

# Molecular cloning and functional characterization of the mouse organic-anion-transporting polypeptide 1 (Oatp1) and mapping of the gene to chromosome X

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We have cloned a murine member of the organic-anion-transporting polypeptide (Oatp) family of membrane-transport proteins from mouse liver. The cloned cDNA insert of 2783 bp with an open reading frame of 2011 bp codes for a 12-transmembrane 670-amino-acid protein with highest amino acid identity with the rat Oatp1. When expressed in *Xenopus laevis* oocytes, the mouse Oatp exhibited the same substrate specificity as the rat Oatp1. Besides the common Oatp substrates bromosulphophthalein, taurocholate, oestrone 3-sulphate and ouabain, the new mouse Oatp also mediates transport of the Oatp1-specific magnetic-resonance-imaging agent gadoxetate. The Oatp2-specific cardiac glycoside digoxin, however, is not trans-

ported. Kinetic analyses performed for taurocholate and oestrone 3-sulphate revealed apparent  $K_m$  values of 12  $\mu\text{M}$  and 5  $\mu\text{M}$  respectively. Northern-blot analysis demonstrated a predominant expression in the liver with an additional moderate expression in the kidney. Taken together, the amino acid identity, the functional characteristics and the tissue distribution suggest that we have isolated the murine orthologue of the rat Oatp1, and consequently the identified protein will be called Oatp1. Using fluorescence *in situ* hybridization, the murine Oatp1 gene was mapped to chromosome XA3-A5.

Key words: bile salt, liver, steroid conjugate.

## INTRODUCTION

An important function of the liver is the elimination of a wide variety of mainly amphipathic xenobiotics. The first step of this task – uptake of these anionic, neutral and cationic compounds across the sinusoidal membrane – is mediated by  $\text{Na}^+$ -dependent and  $\text{Na}^+$ -independent transport systems [1]. Several  $\text{Na}^+$ -independent organic-anion-transporting polypeptides (Oatps) have been cloned from rat liver [2], brain [3,4] and retina [4], as well as from human liver (OATP or OATP-A and OATP-C or LST-1) [5,6] and brain (OATP-B) [7]. In addition, OAT-K1, a methotrexate transporter with high similarity to the Oatps, has been isolated from rat kidney [8]. All these members of the Oatp gene family are found in many different tissues besides the ones they were cloned from. Oatp1 is expressed, in addition to the sinusoidal membrane of hepatocytes [9], in the apical membrane of the proximal tubule [10] and in the apical membrane of the choroid plexus [11]. Oatp2 protein was detected in rat liver hepatocytes, endothelial cells of the blood/brain barrier [12–14] and the choroid plexus [12]. On the basis of Northern-blot analysis, Oatp3 is expressed in the kidney, liver and the retina [4]. mRNA for the human OATP (OATP-A) has been demonstrated in brain, lung, liver, kidney and testis [5].

Besides this broad tissue distribution, these proteins exhibit also a broad partially overlapping substrate specificity and are able to transport anionic compounds such as, for example, bile salts, bromosulphophthalein and steroid conjugates, glycosides

such as ouabain and digoxin, as well as the cation *N*-propylajmalinium (summarized in [1]). In addition, several peptidomimetic drugs such as enalapril [15], temocaprilat [16] and CRC220 [17], as well as cyclic (BQ123) [14] and linear (deltorphan) [18] peptides, are substrates of this growing family of membrane-transport proteins. A comparison between Oatp1, Oatp2 and the human OATP (OATP-A) revealed that the magnetic-resonance-imaging agent gadoxetate was only transported by Oatp1 [19], whereas digoxin was a specific compound for Oatp2 [3]. The human OATP (OATP-A), on the other hand, was unique in being the only Oatp that could mediate uptake of the type II cations *N*-methylquinine and *N*-methylquinidine [19].

To delineate the physiological importance of the individual family members in the different tissues, mouse knockout models of the Oatps are needed. As a first step in the identification and characterization of the mouse *oatp* gene family we report here the isolation, functional characterization and chromosomal mapping of the mouse Oatp1.

