Family of human oxysterol binding protein (OSBP) homologues: a novel member implicated in brain sterol metabolism

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Abstract **Oxysterol binding protein (OSBP) is a cytosolic protein that undergoes ligand-induced binding to the Golgi apparatus and has been implicated in the regulation of cellular cholesterol metabolism. In the yeast *Saccharomyces cerevisiae* an OSBP homologue is involved in membrane trafficking through the Golgi complex. Prompted by the multitude of OSBP-related genes in the yeast genome, we carried out a search for human expressed sequence tags (ESTs) displaying homology to the sterol-binding domain of OSBP. This revealed a minimum of six novel OSBP-related proteins, designated ORP-1 to ORP-6. ORP cDNA probes were generated by reverse transcription-PCR from human liver mRNA, and used for Northern blot analysis of human tissue transcript panels. This verified that each of them represents a different gene product and showed that they display distinct tissue-specific expression patterns. The ORP-1 and -2 mRNA expression levels were similar to or higher than that of OSBP while the ORP-3 to -6 mRNAs were detected at lower levels in specific tissues. The most abundantly expressed new gene, ORP-1, was transcribed at strikingly high levels in the cortical areas of human brain and displayed sterol-regulated expression in a cultured human neuroblastoma cell line. This indicates that ORP-1 may play an important role in maintaining the sterol balance in cells of the central nervous system. Together with OSBP, the identified gene products constitute a novel human protein family that may provide a link between organellar sterol status and membrane dynamics.**—Laitinen, S., V. M. Olkkonen, C. Ehnholm, and E. Ikonen. **Family of human oxysterol binding protein (OSBP) homologues: a novel member implicated in brain sterol metabolism.** ***J. Lipid Res.*** **1999:** 40: **2204–2211.**

Supplementary key words cholesterol metabolism • membrane transport • hydroxycholesterol • brain cholesterol

Oxygenated derivatives of cholesterol have multiple biological activities, including perturbation of cellular cholesterol homeostasis, disturbance of cellular membrane structure, and cytotoxic effects. Because of their toxicity, oxysterols pose a potential threat to human health and have been suggested to promote atherosclerosis, aging, and cancer (for review see refs. 1, 2). Their physiological functions remain largely unknown but they have been most often associated with the regulation of cellular cholesterol metabolism (3).

One way to obtain insight into the cellular roles of oxysterols is via the identification of the cellular receptors or carrier proteins through which oxysterols exert their functions. An important breakthrough in the field was the recent appreciation that members of the nuclear receptor superfamily are involved in mediating the functions of oxysterols. LXR was found to bind oxysterols that exist in the liver when there is a surplus of cholesterol and function as a ligand-activated transcription factor to up-regulate cholesterol catabolism to bile acids (4, 5). Another member of this family, PPARγ, functions as a receptor for oxidized metabolites of cholesteryl esters and regulates macrophage gene expression upon uptake of oxidized low density lipoprotein (LDL) (6, 7).

However, the first protein characterized as oxysterol binding and hence named oxysterol binding protein (OSBP) (8, 9) does not belong to the group of steroid hormone receptors that display direct binding to DNA. Instead, it is a cytoplasmic protein that, upon oxysterol binding, translocates from the cytosol to the membranes of the Golgi apparatus (10). After cloning of the OSBP cDNA (11, 12), considerable effort has been directed towards understanding how the membrane association of OSBP is mediated and regulated. We know that the membrane interaction is attributed to the N-terminal pleckstrin homology (PH) domain in the protein, which most probably associates with phosphatidylinositol-4,5-bisphosphate or a related phosphatidylinositide on Golgi membranes (13, 14). The C-terminal part of the protein is responsible for

Abbreviations: OSBP, oxysterol binding protein; ORP, oxysterol binding protein-related protein; EST, expressed sequence tag; PCR, polymerase chain reaction; PH, pleckstrin homology; LDL, low density lipoprotein; FBS, fetal bovine serum; LPDS, lipoprotein-deficient serum; CNS, central nervous system.

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oxysterol binding and when this is removed the remainder of the protein localizes to the Golgi spontaneously (10).

Several studies have implicated OSBP in the regulation of cellular cholesterol balance. Stably transfected cells overexpressing OSBP were shown to display a marked decrease in cholesteryl ester synthesis and an increase in cholesterol biosynthesis as well as up-regulated mRNA levels of the sterol-regulated genes 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, HMG-CoA-synthase, and LDL receptor (13). Interestingly, the PH-domain deletion mutant did not translocate to the Golgi complex nor did it display increased cholesterol synthesis or decreased acyl-CoA:cholesterol acyltransferase (ACAT) activity. Moreover, the disruption of the Golgi apparatus was found to reduce the action of oxysterols on cholesterol synthesis (15). These results suggest that the correct localization of OSBP to the Golgi complex is necessary to induce alterations in cholesterol balance. On the other hand, the Golgi localization of OSBP may also be coupled to the regulation of protein traffic through this organelle as overexpression of the PH domain prevented forward transport of a cargo protein (14).

