestasis in rat liver. *Hepatology* 2001;33:783–791. [PubMed: 11283840]

4. Cai S-Y, Soroka CJ, Ballatori N, Boyer JL. Molecular characterization of a multidrug resistance-associated protein, Mrp2, from the little skate. *Am J Physiol Regul Integr Comp Physiol* 2003;284:R125–R130. [PubMed: 12388433]
5. Lang T, Hitzl M, Burk O, Mornhinweg E, Keil A, Kerb R, et al. Genetic polymorphisms in the multidrug resistance-associated protein 3 (ABCC3, MRP3) gene and relationship to its mRNA and protein expression in human liver. *Pharmacogenetics* 2004;14:155–164. [PubMed: 15167703]
6. Maher JM, Cheng X, Slitt AL, Dieter MZ, Klaassen CD. Induction of the multidrug resistance-associated protein family of transporters by chemical activators of receptor-mediated pathways in mouse liver. *Drug Metab Dispos* 2005;33:956–962. [PubMed: 15833929]
7. Moffit JS, Aleksunes LM, Maher JM, Scheffer GL, Klaassen CD, Manautou JE. Induction of hepatic transporters multidrug resistance-associated proteins (Mrp) 3 and 4 by clofibrate is regulated by peroxisome proliferator-activated receptor alpha. *J Pharmacol Exp Ther* 2006;317:537–545.
8. Chen W, Cai S-Y, Xu S, Denson LA, Soroka CJ, Boyer JL. Nuclear receptors RXR α are repressors for human MRP3 expression. *Am J Physiol Gastrointest Liver Physiol* 2007;292:G1221–G1227. [PubMed: 17272513]
9. Zaja R, Klobucar RS, Smital T. Detection and functional characterization of Pgp1 (ABCB1) and MRP3 (ABCC3) efflux transporters in the PLHC-1 fish hepatoma cell line. *Aquat Toxicol* 2007;81:365–376. [PubMed: 17313982]
10. Ballatori N. Biology of a novel organic solute and steroid transporter, OSTalpha-OSTbeta. *Exp Biol Med* 2005;230:689–698.
11. Ballatori N, Rebbor JF, Connolly GC, Seward DJ, Lenth BE, Henson JH, et al. Bile salt excretion in skate liver is mediated by a functional analog of Bsep/Spgp, the bile salt export pump. *Am J Physiol Gastrointest Liver Physiol* 2000;278:G57–G63. [PubMed: 10644562]
12. Cai S-Y, Wang L, Ballatori N, Boyer JL. Bile salt export pump is highly conserved during vertebrate evolution and its expression is inhibited by PFIC type II mutations. *Am J Physiol Gastrointest Liver Physiol* 2001;281:G316–G322. [PubMed: 11447010]
13. Cai S-Y, Wang L, Soroka CJ, Ballatori N, Boyer JL. An evolutionarily ancient Oatp: insights into conserved functional domains of these proteins. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G702–G710. [PubMed: 11897630]
14. Mattingly C, Parton A, Dowell L, Rafferty J, Barnes D. Cell and molecular biology of marine elasmobranchs: *Squalus acanthias* and *Raja erinacea*. *Zebrafish* 2004;1:111–120. [PubMed: 18248223]
15. Barnes DW, Parton A, Tomana M, Hwang J-H, Czechanski A, Collodi P. Stem cells from cartilaginous and bony fish. *Methods Cell Biol.* 2007(in press)
16. Schirmer K. Proposal to improve vertebrate cell cultures to establish them as substitutes for the regulatory testing of chemicals and effluents using fish. *Toxicology* 2006;224:163–183. [PubMed: 16765501]
17. Parton A, Forest D, Kobayash H, Dowell L, Bayne C, Barnes D. Cell and molecular biology of SAE, a cell line from the spiny dogfish shark, *Squalus acanthias*. *Comp Biochem Physiol C Toxicol Pharmacol* 2007;145:111–119. [PubMed: 16949345]
18. Forest D, Nishikawa R, Kobayashi H, Parton A, Bayne CJ, Barnes DW. RNA expression in a cartilaginous fish cell line reveals ancient 3' noncoding regions highly conserved in vertebrates. *Proc Natl Acad Sci USA* 2007;104:1224–1229. [PubMed: 17227856]
19. Helmrich A, Barnes D. Zebrafish embryonal cell culture. *Methods Cell Biol* 1999;59:29–37. [PubMed: 9891353]
20. Zhang DW, Gu HM, Vasa M, Muredda M, Cole SPC, Deeley RG. Characterization of the role of polar amino acid residues within predicted transmembrane Helix 17 in determining the substrate specificity of multidrug resistance protein 3. *Biochemistry* 2003;42:9989–1000. [PubMed: 12924948]
21. Lee YM, Cui Y, Konig J, Risch A, Jager B, Drings P, et al. Identification and functional characterization of the natural variant MRP3-Arg1297His of human multidrug resistance protein 3 (MRP3/ABCC3). *Pharmacogenetics* 2004;14:213–223. [PubMed: 15083066]
22. Chandra P, Zhang P, Brouwer K. Short-term regulation of multidrug resistance-associated protein 3 in rat and human hepatocytes. *Am J Physiol Gastrointest Liver Physiol* 2005;288:1252–1258.