## MATERIALS AND METHODS

### Materials

[<sup>3</sup>H]Digoxin (555 GBq/mmol), [<sup>3</sup>H]oestrone 3-sulphate (1.81 TBq/mmol), [<sup>3</sup>H]ouabain (758.5 GBq/mmol) and [<sup>3</sup>H]taurocholic acid (96.2 GBq/mmol) were obtained from Du Pont NEN (Boston,

Abbreviations used: Oatp, organic-anion-transporting polypeptide; RT-PCR, reverse-transcription PCR; SSC, standard saline citrate (0.15 M NaCl/0.015 M sodium citrate); FISH, fluorescence *in situ* hybridization; DAPI, 4,6-diamidino-2-phenylindole.

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The nucleotide sequence data reported will appear in DDBJ, EMBL and GenBank Nucleotide Sequence Databases under the accession number AF148218.

	1									80
mouse Oatp1	MEETEKVKVAT	QEGRFFSKMK	VFLMSLTCAY	LAKSLSGVYM	NSMLTQIERQ	FGIPTSVVGF	ITGSFEIGNL	LLIVFVSYFG		
rat Oatp1	MEETEKKIAT	QEGRLFSKMK	VFLLSLTCAC	LTKSLSGVYM	NSMLTQIERQ	FDISTSVAGL	INGSFEIGNL	FFIVFVSYFG		
Consensus	MEETEKK-AT	QEGR-FSKMK	<u>VFL-SLTC</u> A-	<u>L-KSLSGVYM</u>	<u>NSMLTQIERQ</u>	<u>F-I-TSV-G-</u>	<u>I-GSFEIGNL</u>	<u>--IVFVSYFG</u>		
	81				*	*				160
mouse Oatp1	RKLHRPIIIG	VGCVVMGLGC	FLMASPHFLM	GRYKYETTIS	PTSNLSSNSF	LCIENRTQTL	KPTQDPTECV	KEIKSLMWIY		
rat Oatp1	TKLHRPVVIG	IGCVIMGLGC	LLMSLPHFFM	GRYKYETTIS	PTGNLSSNSF	LCMENRTQTL	KPTQDPAECV	KEMKSLMWIC		
Consensus	-KLHRP--IG	-GCV-MGLGC	-LM--PHF-M	GRY-YETTIS	PT-NLSSNSF	LC-ENRTQTL	KPTQDP-ECV	KE-KSLMWI-		
	161									240
mouse Oatp1	VLIGHTMRGI	GETPIMPLGI	SYIEDFAKSE	NSPLYIGILE	MGKIVGPIIG	LLGGSFFARV	YVDIGSVNTD	DLTITPTDTR		
rat Oatp1	VMVGNIIIRGI	GETPIVPLGI	SYIEDFAKSE	NSPLYIGILE	MGKVAGPIFG	LLGGSYCAQI	YVDIGSVNTD	DLTITPSDTR		
Consensus	V--GN--RGI	GETPI-PLGI	SYIEDFAKSE	NSPLYIGILE	<u>MGK--GPI-G</u>	<u>LLGGS--A--</u>	<u>YVDIGSVNTD</u>	<u>DLTITP-DTR</u>		
	241									320
mouse Oatp1	WVGAWWIGFL	VCAGVNILTS	IPFFFFPKTL	PKKELQDNVD	VTKYEKVEKH	RERAKKENLG	ITKDFLPMK	SLCCNPIYML		