Interestingly, several *Saccharomyces cerevisiae* genes related in sequence to OSBP have been identified (16, 17). Their functions are still poorly understood. The genes do not appear to be essential, and there is only suggestive data on their role in the biosynthesis of the yeast sterol ergosterol (16). However, one of the yeast genes, *KES1*, has been linked to the biogenesis of Golgi-derived transport vesicles (18). Another OSBP homologue Osh1p, contains a PH domain that, analogous to that of OSBP, is sufficient to target fusion proteins to mammalian Golgi membranes (14). These findings strengthen the idea that OSBP and its yeast homologues may play a role in integrating functions that relate organelle lipid composition to membrane traffic.

As OSBP seems to carry out a fundamental, conserved function for which the yeast assigns several proteins, it is likely that OSBP has a number of homologues also in humans. In order to outline the human OSBP family members, we searched for OSBP-related human sequences in the EST (expressed sequence tag) database. This led us to identify six novel human OSBP-related cDNAs. We prepared probes specific for these sequences by PCR (polymerase chain reaction) and studied the gene expression patterns in mRNAs extracted from human tissues. Each of the cDNAs was found to exhibit a unique tissue distribution demonstrating that they code for separate proteins. One of the novel transcripts, ORP-1, is present at high levels in the brain, shows sterol regulatable expression, and may thus be an important controller of cholesterol homeostasis in the central nervous system (CNS).

MATERIALS AND METHODS

Generation of the ORP cDNA fragments

Primers for the amplification of the ORP cDNA fragments by PCR were designed to generate similar-sized specific amplification products of each ORP, with either BamHI or EcoRI restriction sites at the ends, to facilitate subcloning into pBluescript KS(−) (Stratagene). The primer sequences and sizes of the amplification products are given in **Table 1**. Human liver first strand cDNA was used as a template and the thermal cycling protocol was 94°C 1 min, 58°C 30 s, and 72°C 1 min for 35 cycles. In the case of ORP-5, the product had to be reamplified under identical conditions in order to obtain enough material for subcloning. The cloned fragments were verified by dideoxy sequencing to correspond to the ORPs identified in the database search.

RNA extraction and Northern blot hybridization

Total RNA was extracted from human liver using the guanidine isothiocyanate method (19), and mRNA was purified with Oligotex-dT mRNA Mini Kit (Qiagen). Oligo-dT primed cDNA was synthesized using AMV-reverse transcriptase (Promega). RNA from human neuroblastoma and hepatocellular carcinoma cells was extracted using the RNAeasy kit (Qiagen), electrophoresed in 1% formaldehyde agarose gel, and either visualized with ethidium bromide staining (10 μg total RNA) or transferred to a Hybond-N membrane (Amersham) (20 μg total RNA from neuroblastoma cells or 1 μg mRNA from hepatocellular carcinoma cells). This filter and three Multiple Tissue Nothern (MTN) Blot filters (Clontech) were hybridized using ExpressHyb solution (Clontech); the filters were stripped between hybridizations according to the instructions of the manufacturer. The human β-actin cDNA control probe was from Clontech. The probes were labeled to a specific activity of $1.5–3 \times 10^8$ cpm/ug with ^{32}P-dCTP (Amersham) using the random primed DNA labeling kit (Boehringer Mannheim), and the probe was used in the concentration of 1.5×10^7 cpm/10 ml of ExpressHyb solution. After hybridization, the filters were washed in 2 × SSC, 0.05% SDS for 45 min (30 min and 15 min, exchanging the wash

TABLE 1. Primers used for the amplification of the ORP cDNAs and lengths of the generated fragments

Fragment Name	5′ primer	3′ primer	Fragment Length
OSBP	gtcgcggatccaaaagcagggtcatgctgtgg	ctcgcggatcctcagaaaatgtccgggcatgagct	401
ORP-1	tccggaattcgagaagaagaacagcaaacag	tccggaattctttaataaatgtcaggcaaattg	446
ORP-2	tcgcggatccgagctggagacaggcatg	cgcggatcctcacagtggccactcgag	351
ORP-3	tccggaattcatcggctgtttgggaaatg	tccggaattcacccaagagtcatcgtcg	353
ORP-4	tcgcggatcctcagctgcgcactacgtg	tcgcggatcctggcaccggtcattggtct	254[a]
ORP-5	tcgcggatccggagaacaagagtctgatgt	tcgcggatcctgatgcagaagccgtcc	394
ORP-6	tcgcggatccaactgatgatccatatgagc	tcgcggatccataatgttctatccatcttc	388

[a] The fragment is smaller than the others as the amplification of a longer ORP-4 fragment failed, likely due to sequencing errors in the database ESTs resulting in primer mismatch.

