Prosaposin: promoter analysis and central-nervous-system-preferential elements for expression in vivo

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The expression of prosaposin is temporally and spatially regulated at the transcriptional and post-translational levels. In vitro, the mouse prosaposin promoter contains functional RORE [retinoic acid-receptor-related orphan receptor α subunit (RORα)-binding element], Sp1 and U (unknown) sites within 310 bp directly 5’ to the transcription start site and additional elements within 2400 bp 5’ to the transcription start site. To elucidate promoter regions important to tissue-preferential expression in vivo, transgenic mice were created with 5’-flanking deletions of the prosaposin gene fused to a luciferase reporter. Nearly exclusive expression was observed in cerebrum, cerebellum and eyes of adult transgenic mice containing constructs with 234–310 bp of 5’-flanking DNA. This central nervous system (CNS) expression was due to the presence of RORE and overlapping Sp1 sites in this region. Internal deletion of RORE and the Sp1 cluster from the longer constructs with 2400 bp of 5’-flanking DNA significantly diminished expression in the CNS. The appearance of substantial visceral tissue (e.g. liver, spleen, lung, kidney, thymus and heart) expression was obtained with transgenic mice bearing constructs with 742–2400 bp of 5’-flanking DNA. The cellular localization of luciferase reporter-gene expression from these constructs corresponded closely with that for prosaposin. These results define important CNS and visceral regulatory regions in the promoter in vitro and may be sufficient to account for the majority of prosaposin’s tissue-preferential expression.

Key words: luciferase, RORE, Sp1, transcription, transgenic mice.

INTRODUCTION

Prosaposin is a multifunctional protein with essential intra- and extra-cellular functions. Intracellularly, prosaposin (522 amino acids), the precursor, is targeted to lysosomes via the mannose-6-phosphate-receptor-dependent and/or -independent systems where it is proteolytically processed to smaller polypeptides (80 amino acids), termed saposins [1–4]. These four tandemly encoded saposins A, B, C and D interact with glycosphingolipid hydrolases and/or their substrates to enhance lysosomal hydrolytic activities. Their physiological importance is shown by severe phenotypes resulting from inherited mutations at the prosaposin locus that lead to deficiencies of prosaposin or individual saposins, and glycosphingolipid storage [5–10]. Extracellularly, the intact prosaposin precursor functions ex vivo or in vivo as a neurite-outgrowth factor or nerve-regeneration factor respectively [11–14]. The neurite-outgrowth property has been localized to the saposin C region of prosaposin, particularly to a 21-amino acid linear sequence in the N-terminal half of saposin C [11,13,15].

In addition to these multifunctional properties, prosaposin is temporally and spatially regulated at the transcriptional and post-translational levels [3,16]. Concordant high-level expression of its mRNA and protein has been shown in neurons of the central nervous system (CNS), particularly in Purkinje cells of the cerebellum in adult mice, and in various epitheloid and reproductive cell types in adult mice and rats [16–18]. The expression of prosaposin also was induced by p21, a cyclin-dependent kinase inhibitor, and serum starvation during cell-growth arrest [19]. Prosaposin is a major protein produced by Sertoli cells and has been termed sulphated glycoprotein 1 [18]. Post-translationally, the proteolytic processing of prosaposin also has cell-type specificity, particularly in the CNS [3,16]. This variation in the proteolytic processing to mature saposins is cell-differentiation-specific for human and mouse neuronal-like cell lines in culture [3].

To initiate evaluation of the transcriptional control of the prosaposin locus, the genomic structure, particularly of the 5’ end of the gene, was defined [20]. The human and murine genes contain 14 exons and 13 introns [21–23]. An alternatively spliced 9 bp ‘exon’ is present in the saposin B region that appears to have tissue selectivity for splicing [21,24]. Studies in vitro indicate that the mouse prosaposin promoter is TATA-less, with positive and negative regulatory elements in the first 2400 bp 5’ to the transcription start site [20]. An important region within the first 310 bp 5’ to the transcription-initiation site contains functional binding elements for RORE [retinoic acid-receptor-related orphan receptor α subunit (RORα)-binding element], Sp1 and at least one additional unidentified region. These three regions modulate the expression of prosaposin in vitro [25].