rat Oatp1	WVGAWWIGFL	VCAGVNILTS	IPFFFFPKAL	PKKGQENVA	VTKDGKVEKY	GGQARENLG	ITKDFLPMK	RLFNCPIYML		
Consensus	WVGAWWIGFL	VCAGVNILTS	IPFFF-PK-L	PKK--Q-NV-	VTK--KVEK-	---A--ENLG	ITKDFL-FMK	-L-CNPIYML		
	321									400
mouse Oatp1	FSLTSVLQIN	GFASTFTFLP	KYLEQQYGKS	TSEAVFLIGV	YSLPPVCLGY	LISGFIMKKF	KITVKKAAYI	AFGLSLSEYF		
rat Oatp1	FILTSVLQVN	GFINKFTFLP	KYLEQQYGKS	TAEAIFLIGV	YSLPPICLGY	LIGGFIMKKF	KITVKKAAYL	AFCLSVFEYL		
Consensus	<u>F-LTSVLQ-N</u>	<u>GF--FTFLP</u>	<u>KYLEQQYGKS</u>	<u>T-EA-FLIGV</u>	<u>YSLPP-CLGY</u>	<u>LI-GFIMKKF</u>	<u>KITVKKAAY-</u>	<u>AF-LS--EY-</u>		
	401									480
mouse Oatp1	IFLCNYLLTC	DNFPVAGLTT	SYKGVQHPLY	GEKNVLADCN	TRCSCLTDTW	DPVCGDNGLA	YMSACLAGCE	KSVGTGTNMV		
rat Oatp1	LFLCHFMLTC	DNAAVAGLTT	SYKGVQHQLH	VESKVLADCN	TRCSCSTNTW	DPVCGDNGVA	YMSACLAGCK	KFVGTGTNMV		
Consensus	-FLC--LTC	DN--VAGLTT	SYKGVQH-L-	-E--VLADCN	TRCSC-T-TW	DPVCGDNG-A	YMSACLAGC-	K-VGTGTNMV		
	481									560
mouse Oatp1	FQNCSCIGSS	GNSSAVLGLC	KKGPECDNKL	QYFLIKSVFS	SFIFSLAaip	GYMVLLRCVK	SEEKSIGVGL	HAFPIRLLAG		
rat Oatp1	FQDCSCIQSL	GNSSAVLGLC	KKGPECANRL	QYFLILTIII	SFIYSLTAIP	GYMVFLRCVK	SEEKSLGVGL	HTFCIRVFAG		
Consensus	<u>FQ-CSCI-S-</u>	<u>GNSSAVLGLC</u>	<u>KKGPEC-N-L</u>	<u>QYFLI-----</u>	<u>SFI-SL-AIP</u>	<u>GYMV-LRCVK</u>	<u>SEEKS-GVGL</u>	<u>H-F-IR--AG</u>		
	561									640
mouse Oatp1	IPAPVYFGAL	IDRTCLHWGT	LKCGQPACR	MYDINRFRHI	YLGLPAAVRG	SSFLPAVFIL	ILMRKFHPPG	DIHSPDETELA		
rat Oatp1	IPAPVYFGAL	IDRTCLHWGT	LKCGQRACR	MYDINSFRHI	YLGLPIALRG	SSYLPAVFIL	ILMRKFQPPG	DIDSSATDHT		
Consensus	<u>IPAPVYFGAL</u>	<u>IDRTCLHWGT</u>	<u>LKCGQ-GACR</u>	<u>MYDIN-FRHI</u>	<u>YLGLP-A-RG</u>	<u>SS-LPA-FIL</u>	<u>ILMRKF-FPG</u>	<u>DI-S--T---</u>		
	641			670						
mouse Oatp1	EMKLTEKESE	CTDVCRSPKV	ENDGELKTKL							
rat Oatp1	EMMLGEKESE	HTDVHGSPQV	ENDGELKTKL							
Consensus	EM-L-EKESE	-TDV--SP-V	ENDGELKTKL							