23. Takada T, Suzuki H, Sugiyama Y. Characterization of 5'-flanking region of human MRP3. *Biochem Biophys Res Commun* 2000;270:728–732. [PubMed: 10772892]
24. Staudinger JL, Madan A, Carol KM, Parkinson A. Regulation of drug transporter gene expression by nuclear receptors. *Drug Metab Dispos* 2003;31:523–527. [PubMed: 12695338]
25. Maher JM, Dieter MZ, Aleksunes LM, Slitt AL, Guo G, Tanaka Y, et al. Oxidative and electrophilic stress induces multidrug resistance-associated protein transporters via the nuclear factor-E2-related factor-2 transcriptional pathway. *Hepatology*. 2007(E-pub)
26. Tzeng SJ, Chang WC, Huang JD. Transcriptional regulation of the rat Mrp3 gene promoter by the specificity protein (Sp) family members and CCAAT/enhancer binding proteins. *J Biomed Sci* 2005;12:741–761. [PubMed: 16132117]
27. Cheng X, Klaassen CD. Regulation of mRNA expression of xenobiotic transporters by the pregnane x receptor in mouse liver, kidney, and intestine. *Drug Metab Dispos* 2006;34:1863–1867. [PubMed: 16928788]
28. Kubo K, Seiji S, Saito M. Induction of multidrug resistance-associated protein MRP3 in the liver of rats fed with docosahexaenoic acid. *Biosci Biotechnol Biochem* 2006;70:1672–1680. [PubMed: 16861802]

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 I L H Q H F Q Y C F V T G M R L R T A I
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 V G A I Y R K S L V I T N S A K R S S T
 gtccgggagattgtgaacttgatgtccgtggatgccagaggttcatggatctcaccagc
 V G E I V N L M S V D A Q R F M D L T S
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 F L N M L W S A P L Q I C L A L Y F I W
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 Q Y L G P S V L A G V A V M I L L I P P
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 N A V I A M S R S F Q V Q Q M L H K D
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 S R I K L M N E I L N G M K V L K L Y A

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 W E P S F E Q K V L A I R Q K E L D I L
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 K K A A Y L S A L S T F T W T T A P F I
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 V A L T T F A V Y V T V D E N N V L D A
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 Q K A F V S L S L F N I L R F P L N M L
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 P Q V I S S V V Q A T V S L N R L Q K F
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 L S H D E L D P T S V D R Q K T A T G H
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 A I T V L N G T F S W G K S D P V V L D
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 G I S L T V P Q G S L L A V V G H V G C
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 G K S S L V S A L L G E M E K L E G R V
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 A I E G T V A Y V P Q Q A W I R N A S L
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 K D N I V F G E S L N E Q K Y Q Q V L E
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 A C A L I T D L N V L P G G D Q T E I G
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 E K G I N **L S G G Q K Q R V S** L A R A V
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 Y S D T D V Y L L D D P L S A V D A H V
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 A K H I F D K V I G P E G A L K G K T R
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 V L V T H G V S F L P Q V D Q I V V F V
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 A E F L R N Y A Q R D D V E E D E P T V
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 L D E D E E F L G E D A L S N H V D L S
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 D N E P S A A E A R K L F M R Q I S V I
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 S S D G E A A T W K S T R R R L S E K K
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 K V V E R H P Q T M P E S K R L I Q A E
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 T T E T G R V K L T V F W **Q Y L K A V S**
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P F I S V V I C P L Y C C Q N A A A I G
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 A N F W L S D W T N D P V V N G T Q H R
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 T N M R V G V **Y A A L G F T Q G V V V**
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T S S P F L A L G G L G A A R Q L H A R
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 L L D N K L H T P Q A F F D T T P I G R
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I I N R F G K D V H V I D E V I L T F
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T P W F L L L L L L L L F V Y F F V Q S
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F Y V A T E R Q L K R L E S V S R S P I
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K S F I L M N D T K V D A N Q K S Y Y P
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tgtgacagcgggaatacaatccatacgggcagtttgctgttttaataaaacaagattttt
gctaaaaaaaaaaaaaaaaa

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FIG. 1.
Amino acid and nucleotide sequences of shark Mrp3. Transmembrane regions are shaded.
Walker A and B regions are underlined. The ABC protein signature sequences are underlined
and bolded. The two predicted extracellular N-glycosylation sites are bolded.