To elucidate the in vivo elements responsible for the tissue-specific expression of prosaposin, transgenic animals bearing fragments 5’ to the prosaposin coding sequence were coupled to the luciferase reporter gene. Here, the results of analyses in transgenic mice delineate important prosaposin promoter regions for CNS and/or visceral expression.

MATERIALS AND METHODS

Materials

The following were from commercial sources: GeneClean DNA purification kit (Midwest Scientific Laboratory, Valley Park, MO, U.S.A.); Magna Charge Nylon transfer membrane (Micron Separation, Westbond, MA, U.S.A.); random-primer labelling
prosaposin antiserum was described previously [3]. Rabbit anti-mouse Corporation, Durham, NC, U.S.A.) and anti-luciferase anti-rabbit IgG F(ab)\(^2\) goat antibody (Organon Teknika U.S.A.). Molecular Dynamics ImageQuant software (Molecular Dynamics, Sunnyvale, CA, U.S.A.); Antifade propidium iodide (Amersham Life Science, Arlington Heights, IL, U.S.A.); Bradford reagent system (Bio-Rad, Hercules, CA, U.S.A.); isotope \(\left[{ }^{35}S\right]\)UTP (Amersham Life Science, Arlington Heights, IL, U.S.A.); restriction enzymes (New England Biolabs, Beverly, MA, U.S.A.); luciferase assay system and pGL2B luciferase reporter vectors (Promega, Madison, WI, U.S.A.); Molecular Dynamics ImageQuant software (Molecular Dynamics, Sunnyvale, CA, U.S.A.); Antifade/propidium iodide (Oncor, Gaithersburg, MD, U.S.A.); FITC-conjugated anti-rabbit IgG F(ab')\(^2\), goat antibody (Organon Teknika Corporation, Durham, NC, U.S.A.) and anti-luciferase antibody (Biogenesis, Sandown, NH, U.S.A.). Rabbit anti-mouse prosaposin antisem was described previously [3].

Transgene constructs

The firefly luciferase reporter-gene vector pGL2B was used for transgenic constructs. Based on the 5′-flanking sequence of the mouse prosaposin gene, a series of PCR primers containing restriction-enzyme sites on the 5′ ends were designed for making deletion constructs [20]. Following restriction digestion, the PCR products were cloned into the HindIII and XhoI sites of pGL2B upstream of the luciferase gene. Six deletion fragments of the proximal promoter of the prosaposin gene were used and termed −43 LUC, −234 LUC, −305 LUC, −310 LUC, −741 LUC and −2400 LUC (Figure 1). An internal deletion construct was generated by cloning the PCR fragment containing nucleotides from −2400 to −311 into the 5′ upstream region of −43 LUC. This construct was termed 2400ALUC and did not contain RORE and the Sp1-cluster regions (Figure 1).

Generation of transgenic mouse lines

Each transgene construct was digested with BamHI and SmaI to include the downstream luciferase polyadenylation signal and the upstream prosaposin promoter sequences. The linearized insert was isolated by agarose gel electrophoresis, eluted and further purified twice with the GeneClean DNA purification kit. Following dialysis overnight at 4 °C in 10 mM Tris/HC1 (pH 7.4) containing 0.1 mM EDTA, the DNA (1–5 ng/μl) was injected into the pronuclei of fertilized eggs of B6C3 F\(_1\) mice using standard procedures [26]. Transgenic pups (21 days) were identified by Southern blotting from tail clips and bred with wild-type B6C3 mice to obtain hemizygotes.