**Figure 1** Amino acid sequence of the mouse Oatp aligned with the orthologous transporter from the rat

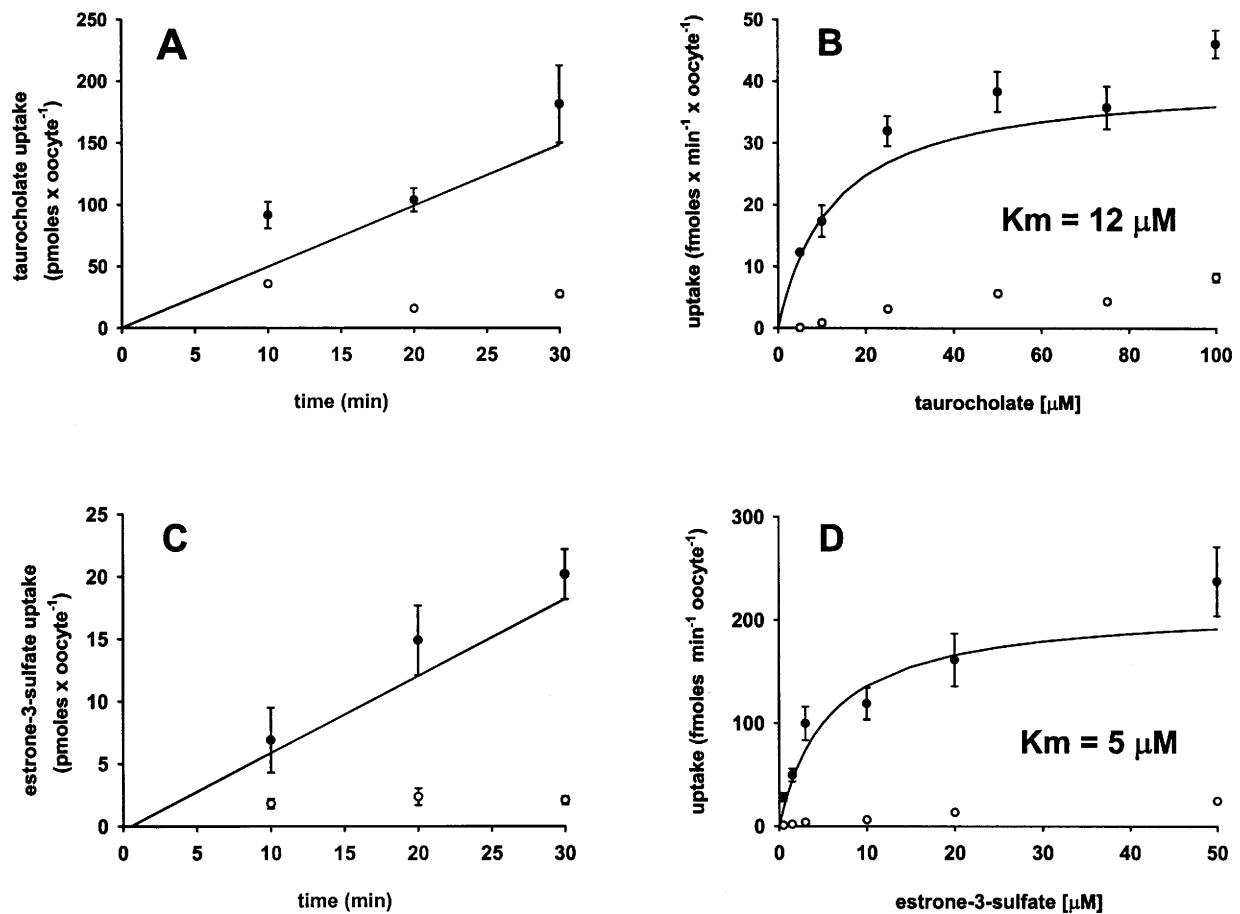
Conserved N-glycosylation sites at positions 124 and 135 is shown by asterisks. Putative transmembrane domains were determined with the TopPred2 membrane prediction server at the Stockholm University ([www.biokemi.su.se/~server/toppred2/](http://www.biokemi.su.se/~server/toppred2/)) [27] and are underlined.

MA, U.S.A.). [<sup>35</sup>S]Bromosulphophthalein was prepared as described by Kurisu et al. [20] at a specific radioactivity of 55.5 GBq/mmol). [<sup>153</sup>Gd]Gadoxetate (107.51 GBq/mmol) was kindly provided by Schering AG (Berlin, Germany). All other chemicals and reagents were of the highest degree of purity and were readily available from commercial sources.

### Library construction and screening

Total RNA was isolated from livers of female Balb/c mice as described in [21], and mRNA was prepared with the PolyATtract mRNA isolation system (Promega Corp., Madison, WI, U.S.A.). The cDNA library was constructed using an oligo(dT) primer with the Superscript kit (Gibco BRL, Gaithersburg, MD, U.S.A.). To screen the library by hybridization an Oatp cDNA probe was generated using reverse-transcription PCR (RT-PCR). A 100 ng portion of mRNA was reverse-transcribed and amplified with the Access RT-PCR System (Promega Corp.) and