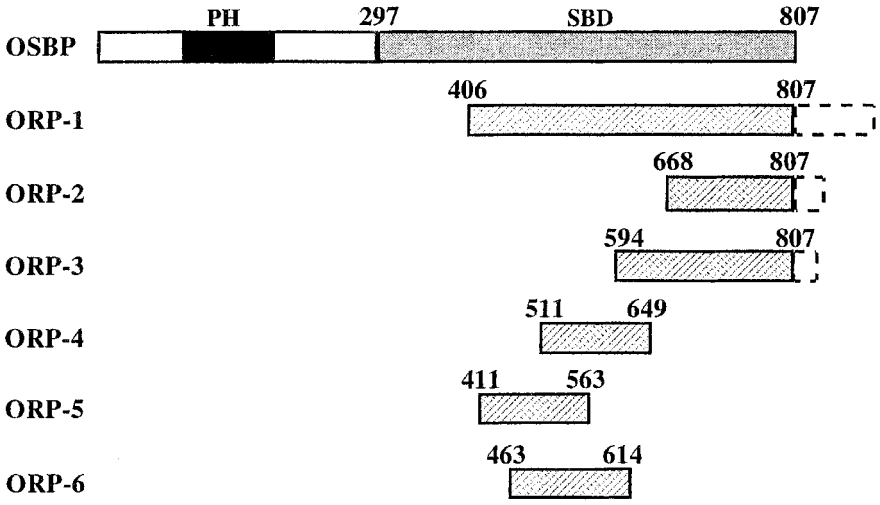

Fig. 1. Alignment of the translates of the novel EST contigs with the OSBP amino acid sequence. The pleckstrin homology domain of OSBP (PH) is indicated by a black bar, and the sterol binding domain (SBD) by a grey bar. The numbers refer to the amino acid numbers of OSBP. The numbers at the beginning and end of each ORP (indicated by striped bars) show the positioning of the best match of the ORP translate relative to OSBP. The dotted line indicates the extension of the ORP to the sequence corresponding to the 3′ untranslated region of OSBP.

solution once) at room temperature, followed by a 20-min wash in 2 × SSC, 0.1% SDS at 50°C, and exposed on Kodak X-Omat films for 2 or 7 days.

Cell culture and manipulation of cellular sterol levels

The human neuroblastoma-derived SHEP cell line (kindly provided by Dr. Marc Billaud, Laboratoire de Genetique, Domaine Rockefeller, Universite Claude Bernard Lyon 18, Lyon, France), representing a variant with an epithelial-like phenotype from the SKNSH neuroblastoma line, was cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mm glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. The human hepatocellular carcinoma HuH7 cells were cultured as described previously (20). Cells grown to 70% confluency were subjected to the following treatments: *1*) cultured in the continued presence of 10% FBS for an additional 48 h, or *2*) delipidated by shifting to medium containing 5% lipoprotein-deficient serum (LPDS) for 48 h, or *3*) subjected to LDL loading by first delipidating in 5% LPDS for 24 h and then adding LDL at 50 μg/ml for an additional 24 h, or *4*) subjected to 25-hydroxycholesterol treatment by delipidating in 5% LPDS for 44 h and then adding 5 μg/ml of 25-hydroxycholesterol (Sigma) for 4 h. After the treatments the cells were harvested for RNA extraction. LDL was isolated from fresh human pheresis plasma by standard sequential untracentrifugation techniques using solid KBr to adjust the density (d 1.019–1.063 g/ml) (21). To prepare LPDS, lipoproteins were removed from FBS as described by Krieger (22). The cholesterol content of the cells was assayed spectrophotometrically using a cholesterol oxidase assay kit (Boehringer Mannheim), and normalized for total cellular protein measured according to Lowry et al. (23). The intensities of the RNA signals were quantified by Phosphorimager analysis using the Fujifilm BAS-1500 apparatus.

RESULTS

Search for human OSBP homologues

The National Center for Biotechnology Information (NCBI) dbEST database was searched for human cDNA sequences homologous to the sterol binding region of OSBP (amino acid residues 297–807, see **Fig. 1**) using the tBLASTn program. The search was confined to the sterol binding region because we found the *S. cerevisiae* homologues of OSBP to consist of three long (YDL019C, Osh1p, and YHR073W, 996–1283 amino acids in length) and four short (Kes1p, YKR003W, Hes1p, and YHR001W, 434–448 amino acids) members of the family, the latter ones spanning only the C-terminal sterol binding domain. Furthermore, among the long OSBP homologues the conservation was highest in the sterol binding domain (36–39% identical residues, data not shown).