Southern-blot analysis

Genomic DNA was isolated [27] and digested overnight with HindIII and PvuII. The fragments were resolved on 0.8 % (w/v) agarose gels and transferred to Nylon membranes (Magna Charge Nylon transfer membrane). The \(\left[{ }^{32}P\right]dCTP\) randomly labelled 310 bp fragment immediately 5′ to the transcription-initiation site was used as the probe. Hybridizations were overnight in 50 % (v/v) formamide, 6 × SSC (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate), 5 × Denhardt’s (0.02 % Ficoll 400/0.02 % polyvinylpyrrolidone/0.002 % BSA) and 0.3 mg/ml herring sperm DNA. The filters were washed in 0.2 × SSC/0.1 % (w/v) SDS, and exposed to X-ray film. The copy number of each transgenic line was determined by comparison of blot hybridization intensities of the digested transgene fragment with those obtained with the endogenous prosaposin genomic fragment. The autoradiograms were quantified by densitometric scanning using Molecular Dynamics ImageQuant software.

Luciferase activity and protein concentration

Tissues were harvested from non-transgenic and transgenic mice between 6 and 10 weeks of post-natal age and included cerebrum, cerebellum, kidney, liver, lung, spleen, eye, heart, thymus, testes and uterus/ovary. Organs were pulverized under liquid N\(_2\) and homogenized in reporter lysis buffer (Promega). The supernatants from tissue homogenates were assayed for luciferase activity using the Promega assay system and a Monolight 2010 luminometer (Analytical Luminometer Laboratory, San Diego, CA, U.S.A.). The linearity of the luciferase assay was verified for all samples assayed under the conditions used in these experiments. The protein concentration of each supernatant was determined using the Bradford reagent system.

In situ hybridization

A 0.75 kb luciferase cDNA fragment, released by EcoRI and EcoRV from pGL2B, was cloned into pBluescript vector and used to generate luciferase riboprobes. Radiolabelling of luciferase and prosaposin riboprobes was as described in [16]. The frozen sections (7 μm) of transgenic mice with the −310 LUC or −2400 LUC constructs were postfixed in 4 % (w/v) paraformaldehyde, treated with proteinase K, and then incubated in Tris/glycine and acetylated. Prehybridizations were at 42 °C for 15 min. The sections were hybridized (42 °C overnight) with 4 × 10\(^5\) c.p.m./ml of the complementary antisense or sense probes. After hybridization, the slides were washed with SSC/dithiothreitol and then incubated in RNase A and RNase T1. A series of high-stringency washes was employed as described in [16]. After dehydration with graded ethanol washes, the slides were dipped in Kodak D19 developer and fixer. All slides were counterstained with haematoxylin and eosin. The sections with sense probes were the negative controls.

Immunofluorescence

The embedded tissue sections were incubated in 0.2 % (v/v) Tween 20 in PBS at room temperature (10 min) and then covered (30 min) with blocking buffer [3 % (w/v) gelatin in PBS for anti-prosaposin antibody or 10 % (v/v) goat serum for anti-luciferase
antibody]. This was followed by incubation (1 h, 37 °C) with 1/100 diluted primary antiserum in 0.05% (v/v) Tween 20 in PBS (TPBS) for anti-mouse prosaposin or 3% (w/v) BSA/3% (v/v) goat serum for anti-luciferase. The tissue sections were washed three times (15 min) in TPBS at room temperature. Secondary FITC antibody, diluted 1/20 in TPBS, was applied and incubated (1 h, 37 °C). The sections were washed as above. Antifade/propidium iodide was added and the signals detected by fluorescence microscopy.