primers derived from the human OATP (OATP-A) [5] (forward: 5'-TCACTACCTCATTTCCCTCATG-3'; reverse: 5'-AATGCA-TTGAAGTGTATCACA-3') which resulted in the amplification of a 685 bp fragment. After cloning this fragment into the pCR<sup>®</sup>II-TOPO vector (Invitrogen Corp., Carlsbad, CA, U.S.A.) it was sequenced using cycle sequencing (Thermosequenase; Amersham Pharmacia Biotech AB, Uppsala, Sweden) and an ALFexpress (Amersham Pharmacia Biotech AB). The fragment, which was 91 % identical with the rat liver Oatp1 [2], was labelled with deoxycytidine 5'-[ $\alpha$ -<sup>32</sup>P]triphosphate (Amersham Pharmacia Biotech AB; 111 TBq/mmol) and the Rediprime II DNA labelling system (Amersham Pharmacia Biotech AB) and was used to screen replica filters (Hybond-N; Amersham Pharmacia Biotech AB) of the library in ExpressHyb hybridization solution (ClonTech, Palo Alto, CA, U.S.A.) (30 min prehybridization followed by 2 h hybridization at  $1.3 \times 10^6$  c.p.m./ml and 60 °C). After two rounds of screening (final washing conditions were 59 °C with  $1 \times$  standard saline citrate (SSC; 0.15 M NaCl/0.015 M sodium citrate)/0.1 % SDS), a single positive clone was



**Figure 2** Oatp-mediated taurocholate and oestrone 3-sulphate uptake in *Xenopus laevis* oocytes

(A) Time course of uptake of [ $^3$ H]taurocholate (100  $\mu$ M) in Oatp-cRNA-injected (●) and in water-injected (○) oocytes. (B) Kinetics of initial (15 min) [ $^3$ H]taurocholate uptake in Oatp-cRNA-injected (●) and in water-injected (○) oocytes. (C) Time course of uptake of [ $^3$ H]oestrone 3-sulphate ('estrone-3-sulfate'; 100  $\mu$ M) in Oatp-cRNA-injected (●) and in water-injected (○) oocytes. (D) Kinetics of initial (15 min) [ $^3$ H]oestrone 3-sulphate uptake in Oatp-cRNA-injected (●) and in water-injected (○) oocytes. Oocytes were injected with 5 ng of cRNA (●) or water (○) and after 2 days in culture, uptakes were measured at 25 °C. The uptake values represent means  $\pm$  S.E.M. of eight to ten determinations. After subtracting the values of the water-injected oocytes, the data were fitted and plotted either to a straight line using linear regression analysis (A and C) or to the Michaelis–Menten equation using non-linear regression analysis (B and D).

isolated and functionally tested using the *Xenopus laevis* oocyte system. This mouse Oatp cDNA was sequenced on both strands using cycle sequencing and an ALFexpress.

#### Expression of Oatp1 in *Xenopus laevis* oocytes

For *in vitro* synthesis of cRNA, the recombinant plasmid containing the cDNA of the mouse Oatp was isolated using the QIAprep Spin Miniprep kit (Qiagen AG, Basel, Switzerland) and cut with *NotI* (Roche Diagnostics, Rotkreuz, Switzerland). Capped cRNA was synthesized using T7 RNA polymerase (Promega Corp.) as described in [22]. *Xenopus laevis* oocytes were prepared as described previously [23]. After an overnight incubation at 18 °C, healthy oocytes were injected with 5 ng of Oatp1 cRNA or water. After 2 days in culture, uptake of the indicated substrates was measured at 25 °C in a medium containing 100 mM NaCl, 2 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub> and 10 mM Hepes/Tris, pH 7.5, as described in [24].

#### Northern-blot analysis

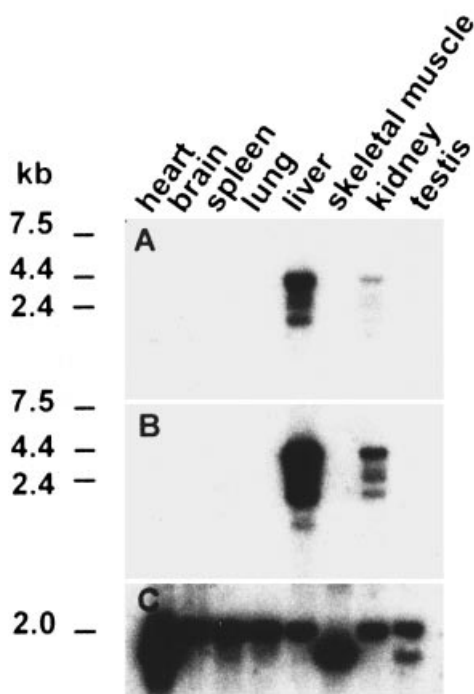
A commercially available Northern blot (ClonTech) was pre-hybridized for 30 min at 60 °C in ExpressHyb hybridization

solution and then hybridized for 60 min at 60 °C with a PCR-amplified  $^{32}$ P-labelled mouse Oatp cDNA probe (nts 1–325). The blot was washed twice for 5 min at room temperature with  $2 \times$  SSC/0.1 % SDS, followed by 15 min at 63 °C with  $0.1 \times$  SSC/