FIG. 2. Schematic of Mrp3. MSD indicates membrane-spanning domains (1, 2, and 3). NBD indicates nucleotide-binding domains (1 and 2).

<i>S. acanthias</i>	MAPLPDSLSLPGMERLCGP--EPGRLWDHNVFNITDSPDLTPCFQ KTILVWIPCVYLWVTF	59
<i>H. sapiens</i>	-----MDALCCSGELGSKFWDNSLSVHTENPDLTPCFQNSILAKVPCIVLWVAL	49
<i>G. gallus</i>	-----MEGCCGK-----CDANLSIHITDSPDLTPCFQNTILANIPRSIYLMTAL	42
<i>X. tropicalis</i>	-----METFCSSGDLSSQFWDNSILLYTINPDVTECFQNTVLANIPCVYLWVAL	49
<i>T. nigroviridis</i>	-----MDAFLQLSGLDE--LWDWNRWYWTNPDLTPCFQNTVLVWVPCIVLWALA	48
<i>S. acanthias</i>	FLVFLYLEQSSRGYIRVTVINNVETVLGVLLNIVCWSDLFYSMDEMANQKARRLAYFISE	119
<i>H. sapiens</i>	PCILLEYLHHHCRGYIILSHASKLWVLGVLLWCVSADLFYSFHGLVHGRAPFPVFFVTF	109
<i>G. gallus</i>	PFLLLYLKRSKRGYIVILMISPFMLFGVLLWCVSADLFYSFHLQSRTPPPVVFVTF	102
<i>X. tropicalis</i>	PFLLLYLKYNRRGYIVLSMISKAKTVFGVLLWCVSADLFYSFHGLVQNSPPPVVFVTF	109
<i>T. nigroviridis</i>	PFCLMLYCHDHRIQMSGFAAKTVLGFLASFGCVFEPHILLRSQEIHQHMVFLLE	106
<i>S. acanthias</i>	LLLGVTMLLATFLIQYERLRGIQSSGVLFFFWLISLLCAVIPFRSKILLALREGGVSDVF	179
<i>H. sapiens</i>	LVVGVTMLLATLLIQYERLQGVQSSGVLIIFWFLCVCAIVPFRSKILLAKAEGEISDP	169
<i>G. gallus</i>	LVISITVLLATLLIQYERLRGVQSSGVLIIFWFLVCAVAPFRSKIMTTTAQNHVNER	162
<i>X. tropicalis</i>	LVLGITHITATVLAQYERLRGVQSSGVLIIFWFLATVCAIIPFRSKVMASACQVITKE	169
<i>T. nigroviridis</i>	IIRSMTVVAVLCIICLERVRCRSGVFLIFWVMAVVSLVPLRAKQLAMDEGFASDIV	166
<i>S. acanthias</i>	RPTTFYIYFVLVLIQFFLCCFSEP PHYFCRLSPDANPCPEASAGFLSHLTFWWTFRMAIQ	239
<i>H. sapiens</i>	RPTTFYIYHVALVISALILACFRKPPFSAKNVDPNHYPETSAQFLSRLFFWWTFRMAIY	229
<i>G. gallus</i>	RSATFYIYFVLLIVELILSCFEKPKPFSPAN' 'SPELTSSFLSRLTFWWTFRMAIL	222
<i>X. tropicalis</i>	RPTTFYIYFVLLIVELILSCFEKARPFSPAN' 'CPESDAGFLSRLTFWWTFRMAIL	229
<i>T. nigroviridis</i>	QYFAFFSYETIQALFLCCFADQFF-V 'LEKNPCFVKDASFLSKILLEWWTGLVVK	227
<i>S. acanthias</i>	GYMHPLEDKDLWSILNKDDSDVIVPKLKEWEKONLAKRRQEVLPNTYPPTSFEEGE	299
<i>H. sapiens</i>	GYMHPLEKOLWSIKEEDRQMVVQCLLEANKRQEKQTARHK-----ASAAPGKNA	280
<i>G. gallus</i>	GYKPLEKDLWSILNEDDISKNIVCKLSREWDKEKAECKQK-----SNHALSHVGDG	275
<i>X. tropicalis</i>	GYMHPLEDKDLWSILNEDDTSNVVITMLIKWEKESNLAK-----PEAVLHSHDEK	281
<i>T. nigroviridis</i>	GYRNPLAEDLWTREEDTCKTIAELQODTAECALIONCVVGGRRQKALASNAALGSR	287
<i>S. acanthias</i>	PHEAEVLFPNKADQEK--SFFLALPKATPYFLMGSALKLCODLLSFVNPQLINMLISE	357
<i>H. sapiens</i>	SGDEVILGARPRPK--SELKAILATGSSFLISACPKLIQDLLSPINPQLISILIRE	336
<i>G. gallus</i>	PEAEVLIIRDGRHNK--SELKAILMRTGPGYFLIGSFFKLIQDLLSFVNPQLISVLISE	333
<i>X. tropicalis</i>	AYSDVLIVDNKKPK--SELKAVILRTGPGYFLIGSFFKLIQDLLSFVNPQLISILIRE	339
<i>T. nigroviridis</i>	LPDQAQLRKLLQKEQSSGFFLLRTTRKKGYYLSLTLCIIFHDAFMAIPQVLSLIGE	347
<i>S. acanthias</i>	IKNKEARITWNGYCSFLMFFSA-ALQTLILHQHFQYCFVTGMRLRTAIVGAIYRKSLVIT	416
<i>H. sapiens</i>	ISNEMAPSNGWGLVAGLMELCS-MMQSLILQHYHNIIVTGVKFTGEMGVYRKALVIT	396
<i>G. gallus</i>	VVDKDAITWNGFLAALMFAC--VLOTILILHQHFQYCFVTGMRLRTGILGVYRKSLVIT	392
<i>X. tropicalis</i>	IKNKDAPSNGWFCNAVIMFLTS-LVOTILILHQHFQYCFVTGMRLRTAITGVYRKSLVIT	396
<i>T. nigroviridis</i>	MRDEDAPLWKGYYFATIMELLS-CLQSLFNHQMYTCFTVGMRLVKTAVMLVYRKSLVIN	406
<i>S. acanthias</i>	NSAKRSSTVGEIVNLMSEVDAQRFMDLTSFLNMLWSAPLQICLALYFLWQYLGFSVLGVA	476
<i>H. sapiens</i>	NEVTRASTVGEIVNLMSEVDAQRFMDLAPFLNMLWSAPLQILAIYFLWQNLGPSVLGVA	456
<i>G. gallus</i>	NSAKRSSTVGEIVNLMSEVDAQRFMDLVTFLNMLWSAPLQICLALYFLWQALGPSVLGVA	452
<i>X. tropicalis</i>	NSAKRSSTVGEIVNLMSEVDAQRFMDLTFNMLWSAPLQICLALYFLWQALGPSVLGVA	456
<i>T. nigroviridis</i>	SASRETCVGEIVNLMSEVDAQRFMDLTFNMLWSAPLQICLALYFLWQALGPSVLGVA	466