To exclude from the search very short sequences, ones of poor quality, and ones whose homology to OSBP may

TABLE 2. Human ESTs showing homology to the sterol binding region of OSBP

Name of Gene Fragment (length, bp)	ESTs within Fragment (order of homology to OSBP)
ORP-1 (1553)	W25938, AA424108, W70050, W70049, AA996189, AA962427, F00113, W19983, AA664137, H24107, H15330, N53619, N51477, AI018595, AA424058, N64837, N56481
ORP-2 (542)	AA628418, T60859, R26640, AA339145
ORP-3 (760)	H73831, T86866, T87553, AA287431
ORP-4 (415)	R87310, AA326182, H14182, R87451
ORP-5 (458)	AA776467
ORP-6 (455)	AA081659
ORP-7 (113)	F06485
ORP-8 (65)	AA248632

The NCBI dbEST database was searched for sequences similar to the OSBP sterol binding region; 40 ESTs were identified, 7 of which represented OSBP itself. Of the remaining 33 ESTs, 29 could be grouped to four contigs (ORP-1–4) and 4 ESTs (ORP-5–8) had no overlaps with other ESTs.

be questionable, the *P* value cutoff level for the sequences was set at 0.0001. The search yielded 40 human ESTs, seven of which clearly represented OSBP itself. The remaining 33 ESTs were analyzed and grouped to contigs using the University of Wisconsin Genetics Computer Group (GCG) software (**Table 2**). This resulted in the identification of 8 novel sequences showing definite homology to OSBP. The novel open reading frames were named ORP-1 to ORP-8 (OSBP-related proteins). However, ORP-7 and -8 were so short (113 bp and 65 bp, respectively) that they were omitted from further analyses. The positioning of the translates of the novel EST contigs in alignment with the OSBP amino acid sequence is shown in Fig. 1. The alignment of the sequences suggests that they represent at least four independent genes. Of the six sequences subjected to further analysis, ORP-2 does not overlap with ORP-4, -5, or -6 and could thus belong to any of these three genes. Likewise, ORP-3 could be part of the same gene as ORP-5.

Tissue expression patterns of the novel genes

To verify that the novel sequences are expressed and to elucidate whether they truly represent distinct genes, a Northern blotting approach was taken. Nucleic acid probes corresponding to the six EST contigs were amplified by PCR using human liver cDNA as a template (Table 1), subcloned into pBluescript and the sequences verified. Human multiple tissue Northern blots were then hybridized with the cDNA probes under standardized conditions. To obtain signals comparable with each other, the probes were designed to be similar in length (Table 1) and labeled to a specific activity of $1.5–3 \times 10^8$ cpm/μg. Incubations during the hybridizations and washings were always similar and the exposure times were 2 and 7 days