RESULTS

Establishment of transgenic mouse lines and analysis of transgene expression

The role of tissue-preferential regulatory elements within 2400 bp of the 5'-flanking region of the mouse prosaposin promoter was investigated in vivo using seven deletion constructs fused to the luciferase reporter gene. Each transgene construct contained 5'-flanking sequence with −43 to −2400 bp 5' to the transcription-initiation site and 98 bp of the 5' untranslated region within the first exon (Figure 1). The −43 LUC construct contained the first 3 Sp1-binding site whereas the −234 LUC construct also had a RORE site. The −310 LUC construct included a Sp1 cluster of three overlapping Sp1 half sites and an unknown (U) transcription-factor-binding site. The −310 LUC and −305 LUC were identical except that −305 LUC had a 5 bp deletion within the Sp1 cluster that eliminated the most 5' Sp1 half site. The −742 LUC and −2400 LUC constructs had increasing 5' lengths of promoter region up to 2400 bp from the transcription start site. To evaluate the function of RORE and the Sp1 cluster in vivo, the internal deletion construct −2400ΔLUC was designed to eliminate RORE and the Sp1-cluster regions. Transgenic founders were made from each of the seven constructs (Figure 1). From each founder, the yield of mouse genotypes conformed to Mendelian expectations, indicating no significant embryonic lethality. The transmission of transgene to germ line occurred in 68% of founders. For all the constructs, excluding −43 LUC, luciferase expression was detected in about 45% of the lines containing transgenes. The absence of transgene expression in other lines could have been due to positional effect [28].

The transgene copy numbers for each construct are listed in Table 1. HindIII/PstIII digests of tail DNA were used to quantify the copy number of integrated constructs. From the endogenous promoter, a 1.1 kb fragment was obtained and variously sized fragments are obtained from the different transgenes (Figure 2). On the Southern blot the 310 bp probe immediately 5' to the prosaposin transcription-initiation site was used to standardize integrands since this fragment hybridizes to all constructs and the endogenous promoter fragment. The denstometric scans from two or three mice were averaged for each transgenic line. The intensities of equal molar amounts of each transgene and the endogenous promoter would be the same since the probes and recognition sequences are identical for all. The copy numbers were calculated as the ratio of the intensity of the transgene relative to that of the endogenous 1.1 kb fragment and presented as the number of transgenes per mouse genome.

To determine the effect on the tissue expression patterns of various 5' regions of the prosaposin gene, the luciferase activities in variety tissues from transgenic mice were evaluated (Table 1). The tissue-selective expression patterns for each construct were consistent, but the expression level varied widely between founder lines. In comparison for each transgenic founder line, the level of expression in and among various tissues was consistent, i.e. either low, intermediate or high levels of luciferase activity expression were maintained proportionately in the various tissues. The results are the means from several animals in each line and for each tissue, and are normalized for gene copy number. The non-transgenic mouse controls had no luciferase activity.

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CNS-preferential expression directed by the $-43$ to $-310$ bp region

From the results in Table 1, the CNS-preferential expression for the constructs $-234$, $-305/-310$, $-742$ and $-2400$ LUC was evident, even with the substantial variation in the levels of promoter activity that were observed between the different transgenic founder lines for each construct. The 234 bp 5'-flanking sequence is sufficient to direct CNS (cerebrum, cerebellum and eye)-preferential expression. With progressive sequence extension to 2400 bp 5' of prosaposin promoter, the luciferase activities were greatly increased. Figure 3 shows the comparison of luciferase expression in cerebrum and cerebellum in one of the transgenic lines from each construct. With the $-305$ LUC construct, the luciferase level was increased over 8-fold by inclusion of the Sp1 sites 5' of RORE. The substantially enhanced activities also were seen in the constructs $-310$ LUC, $-742$ LUC and $-2400$ LUC; the expression levels in cerebrum or cerebellum were increased by over 35- to 100-fold relative to those for the $-234$ LUC construct. The results suggested that the region from $-234$ to $-2400$ bp appears to contain enhancer activities that augment the level of expression in cerebrum and cerebellum. Expression levels for $-310$ LUC were about 4-fold higher in cerebrum and 6-fold higher in cerebellum than those for $-305$ LUC (Table 1 and Figure 3). This may indicate that the enhancer activity in CNS requires all three of the Sp1 half sites in the cluster. However, this could not be proved due to only a single transgenic line with the $-310$ LUC construct being obtained.