<i>S. acanthias</i>	VMILLIPFNNAVIMKSSPQVQOQLKDSRIKLMNEILNGMVLKLYAWEPSPSEKVLAA	536
<i>H. sapiens</i>	FMVLLIFLNGAVVVRMRAPOVKQKMLNDSRIKLMSEILNGIKVLKLYAWEPSPILKQVEG	516
<i>G. gallus</i>	VMVLLIFPNSAVAKTRAFQVEQMYKDSRIKLMNEILGGIKVLKLYAWEPSPSEKVLAA	512
<i>X. tropicalis</i>	VMVLLIFINAFIAMNTAFQVEQMYKDSRIKLMNEILNGIKVLKLYAWEPSPAAKVLAE	518
<i>T. nigroviridis</i>	TVIFIFLNGFLAKRSKLDEIQKFMQDRIILMNEILNGIKVLKLYAWEKALAEQVLGH	526
<i>S. acanthias</i>	RQFELDLKKAAYLSALSTFTWTTAPSIIVALTTFVAVYTVDENNVLDAAKAPVSLSLFNI	596
<i>H. sapiens</i>	RQFELDLKKAAYLSALSTFTWTTAPSIIVALTTFVAVYTVDENNVLDAAKAPVSLSLFNI	578
<i>G. gallus</i>	RKNEILVLRKSAVYNSLSNFAWISSPFLVALTTFVAVYTVDEKNILDAEKAPVSLSLFNI	572
<i>X. tropicalis</i>	RKNEILVLRKSAVYNSLSNFAWISSPFLVALTTFVAVYTVDEKNILDAEKAPVSLSLFNI	578
<i>T. nigroviridis</i>	BEKEERALKKSQLYLSIASFNSSSLIIFAMFVYVMLNRRNVLDAAKAPVSLSLFNI	586
<i>S. acanthias</i>	LRIFLNMMLPQVISVSVQATVSLNRLQKFLSHDELDPSTVDKQKATG---HAITVINGTE	653
<i>H. sapiens</i>	LRIFLNMMLPQVISVSVQATVSLNRLQKFLSHDELDPSTVDKQKATG---HAITVINGTE	638
<i>G. gallus</i>	LKPFTHMLPQVISNIACTSVSLKRIQQLFLSHDELDPSTVDKQKATG---HAITVINGTE	629
<i>X. tropicalis</i>	LRIFLNMMLPQVISNIACTSVSLKRIQQLFLSHDELDPSTVDKQKATG---HAITVINGTE	635
<i>T. nigroviridis</i>	LKTFPSQLPFAISTTMQAMVSLRLGNYLCSGELKADNYSKAPRTSNNHGENVVIENGTE	646
<i>S. acanthias</i>	SWGKSDPVVD--GISITVPGSSLLAVVGHVGGCKSSLVSALLGEMEKLEGRVAIEGTVA	711
<i>H. sapiens</i>	TNAQDLPTLH--SLDIQVFKGALVAVVGPVGGCKSSLVSALLGEMEKLEGRVHMKGSSVA	693
<i>G. gallus</i>	SWGKELKPSIK--DINILVPSGALVAVVGHVGGCKSSLVSALLGEMEKLEGRVAVKGSVA	687
<i>X. tropicalis</i>	SWANNGGAILQKHSINILVPSGSSLLAVVGPVGGCKSSLVSALLGEMEKLEGRVSVRGSVA	695
<i>T. nigroviridis</i>	SWSAAGPCIK--RINVHVPFSGSLVAVVGPVGGCKSSLVSALLGEMEKLEGRVTVKGSVA	704
<i>S. acanthias</i>	YVPOQAWIRNASLKDNIVPSESLEQRYEACALITDNLVPGGDQTEIGENGINLS	771
<i>H. sapiens</i>	YVPOQAWIQNTLQENVLPKALNPKRYEACALLADLENLPGGDQTEIGENGINLS	753
<i>G. gallus</i>	YVPOQAWIQNTLQENVLPKALNPKRYEACALKTDLEVLPGGDQTEIGENGINLS	747
<i>X. tropicalis</i>	YVPOQAWIQNTLQENVLPKALNPKRYEACALVTDLEVLPGGDQTEIGENGINLS	755
<i>T. nigroviridis</i>	YVPOQAWIQNTLQENVLPKALNPKRYEACALVTDLEVLPGGDQTEIGENGINLS	764
<i>S. acanthias</i>	GGQQRVSLARAVYSUTLVYLLDDPLSAVDARVAKHIFDKVIGPEGALRGKTRVLVTHGI	831
<i>H. sapiens</i>	GGQQRVSLARAVYSUTLVYLLDDPLSAVDARVAKHIFDKVIGPEGALRGKTRVLVTHGI	813
<i>G. gallus</i>	GGQQRVSLARAVYSUTLVYLLDDPLSAVDARVAKHIFDKVIGPEGALRGKTRVLVTHGI	807
<i>X. tropicalis</i>	GGQQRVSLARAVYSUTLVYLLDDPLSAVDARVAKHIFDKVIGPEGALRGKTRVLVTHGI	819
<i>T. nigroviridis</i>	GGQQRVSLARAVYSUTLVYLLDDPLSAVDARVAKHIFDKVIGPEGALRGKTRVLVTHGI	821
<i>S. acanthias</i>	SFLPQVDQIVVFNNGKVSEMGSYQELCAQNGAPAEFLRNYAQRDDVEDEPT-----VL	886
<i>H. sapiens</i>	SFLPQVDQIVVFNNGKVSEMGSYQELCAQNGAPAEFLRNYAQRDDVEDEPT-----VL	873
<i>G. gallus</i>	GFLPQVDQIVVFNNGKVSEMGSYQELCAQNGAPAEFLRNYAQRDDVEDEPT-----VL	862
<i>X. tropicalis</i>	SFLPQVDQIVVFNNGKVSEMGSYQELCAQNGAPAEFLRNYAQRDDVEDEPT-----VL	875
<i>T. nigroviridis</i>	-----AAPTRSCSGTARFADFIHTFARTERKSAIQR---AGSRR	859
<i>S. acanthias</i>	EDDEFUGEDALSNDVDSNEPSAKAKALFMROIISVISDGEAATWKSTARRLSEKKKV	946
<i>H. sapiens</i>	DKEALLIEDTLNHTDITDNDPVTVVVKQKFMROLSSALSSDGEQGGRPVPRRHLPSPK	933
<i>G. gallus</i>	EEVLLAETLSIHTDLADNEPVTNEVRKQPLNQLSVIS-----SEGGES	907
<i>X. tropicalis</i>	EEVLLAETLSIHTDLADNEPVTNEVRKQPLNQLSVIS-----SEGGES	934
<i>T. nigroviridis</i>	SNARLSMVFMPFSDLSQEQILIGDGTNSNLQNMFPV-----ETE	901