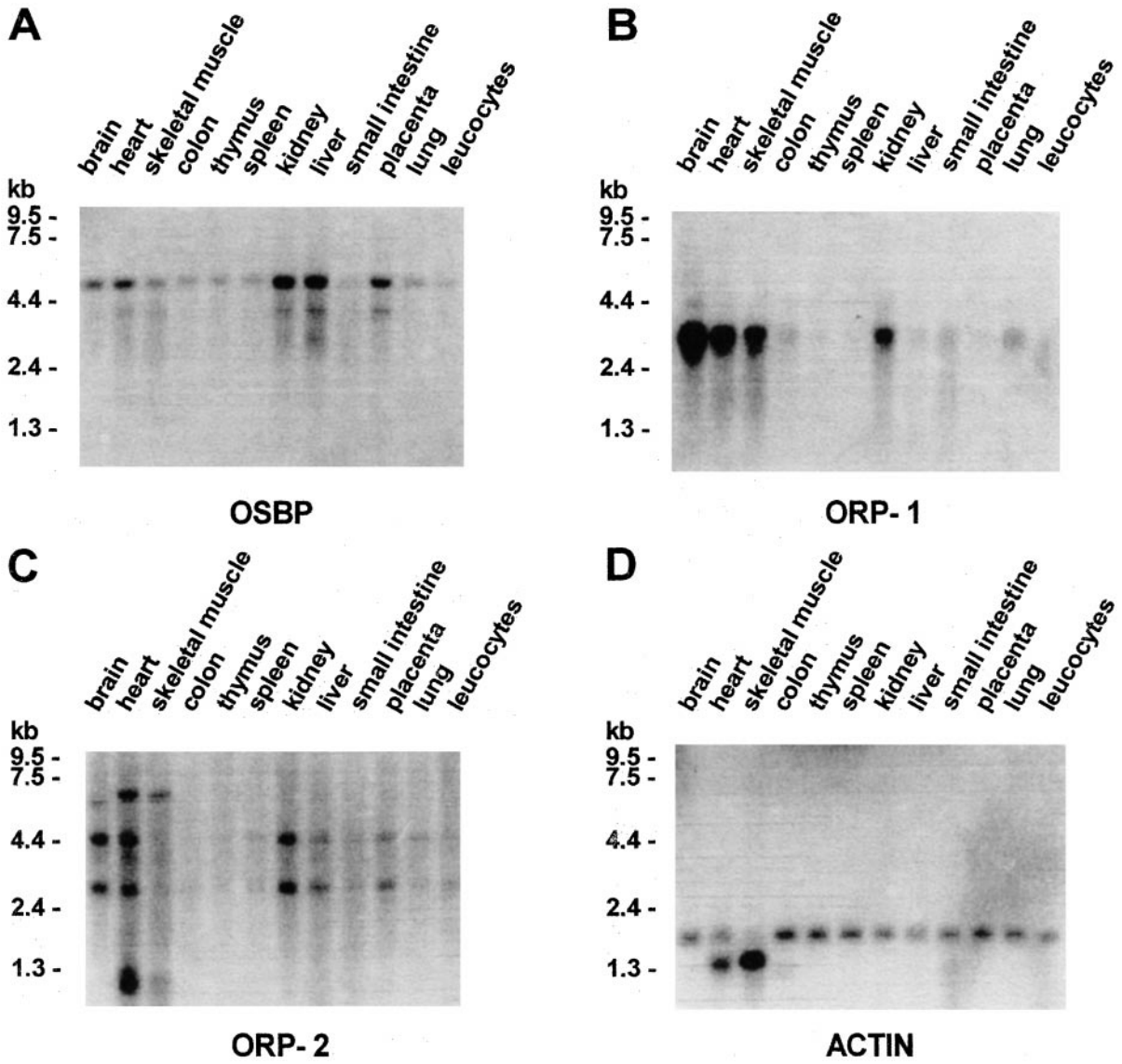

Fig. 2. Expression of OSBP, ORP-1, and ORP-2 in human tissues. Multiple tissue Northern blot filters were hybridized with the cDNA fragments as detailed in Materials and Methods, and the filters exposed for 2 (ORP-1) or 7 (OSBP, ORP-2) days. The molecular weight markers are indicated. Each lane contains approximately 2 μg of poly A^+ RNA, and the actin mRNA levels are shown as loading controls.

(see Materials and Methods). OSBP was included in this analysis for comparison. The 4.6 kb OSBP mRNA was found to be ubiquitously expressed as reported previously (10, 11), being most abundant in kidney and liver (**Fig. 2A**). In contrast, the 3.6 kb mRNA of ORP-1, although detectable ubiquitously, was very abundant in a subset of the tissues: the signal was very strong in the brain (note 2 day exposure vs. 7 day exposure for OSBP), and abundant also in heart, skeletal muscle, and kidney (Fig. 2B). The second new sequence expressed at relatively high levels, ORP-2, displayed a number of different sized mRNAs, suggesting a high degree of differential splicing. The main forms of 4.4 and 2.8 kb were present at highest levels in brain, heart, and kidney. These forms were abundant also in liver and placenta. Additional, probably muscle-specific mRNA forms of 6.6 and 1.2 kb were detectable in heart and skeletal muscle, and a further minor 6.0 kb species was detected in the brain (Fig. 2C).

The remaining four ORPs displayed weaker but clearly distinct and tissue-specific signals. Three mRNAs of ORP-3 (7.0, 4.5, and 3.5 kb) were detectable at very low levels in all the tissues analyzed, the signal of 7.0 kb being most prominent in spleen and white blood cells, which may imply function in B-lymphocytes (**Fig. 3A**). ORP-4 mRNAs were most abundantly expressed in the brain (4.8 and 3.8 kb species) and heart (a 3.5 kb form), and minor mRNA signals were detectable also in colon, kidney, and liver (Fig. 3B). An ORP-5 mRNA of 4.0 kb was found ubiquitously at low levels, showing, however, highest signals in thymus and peripheral blood leukocytes, which indicates predominant expression in T-cells (Fig. 3C). ORP-6 displayed the most distinct signals in brain, heart, and skeletal muscle,