RORE and the Sp1 cluster are located within the region $-43$ to $-310$ bp of the promoter and their roles were defined ex vivo by site-directed mutagenesis [25]. The function of this region was analysed in vivo by comparing the results with the $-2400$ LUC and $-2400\Delta$LUC constructs. As shown in Figures 3 and 4, the expression levels in CNS tissues, including cerebrum, cerebellum and eye, were lower by 67–83% with the deletion construct $-2400\Delta$LUC than with $-2400$ LUC. The luciferase expression in spinal cord was also analysed from most transgenic lines for these two constructs. Unpaired Student’s t tests of five or four transgenic lines, respectively, for constructs $-2400$ LUC or $-2400\Delta$LUC ($P < 0.05$) indicated significantly reduced expression levels due to the deletion of the $-43$ to $-310$ bp region (Table 2). This result indicates that RORE and the Sp1 cluster are necessary for maximal prosaposin expression in CNS. This deletion did not abolish the expression in CNS tissues, suggesting that other elements in the $-310$ to $-2400$ bp region are involved in modulating the basal prosaposin gene expression in the CNS.

Taken together, these results demonstrate the importance of the $-43$ to $-310$ bp region for preferential CNS expression. Both RORE and the Sp1 cluster affect prosaposin expression in the cerebrum, cerebellum, eye and spinal cord. RORE is involved in directing CNS-preferential expression and the Sp1 cluster possibly plays a role in enhancing the expression level. Additional CNS modulator activities reside in the sequences from $-310$ to $-2400$ bp of the 5' region.

Visceral tissue-selective expression on promoter construct

To define prosaposin promoter activity in visceral tissues, luciferase levels were analysed in the transgenic mice. The $-43$ LUC transgenic animals had extremely low to no expression of luciferase in any tissue of any of the transgenic founders, indicating...
Mouse prosaposin promoter analysis

Figure 4 Deletion of RORE and the Sp1 cluster region reduced the luciferase expression in CNS tissues

Five or four transgenic lines for constructs −2400 LUC (black bars) or −2400ΔLUC (hatched bars) were analysed for luciferase activities in cerebrum (A), cerebellum (B), spinal cord (C) and eye (D). Results are means ± S.E.M. from three to seven mice in each transgenic line. The unpaired Student’s t test was used to analyse the significance of luciferase-level differences between constructs −2400 LUC and −2400ΔLUC, each with multiple transgenic lines. P values for t test analysis in each tissue are shown in Table 2. RLU, relative light units.

Table 2 Analysis of luciferase levels in multiple transgenic lines with constructs −2400 LUC and −2400ΔLUC by unpaired Student’s t test

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<th>2400ΔLUC</th>
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<td>Thymus</td>
<td>0.096</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>0.226</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0.502</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>0.043*</td>
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</tbody>
</table>

that the first Sp1-binding site alone is not sufficient to drive prosaposin expression in vivo. Beginning with −234 LUC and increasing the length of the 5′-flanking region up to 2400 bp, an increasing gradient of expression was observed, first in the CNS regions and then, with increasing promoter length, in visceral tissues (Table 1). The visceral organs from the mice with the −234 LUC construct had no or negligible luciferase activity, except for a low level of expression in ovaries and uteri. The −310 LUC/−305 LUC constructs gave minimal expression in visceral organs in two out of five lines, except for in the heart (i.e. lines 1.1 and 2.1). With the −742 LUC and −2400 LUC constructs, consistent levels of expression were noted in visceral organs in all founder lines. In these visceral organs, the largest effects of the 742 and 2400 bp fragments were also in the heart. Substantially lower activities were found consistently in thymus, lung, kidney, liver and spleen. In particular, the liver had the lowest levels of expression of visceral organs on a consistent basis. The deletion construct −2400ΔLUC gave substantially lower expression levels in the CNS, including the cerebellum, cerebrum, eye and spinal cord (Figure 4). Compared with construct −2400 LUC, this internal deletion had no significant effect (P > 0.05) on luciferase expression in visceral organs or reproductive organs (results not shown), except in spleen (Table 2). These findings suggest that the 5′-flanking sequence between −310 to −2400 bp may have modulators or the control of visceral tissue expression.