**Table 1** Substrate specificity of mouse Oatp in *Xenopus laevis* oocytes

Values represent means  $\pm$  S.D. of eight to twelve determinations. *Xenopus laevis* oocytes were injected with 5 ng of mouse Oatp cRNA, and after 2 days in culture, uptake of the indicated substrates was measured for 1 h.

Substrate	Uptake (pmol/oocyte)	
	Oatp1-injected	Water-injected
Bromosulphophthalein (6 $\mu$ M)	1.36 $\pm$ 0.59	0.27 $\pm$ 0.06
Taurocholate (10 $\mu$ M)	1.20 $\pm$ 0.4	0.09 $\pm$ 0.02
Oestrone 3-sulphate (1 $\mu$ M)	1.80 $\pm$ 0.8	0.05 $\pm$ 0.004
Digoxin (0.5 $\mu$ M)	0.04 $\pm$ 0.01	0.06 $\pm$ 0.01
Quabain (25 $\mu$ M)	1.90 $\pm$ 0.4	0.24 $\pm$ 0.1
Gadoxetate (15 $\mu$ M)	1.40 $\pm$ 0.26	0.39 $\pm$ 0.14



**Figure 3** Tissue distribution of Oatp1 by Northern-blot analysis

A commercially available Northern blot (Clontech) containing 2  $\mu$ g of mRNA per lane was hybridized with Oatp1 cDNA (**A**, **B**) or  $\beta$ -actin (**C**). The blot was exposed to autoradiography film at  $-70^{\circ}\text{C}$  for 14 h (**A**), 4 days (**B**) or 2 h (**C**). The different  $\beta$ -actin transcripts detected correspond to the various isoforms of  $\beta$ -actin found in the different tissues.

0.1% SDS. Subsequently the blot was exposed to autoradiography film at  $-70^{\circ}\text{C}$ . After stripping, the blot was hybridized using the same conditions with a human  $\beta$ -actin probe as a control.

### Genomic mapping of the Oatp1 gene

Fluorescence *in situ* hybridization (FISH) was performed on metaphase chromosome preparations obtained from mouse bone-marrow cells according to standard procedures. The full-length mouse Oatp cDNA was labelled with digoxigenin-dUTP (Roche Diagnostics) using the Nick Translation System (Gibco BRL), whereas the X-chromosome painting probe was purchased biotin-labelled (Oncor Appligene, Heidelberg, Germany). Hybridizations were performed following the method described in [25] with some modifications. Labelled probes were mixed with Master Mix 2.1 [55% (w/v) formamide/10% (w/v) dextran in  $1\times\text{SSC}$ ] and denatured at  $78^{\circ}\text{C}$  for 8 min. The sperm slides used for the double-label FISH were incubated in 1% formamide for 5 min and then in 0.002% pepsin for 10 min. The slides were denatured on 70% formamide (in  $2\times\text{SSC}$ , pH 7.0) at  $78^{\circ}\text{C}$  for 5 min, dehydrated in an alcohol series (70, 90 and 100%, 2 min each) and dried on a slide warmer at  $37^{\circ}\text{C}$  for 3 min before application of the denatured hybridization mix.

Hybridization was carried out for 24–48 h in a moist chamber at  $37^{\circ}\text{C}$ . Post-hybridization washing consisted of two steps: 30 min in 50% formamide ( $2\times\text{SSC}$ , pH 7.0) at  $45^{\circ}\text{C}$  and 30 min in 0.1M sodium phosphate buffer, pH 8.0, containing 0.1% Nonidet P-40, at  $37^{\circ}\text{C}$ . The probes were detected by streptavidin-Cy3 (chromosome X) and anti-digoxigenin-FITC (Oatp). The chromosomes were counterstained with 4,6-diamidino-2-phenyl-