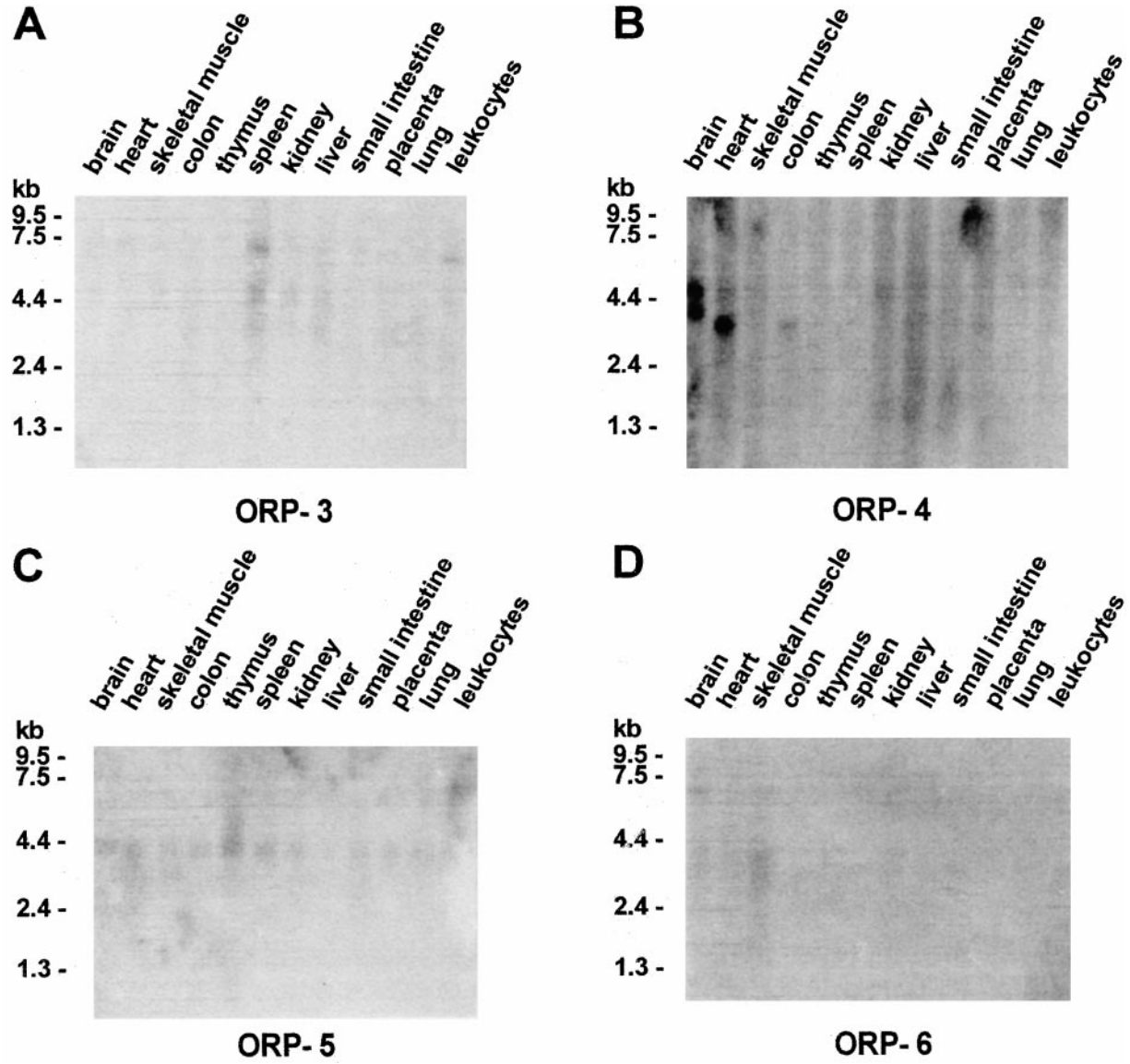

Fig. 3. Expression of ORP-3, -4, -5, and -6 in human tissues. Multiple tissue Northern blot filters were hybridized with the cDNA fragments as detailed in Materials and Methods and exposed for 7 days. Each lane contains approximately 2 μg of poly A^+ RNA; the actin mRNA levels were similar to those shown in Fig. 2D.

and very weak ones in several other tissues. In brain the major mRNA species was 7.2 kb in size, whereas species of 4.0 and 3.5 kb were predominant in skeletal muscle (Fig. 3D).

Because of the strikingly high level of the ORP-1 mRNA in the brain we wanted to study further its expression in subregions of the human central nervous system. This Northern blot analysis revealed the abundant presence of the 3.6 kb mRNA (as well as minor 4.4 and 6.0 kb forms that may also represent cross reactivities of other ORPs expressed in the brain, see above) in all the regions of the brain included in the analysis, i.e., cerebellum, medulla, spinal cord, and cerebral cortex with the frontal and temporal lobes as well as the occipital pole (**Fig. 4**). However, considering the amount of mRNA in each sample as revealed by actin probing, the ORP-1 transcripts were higher in the cortical areas than in the cerebellum and the spinal cord.

ORP-1 mRNA levels are regulated by sterols in neuroblastoma cells

In order to study whether ORP-1 responds at the transcriptional level to changes in cellular sterol balance, we either treated human neuroblastoma SHEP cells under delipidating conditions or increased their sterol load. These cells were found to express endogenously high levels of ORP-1 as reflected by the short exposure times required to visualize the signal in Northern blot analysis of total RNA (**Fig. 5**). Culturing the cells in lipoprotein-deficient serum for 2 days decreased their total cholesterol concentration by 25% (from 68 to 51 μg cholesterol/mg protein) but did not significantly alter the ORP-1 mRNA levels. However, when the partially delipidated cells were loaded with LDL for 24 h, the ORP-1 transcript was up-regulated by 2-fold (Fig. 5). Simultaneously, the cellular cholesterol level returned back to normal (not shown). Short-term treatment of the sterol-depleted cells with 25-hydroxycholesterol (5 μg/ml of 25-hydroxycholesterol for 4 h), a condition known to stimulate the translocation of OSBP to the Golgi apparatus (10, 13), had a minor (1.5-fold) stimulatory effect on the expression of the ORP-1 mRNA (Fig. 5). Considering the importance of the liver in oxysterol metabolism, we also tested whether the amount of ORP-1