<i>S. acanthias</i>	VLRHQTMEESKRLLQAETTETGKRVKLTVPWQYLKAVGPFISVVICFLYCCQNAAGAIGAN	1008
<i>H. sapiens</i>	QVTEAKADG---ALTQESKAAITVELSVFWDYAKAVGLCTTLAICILYVGSAAAIGAN	990
<i>G. gallus</i>	EPPIARRNF-NEKLIQAETTEVGTVKLTVPWQYXMKAVSEVISLIICFLYCCQNAASIGAN	966
<i>X. tropicalis</i>	ENLVAKQPF-TEKLIQTETETGKRVKMTVPWQYMKAVGLAISVFICFLYSCQNAAGAIGAN	993
<i>T. nigroviridis</i>	EEQVREDLG---KLTIVVDKARTGEVRLTEMYKKYFNTIGLAIIPFIIFLYAFIQGVSLAYN	958
<i>S. acanthias</i>	FWLSDWTNDPVPVNGIOHRINMRVGVYAALGFTQGVVVMISFTLALGGLGAARQLHARLE	1066
<i>H. sapiens</i>	VWLQANTNDAMADSRQNTSLRLGVYAALGILQGFVWLAAMAMAAGGIQAAEVLHQALL	1050
<i>G. gallus</i>	VWLSDWTNEPVIINGTQNTSMRIGVYAALGLLQGFIVFVSSFTLAMGGINAARKLHTALE	1028
<i>X. tropicalis</i>	VWLSDWTNEPVIINGTQNTQCMRGGVYAALGILQGLLYMTBESSLAIAIGAAARKLSALL	1053
<i>T. nigroviridis</i>	YWLQNMADNIVNGTQIDTDLKLTVPFALGFVQGVSIFGTTVAISICIIKASHLHMDLE	1018
<i>S. acanthias</i>	DNKHLTPCAFFDTTPIGRIINRSKQDVHVIDEVIPLTFQMFLLSTFFNSLXTMIVIMASTE	1126
<i>H. sapiens</i>	HNNIRSPQSFEDTTESGRILNCSKDIYVDEVILAPVIMLLNSFFNAISLIVIMASTE	1110
<i>G. gallus</i>	ENKENTPQSFYDTTPIGRIINRSKDIYVIDEVIPEIILMFLGTFEASLXTMIVIVASTE	1086
<i>X. tropicalis</i>	DNKHTPQSFYDTTPIGRIINRSKDIYVIDEVIDEILMFLATFTTSLXTMIVIVASTE	1113
<i>T. nigroviridis</i>	MNVLRSPMSFECTESGNLNSRAKEIDAIDCMVREGKMMLSYAKKLEVCIIIVMASTE	1078
<i>S. acanthias</i>	WFILLILPLILVYFVQRFYVATSRQLKRLESVSRSPYISHPSETITGSSVIRAYEKKEK	1186
<i>H. sapiens</i>	LETVVILPLAVLYTLVQRFYAATSRQLKRLESVSRSPYISHPSETITGASVIRAYNRSRD	1170
<i>G. gallus</i>	LEAVVVVPLAVLYYFVQRFYVATSRQLKRLESVSRSPYISHPSETITGASVIRAYQRERS	1146
<i>X. tropicalis</i>	LEAVVVIPLAIAVIFVQRFYVATSRQLKRLESVSRSPYISHPSETITGASIIIRAYQRONS	1173
<i>T. nigroviridis</i>	FAAVIILPLAIIYACVQSPFYVATSCQLRLEAVSREPIYTHINETVQASVIRAFCEQPR	1138