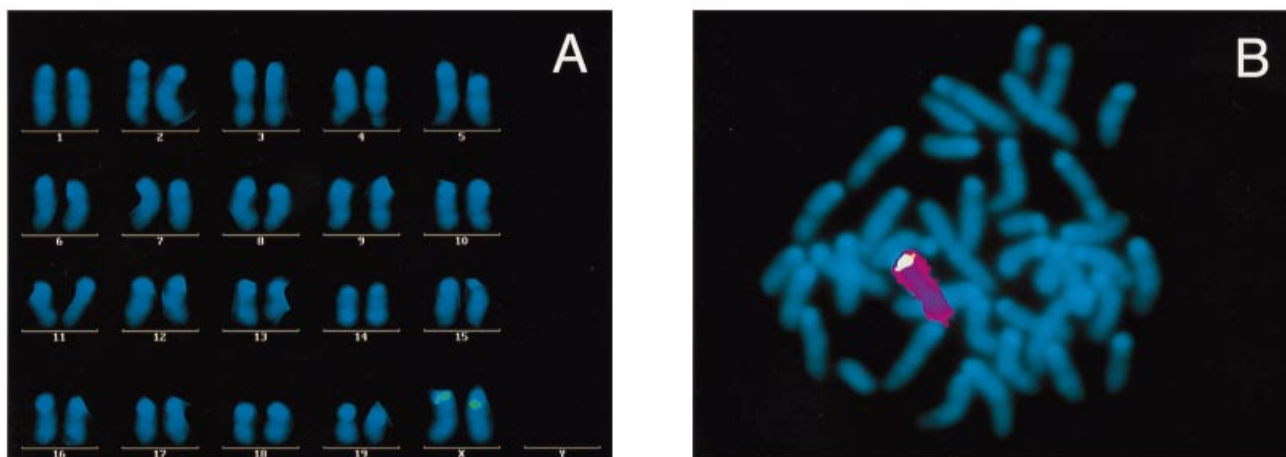
indole (DAPI) (0.6  $\mu\text{g}/\text{ml}$  of PBS) for 10 min at room temperature and cover-slipped in Vectashield (Vector Laboratories, Burlingame, CA, U.S.A.). Slides were stored at  $4^{\circ}\text{C}$  in the dark. Metaphase chromosomes were analysed under an Axiophot microscope (Zeiss, Oberkochen, Germany) and pictures were taken by digitizing the microscopic image with the computer program ISIS3 (Metasystems, Altlußheim, Germany).

### RESULTS AND DISCUSSION

To identify and isolate the first mouse orthologue of the Oatp family [1,26], we first generated and cloned an RT-PCR fragment using mouse liver mRNA and OATP (OATP-A) primers. Sequence analysis revealed a 91% identity of this fragment with the rat Oatp1 [2]. Screening a mouse liver cDNA library with this cDNA fragment as a probe we isolated a mouse Oatp cDNA consisting of 2783 bp with an open reading frame of 2011 bp coding for a protein of 670 amino acids (Figure 1). According to the TopPred2 program of Stockholm University [27], the protein is predicted to have 12 transmembrane domains. On the basis of the extracellular location of the two putative N-linked glycosylation sites at positions 124 and 135, which are conserved among the Oatp family [26], the N- and the C-terminal end of Oatp are proposed to be cytoplasmic (Figure 1). The amino acid sequence of the mouse Oatp was compared with that of the other Oatp family members. The highest identity was found for rat Oatp1 (81%) [2], followed by Oatp3 (79%) [4], Oatp2 (78%) [3], OAT-K1 (74%) [8] and the human OATP (OATP-A) (68%) [5]. A search of the available databases identified, in addition to the prostaglandin transporters from rat (36% identity) and man (35% identity), the human OATP-C (41% identity) [6] and OATP-B (33% identity) [7], as well as several related mouse expressed sequence tags, none of them identical with the here-described Oatp. Given the similar identity with the rat Oatp1 (81%), Oatp3 (79%) and Oatp2 (78%), amino acid sequence comparison alone was not sufficient to determine with certainty which orthologue was cloned. Therefore, functional characteristics and tissue distribution had to be compared as well.