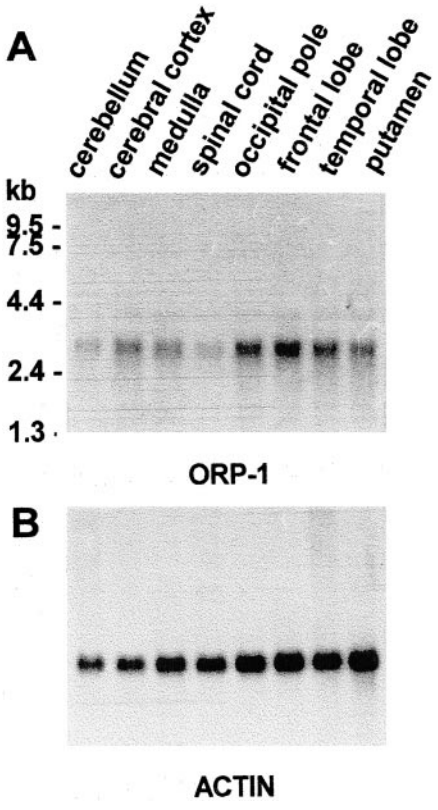

Fig. 4. Expression of ORP-1 mRNA in different parts of the human cerebrum, cerebellum, and spinal cord. The human brain multiple tissue Northern blot filter was hybridized with the ORP-1 cDNA fragment as detailed in Materials and Methods and exposed for 6 h. Each lane contains about 2 μg of poly A^+ RNA. The actin mRNA levels are shown as loading controls.

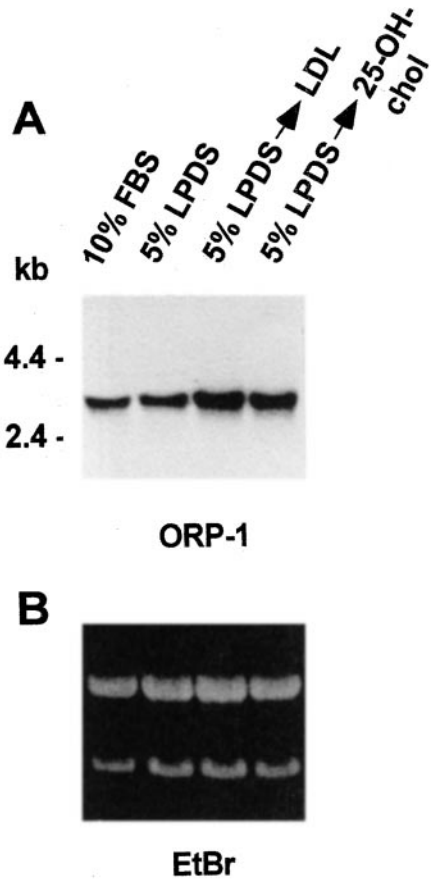

Fig. 5. Expression of ORP-1 mRNA in neuroblastoma cells upon manipulation of cellular sterol balance. SHEP cells were either cultured in 10% FBS (48 h), delipidated using 5% LPDS (48 h), sterol loaded by incubating with 50 μg/ml LDL for 24 h after delipidation of 24 h, or oxysterol treated by incubating with 5 μg/ml of 25-hydroxycholesterol for 4 h after delipidation of 44 h. Total cellular RNA was extracted and 20 μg analyzed by Northern blot hybridization using the ORP-1 cDNA as a probe. The filter was exposed for 16 h. Ethidium bromide staining of the RNA samples (10 μg) visualizing the 18S and 28S ribosomal RNA subunits is shown as a loading control.

mRNA detected in hepatocytes could be up-regulated by sterol loading analogously to neuronal cells. However, similar treatments of human hepatocellular carcinoma HuH7 cells as carried out for SHEP cells did not result in significant alterations of ORP-1 mRNA levels (data not shown). Our results thus show that ORP-1 transcription is sterol-responsive in cells of neuronal origin and further substantiates our hypothesis of the tissue specificity of the ORPs. Moreover, they suggest that human neuroblastoma cells expressing ORP-1 at high levels provide an experimental model to address the role of endogenous ORP-1 in neuronal sterol balance.

DISCUSSION

In the present study, we have used a strategy of computer-based homology probing to delineate a family of human OSBP-related proteins. As the coverage of transcripts in human EST databases rapidly expands, this type of strategy is likely to become increasingly popular in the initial assessment of protein families. Systematic search for homologous ESTs, construction of EST contigs and their probing at the tissue transcript level by hybridization or quantitative PCR-based methods should yield information on the most appropriate tools to work with in a physiologically relevant context.