<i>S. acanthias</i>	FILMNDTKVDANQKSYYPGIVSNRWLGIRIEFIGNCIVLFAALFAVIGRHDLDPGIVGLS	1246
<i>H. sapiens</i>	FEIISDTKNVDANQKSYYPGIIISNFWLSRGVEEVGNCVVLFAALFAVIGRSSNPGLVGLS	1230
<i>G. gallus</i>	FIDISILKVDENQKSYYPGIIISNRWLGIRIEFVGNVIVLFAALFAVIGKSSINAGLVGLS	1206
<i>X. tropicalis</i>	FIVLSNKNVDENQKSYYPGIVSNRWLGVEVEEVGNCVVLFAALFAVIGREHLSPGLVGLS	1233
<i>T. nigroviridis</i>	FIDQANKRVDFNQTSYFRRFATNWLAVNLEFIGNGVVLAAAILSNRNTLSPGIVGLA	1198
<i>S. acanthias</i>	VSYALQVTMSLNMMVFMITSOLESNIVAVERVKEYSETETEAPWVIESNRPFKEWETCNV	1306
<i>H. sapiens</i>	VSYSLQVTFALNMMIIRMSOLESNIVAVERVKEYSKTETEAPWVVEGSRPFEGNPPRGEV	1290
<i>G. gallus</i>	VSYALQVTMALNMMVITSOLESNIVAVERIKKEYSETETEAPWIIEDKRPADWPSRGEL	1266
<i>X. tropicalis</i>	VSYALQVTMSLNMMVFMITSOLESNIVAVERVKEYAENETEAPWHIEDTKIPEIDWPSKGEV	1293
<i>T. nigroviridis</i>	VSHSLQVTAIISIVISWTEVNNIVSVERVNEVADAKRASATVEGSSLPMDNPLKGTI	1258
<i>S. acanthias</i>	EFNYSVRYRPGLDLVLDLQCSVHOGKVGIVGRTGAGKSEMTLCIFRILEAAKGEIRI	1366
<i>H. sapiens</i>	EFKNYSVRYRPGLDLVLRDLSEHVHGGEKVGIVGRTGAGKSEMTLCIFRILEAAKGEIRI	1350
<i>G. gallus</i>	EFVYSVRYRPGLDLVLDLNRVHOGKIDIVGRTGAGKSEMTLCIFRILEAAKGEIRI	1326
<i>X. tropicalis</i>	ELSNYSVRYRPGLDLVLDLNLKNGGKVGIVGRTGAGKSEMTLCIFRILEFNEGIVKI	1353
<i>T. nigroviridis</i>	EFQENGLQYRKGLELALKGITINIEREKVGIVGRTGAGKSSALGIFRILEAAKGTIF	1318
<i>S. acanthias</i>	DGVKTIADIGLHDLRSRLTIIPQDPVLFSGTLRMNLDPPFEQVTEEVNNALELSHLKQFVH	1426
<i>H. sapiens</i>	DGLNVADIGLHDLRSRLTIIPQDPHILFSGTLRMNLDPPFGSYSEEDINWALELSHLHTFVS	1410
<i>G. gallus</i>	DGVRTSEIGLHDLRSRLTIIPQDPVLFSGTLRMNLDPPFNKYSDEEVWKALELSHLKRFVS	1386
<i>X. tropicalis</i>	DNVNISEIGLCOLRSRLTIIPQDPVLFSGTLRMNLDPPFNKYSDEIWKALELSNLMKFVA	1413
<i>T. nigroviridis</i>	DGVNTADIGLHDLRSRLTIIPQDPVLFSGTLRMNLDPPDTITDCLVRSLEHAHKTPTVA	1378