The broad substrate specificities of the different members of the Oatp family have been investigated and described in several reports [1,3,5,28–30]. Besides common compounds such as, e.g., taurocholate, which are transported by Oatp1, Oatp2 and Oatp3, there are also specific compounds, which can discriminate at least between Oatp1 and Oatp2. In a recent study it has been shown, that the magnetic-resonance-imaging agent gadoxetate is exclusively transported by Oatp1, but not by Oatp2 or the human OATP (OATP-A) [19]. Oatp2, on the other hand, has been shown to be unique, so far, in being the only known Oatp that transports the cardiac glycoside digoxin [3]. For Oatp3, unfortunately, the picture is not yet as clear, because it has only been shown to mediate transport of taurocholate and, similar to Oatp1 and Oatp2 [4,31], of the thyroid hormones tri-iodothyronine ( $^3\text{T}_3$ ) and thyroxine ( $^4\text{T}_4$ ) [4].

Thus we tested uptake of several of these compounds in Oatp-cRNA-injected *Xenopus laevis* oocytes. All substrates transported by the rat Oatp1, such as bromosulphophthalein, taurocholate, oestrone 3-sulphate, ouabain and gadoxetate, were also transported by the cloned mouse Oatp (Table 1), whereas the Oatp2-specific cardiac glycoside digoxin [3] was not a substrate. In particular, gadoxetate, which has been shown to be exclusively transported by the rat Oatp1 [19] was also transported by the mouse Oatp. On the basis of this defined substrate specificity, the highest degree of amino acid identity with the rat Oatp1 and the tissue distribution (see below), the isolated mouse Oatp



**Figure 4** Chromosomal mapping of the mouse *Oatp1* gene to chromosome XA3-A5

(A) Metaphase chromosomes prepared from mouse bone-marrow cells were hybridized with a full-length *Oatp1* cDNA probe and detected using an anti-digoxigenin-FITC antibody (green signal). The chromosomes were counterstained with DAPI. After digitizing using the computer program ISIS3 (Metasystems), the chromosomes were karyotyped. (B) To confirm the X-chromosomal location, a two-colour FISH analysis was performed with the *Oatp1* cDNA (green signal) and an X-chromosome painting probe (red signal). In places where the two signals are superimposed, the resulting output signal is white, and the green signal is no longer visible.

was assigned to be the orthologue of the rat *Oatp1* and therefore will be also called *Oatp1*. After determination of the initial linear range (Figures 2A and 2C), kinetic analyses were performed for the common bile salt taurocholate and the steroid conjugate oestrone 3-sulphate, which are both transported by several *Oatps*. As shown in Figures 2(B) and 2(D), the apparent  $K_m$  value for taurocholate is  $12 \mu\text{M}$ , whereas the affinity for oestrone 3-sulphate is slightly higher, with an apparent  $K_m$  value of  $5 \mu\text{M}$ .

Northern analysis was used to determine the tissue distribution of the mouse *Oatp1*. Similar to the situation for other members of the *Oatp* family [2,3,5] also in the mouse, several transcript sizes of 4.4, 2.8, 2.4 and 1.5 kb could be detected (Figure 3A). However, in contrast with the rat *Oatp1*, expression of the mouse *Oatp1* is restricted to the liver and the kidney. Even after a 4 day exposure of the Northern blot (Figure 3B), no additional signals were detected. This is not the result of unequal loading of mRNA, since hybridization with  $\beta$ -actin as a control probe revealed signals in all lanes (Figure 3C). These results clearly demonstrate that the mouse *Oatp1* is mainly a liver carrier, which is also, but to a lesser extent, expressed in the kidney. *Oatp3*, on the other hand, shows expression mainly in the kidney [4] and hardly any signal in the liver. Therefore we concluded that the cloned carrier indeed represents the mouse orthologue of the rat *Oatp1*.

Finally, as illustrated in Figure 4, using FISH, the mouse *Oatp1* gene was localized to chromosome X near the centromere which corresponds to locus XA3-A5. The computerized karyotyping (Figure 4A) was confirmed by co-localization of the *Oatp1* probe and an X-chromosomal painting probe (Figure 4B). This represents a new localization for an *Oatp*, since the only known locus of the human OATP (OATP-A) is on 12p12 [32], which is not syntenic to the X chromosome in the mouse.

In conclusion, the first member of the *Oatp* family was isolated from mouse liver. Its amino acid similarity, together with the functional characterization and the tissue distribution, strongly suggest that it is the orthologue of *Oatp1*. On the basis of this information it will be possible to characterize the cor-

responding gene and to investigate potential species differences, for example with respect to the elimination of xenobiotics.

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