Our analysis yielded a minimum of six novel human homologues of OSBP, named ORP-1 to -6. Northern blot analysis was used to verify that each of them represents a different gene product and served to establish distinct tissue-specific expression patterns for them. Some of the mRNAs had a relatively ubiquitous expression pattern reminiscent of OSBP (ORP-2, ORP-3, and ORP-5) while others (ORP-1 and ORP-4) showed prominent signals only in a subset of the tissues analyzed. It is noteworthy that the intensities of the signals obtained in Northern blot hybridizations reflected the number of ESTs originally assigned for the ESTs in our search (see Table 2). This suggests that it is unlikely that we would have missed abundantly expressed members of the OSBP family in our search unless they have very restricted expression patterns. Thus, specialized tissues not represented by transcripts in the EST databases could constitute sources for yet novel members of the OSBP family that at present harbors seven members in man.

The mere expression patterns of the ORPs do not allow far-reaching conclusions concerning their cellular functions. However, our findings are in accordance with the working hypothesis of OSBP family members as proteins that relate organellar lipid balance and membrane traffic. First, we found the highest expression levels of several ORPs in tissues that are characterized by active membrane transport processes, such as the brain, kidney, and liver. Second, we were able to show that ORP-1, the most abundant family member in the brain, was subject to transcriptional regulation by sterol loading in a neuronal cell line.

The changes in the ORP-1 mRNA levels remained moderate however, in spite of our attempts to maximize the differences by varying the time scale or concentration range of the sterol depletion and loading conditions (S. Laitinen and E. Ikonen, unpublished results). This is probably due to the problem that the treatments do not accurately mimic the in vivo conditions as LDL-cholesterol is not taken up efficiently by neurons across the blood–brain barrier (24). Another problem may be the choice of oxysterol used in the treatment. Although 25-hydroxycholesterol binds tightly to OSBP and regulates its subcellular localization, it may not be the optimal oxysterol ligand for the other members of the family. In the liver and in nonhepatic tissues, cholesterol is metabolized to 27-hydroxycholesterol which serves important regulatory functions in the formation of bile acids (25, 26). On the other hand, the main oxysterol synthesized in the brain has been reported to be 24(S)-hydroxycholesterol (27). Production of this oxysterol may play an important role in maintaining the cholesterol homeostasis in the CNS (27–29), and it could be envisaged that ORP-1 may have a function in the neuronal turnover of this compound. The widespread distribution of ORP-1 expression in several areas of the brain would fit with its rather general role in neuronal lipid homeostasis.

Considering the tissue distribution of cholesterol, with the largest pool and concentration in the brain, it is not surprising that the brain was found to express several ORPs at high levels. Although most of the cholesterol in the brain seems to originate from local synthesis (30, 31) ORPs are probably not involved in direct control of the mevalonate pathway as an OSBP homologue has also been identified in *Drosophila* which is incapable of synthesizing sterols (32). It may be more likely that ORPs relay information to membrane machineries about alterations in cellular sterol balance. Further support for the in vivo connection between ORPs and the cellular cholesterol status comes from our observation that there are alterations in ORP-1 transcript levels in Niemann-Pick disease, an inherited neurovisceral disorder where excess endocytosed cholesterol accumulates intracellularly (33) (S. Laitinen and E. Ikonen, unpublished results).

In conclusion, we have identified six new human genes showing significant homology to OSBP. Together with OSBP, the gene products constitute a novel human, differentially expressed protein family. Analysis of ORP-1, the family member expressed most abundantly in the central nervous system, demonstrates that the mRNA is up-regulated by sterol loading of neuronal cells, and suggests that all of the novel family members are likely to be implicated in cellular sterol metabolism. The explanation for the multitude of evolutionarily conserved ORPs and their selective tissue distribution awaits characterization of the full-length gene products and their comparative functional analysis.

Noted added in proof: ORP-4 is identical to the recently published *HeLa metastatic* gene, HLM (Fournier, M. V., et al. 1999. *Cancer Res.* **59:** 3748–3753). Furthermore, ORP-2 and ORP-3 are the same as the predicted proteins KIAA0772 (AB018315.1) and KIAA0704 (AB014604.1), respectively, in GenBank. ORP-1 is closely homologous to the mouse mRNA AB017026.

We are grateful to Marc Billaud for supplying the SHEP cell line and Matti Jauhiainen for help in preparing the lipoprotein-deficient serum and LDL. We thank Pirjo Ranta and Ritva Nurmi for skillful technical assistance. This work was supported by the Academy of Finland (E. I. grants 37661 and 43184; V.M.O. grants 36282 and 42163) and the Sigrid Juselius Foundation.

Manuscript received 2 April 1999 and in revised form 11 August 1999.

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