<i>S. acanthias</i>	TLPAGLEHECSEGGENLSVGQRQLVCLARALLRKTRILILDEATAAVDLETDDLQSTIR	1486
<i>H. sapiens</i>	SQPAGIDFQCSEGGENLSVGQRQLVCLARALLRKSRILVLDATAAIDLETDLNLIQATIR	1470
<i>G. gallus</i>	SQPSMLEFECSEGGENLSVGQRQLVCLARALLRKTRVLILDEATAAIDLETDDLQMTIR	1446
<i>X. tropicalis</i>	GQPSQLEYECSEGGENLSVGQRQLVCLARALLRKTRILILDEATAAIDLETDDLQMTIR	1473
<i>T. nigroviridis</i>	NLPDKLNRECSEGGENLSLQQRQLVCLARALLRKTRILVLDATAAVDLETDDLQSTIR	1438
<i>S. acanthias</i>	TQFEGCTVLTIAHRLNTIMDYTRVLVLDKGSIAEFDTPSNLITQKGIFYSMANDAGLA	1544
<i>H. sapiens</i>	TQFDTCVLTIAHRLNTIMDYTRVLVLDKGVVAEFDSPANLIAARGIFYGMARDAGLA	1528
<i>G. gallus</i>	TQFEDCTVLTIAHRLNTIMDYTRILVLENGTIAEFDTPANLIASKGIFYGMARDAGEV	1504
<i>X. tropicalis</i>	TQFEDCTVLTIAHRLNTIMDYTRVLVLDKGRFAEFDTPNLIALNGIFYGMARDAGLA	1531
<i>T. nigroviridis</i>	TQFEDCTVLTIAHRLNTIMDYTRVIVMDRGHISFMDSPGNLIAQRGCFYRMCREAGLV	1496

FIG. 3.

Multiple alignments of MRP3/Mrp3 amino acid sequences among representative species. *S. acanthias*, spiny dogfish shark; *H. sapiens*, human; *G. gallus*, chicken; *X. tropicalis*, frog; *T. nigroviridis*, pufferfish. Transmembrane regions in shark are underlined. Amino acids common with shark are shaded.

FIG. 4.

Induction of Mrp3 mRNA in SAE shark embryo cells *in vitro*. Cultures were incubated with the indicated compounds, and mRNA abundance was determined by qPCR as described in “Materials and Methods” section. Briefly, SAE cells were incubated for 4 days in basal LDF nutrient medium containing amino acids, 10 nM selenous acid, 10 µg/mL transferrin, and 2 mM GlutaMax. Following this, other medium supplements (2% FBS, insulin, 50 ng/mL FGF, 50 ng/mL EGF, and 1:1000 CDL) were added to individual wells. A control was also included, in which none of the medium supplements were added. After incubation for 24 h, total RNA was isolated from each well, and cDNA was synthesized. The expression of shark Mrp3 was compared by qPCR with shark EF-1 α as the normalizer. Data were analyzed with MX pro software (Stratagene).

Table 1MRP3/Mrp3 Amino Acid Identities Between the Shark *S. acanthias* and Other Species

<i>Species</i>	<i>Protein length (amino acids)</i>	<i>Identity with shark (%)</i>	<i>Sequence identification</i>
<i>Homo sapiens</i>	1528	64	NP_003777
<i>Gallus gallus</i>	1504	72	XP_420102
<i>Xenopus tropicalis</i>	1531	71	ENSXETP00000026744
<i>Tetraodon nigroviridis</i>	1496	49	CAG03315.1
<i>Leucoraja erinacea</i>	201 ^a	86	EF991935
<i>Gasterosteus aculeatus</i>	1541	71	ENSGACP00000007835
<i>Oryzias latipes</i>	1538	69	ENSORLP00000025690
<i>Bos taurus</i>	1531	64	XP_612461
<i>Canis familiaris</i>	1523	64	XP_548204
<i>Mus musculus</i>	1522	64	AAX39010.1
<i>Rattus norvegicus</i>	1522	63	O88563
<i>Poeciliopsis lucida</i>	341 ^a	55	DQ842515
<i>Ciona intestinalis</i>	1593	54	ENSCINP00000003888

Protein length of *S. acanthias* Mrp3 (EU250283) is 1544 amino acids.

^a *L. erinacea* (little skate) and *P. lucida* (topminnow) are partial sequences.

Table 2

Percent Identity in Defined Domains of Human and Shark MRP3/Mrp3

<i>Shark domain</i>	<i>Amino acid number (shark)</i>	<i>Number of identical amino acids</i>	<i>Percentage identical (shark/human) (%)</i>
N-terminal	1–43	16	37
MSD1	44–203	96	60
Interdomain region	204–316	51	45
MSD2	317–491	113	65
NBD1	492–976	310	64
MSD3	977–1263	184	64
NBD2	1264–1544	229	82

Interdomain region is between MSD1 and MSD2. NBD2 includes C-terminal of the protein (see Fig. 2).

MSD, membrane-spanning domain; NBD, nucleotide-binding domain.

Table 3

Amino Acid Polymorphisms and Site-Specific Mutation Comparisons in Human and Shark Mrp3

<i>Amino acid (human)</i>	<i>Position</i>	<i>Polymorphism</i>	<i>Corresponding amino acid (shark)</i>
Lysine	13	Asparagine ^a	Arginine
Histidine	68	Tyrosine ^a	Valine
Serine	346	Phenylalanine ^a	Tyrosine
Glutamine	513	Lysine ^a	Lysine
Leucine	547	Glutamine ^b	Isoleucine
Arginine	1297	Histidine ^a	Arginine
Glycine	1423	Arginine ^a	Glycine
<i>Site-directed changes</i>			
Serine	1229	Alanine ^c	Serine
Serine	1231	Alanine ^c	Serine
Tyrosine	1232	Phenylalanine ^c	Tyrosine
Serine	1233	Alanine ^c	Alanine
Glutamine	1235	Alanine ^c	Glutamine
Asparagine	1241	Alanine ^c	Asparagine

^aLang *et al.*⁵^bLee *et al.*²¹^cZhang *et al.*²⁰