Tissue-specific localization of luciferase and prosaposin expression

Since the transgene −310 LUC (line 1.1) showed essentially only CNS expression and −2400 LUC (line 4.2-1) had CNS and visceral expression, the tissues from mice containing these transgenes were used to localize luciferase RNA or protein expression. In similar sections from the same mice, the antisense mouse prosaposin RNA was used for co-localization. In the −310 LUC mice, neurons of the cerebral cortex and spinal cord showed concordant luciferase (Figures 5A and 5B) and prosaposin RNA (results not shown) expression. The latter corresponded to the patterns reported for prosaposin [16]. In the eye
Figure 5  Cellular localization of luciferase expression in transgenic mice

Tissue sections from $-310$ LUC (line 1.1) transgenic mice were used in (A–D), (G) and (H). The heart sections (E and F) were from $-2400$ LUC (line 4.2-1) transgenic mice. The tissues in (A–F) were probed with $^{35}$S-labelled luciferase or prosaposin RNA by in situ hybridization. Luciferase expression in (A) cerebrum and (B) spinal cord: the larger white condensed grains correspond to neurons in the cerebral hemisphere and anterior horns of the spinal cord. The general “white” blush in the outer regions of the spinal cord (B) correspond to non-specific granular probe deposits that are not associated with cell bodies. (C) Differential prosaposin RNA expression in the ganglion cell layer (g) and inner nuclear layer (i) of the eye. (D) Retina of the eye probed with antisense luciferase RNA. The labels are as in (C) and the distribution of prosaposin (C) and luciferase (D) RNA is similar within the same cell types. In (E) and (F) heart sections from $-2400$ LUC transgenic mouse were probed with antisense (E) or sense (F) luciferase RNA. A general pattern of luciferase RNA expression (E) above background (F) was detected. In (G) and (H), immunofluorescence was detected in Purkinje cells (p) of cerebellum from $-310$ LUC transgenic mice using anti-luciferase (H) or anti-mouse prosaposin (G) antibodies.

(Figure 5D), the luciferase mRNA expression was detected in the cells of the ganglion layer (g) and was nearly absent in the inner nuclear layer (i) of retina. These patterns were comparable in distribution with those for prosaposin (Figure 5C). Using anti-luciferase antibody, positive immunofluorescence was detected in the Purkinje cell layer of the cerebellum (Figure 5H). This co-localized to the signal obtained with anti-mouse prosaposin antibody (Figure 5G). An essentially identical pattern of luciferase expression was observed in CNS tissues from mice bearing the $-2400$ LUC transgene.
The visceral tissues from mice carrying the −2400 bp construct (line 4.2) were examined for luciferase expression patterns. The cardiac myocytes showed low-level, diffuse luciferase RNA signals (Figure 5E) compared with the sense control (Figure 5F). This was not observed for prosaposin [16]. Using anti-luciferase antibody, low-level immunofluorescence signals were detectable in macrophages of thymus, spleen and lung (results not shown). Cell-specific luciferase expression in other tissues could not be evaluated since signals were not detected with antisense RNA or antibody to luciferase even though substantial luciferase activity was present consistently.

**DISCUSSION**

As with many lysosomal proteins, prosaposin has been thought to be a housekeeping gene with generalized expression. Previously, we showed great variation in prosaposin mRNA levels among various mouse tissues during development and in adulthood [16]. In particular, expression was high in CNS neurons [16,29], where the greatest levels of expression were found in cerebral neurons and cerebellar Purkinje cells. Sertoli cells of the testes also showed high-level expression. Transfection of deletion/reporter-gene constructs into NS20Y, NIH 3T3 and SF-7 cell cultures showed positive regulation, but with cooperative interacting elements within the first 310 bp 5' to the transcription-initiation site [20]. In addition, DNase I footprinting and site-directed mutagenesis analyses revealed several functional transcription-factor-binding elements within this 310 bp fragment, including Sp1, a Sp1 cluster, RORE and an unknown (U) site [22]. The repertoire of these transcription-factor-interactions was postulated to modulate gene expression [25]. However, the *ex vivo* data did not account for the tissue-preferential expression, since similar patterns of reporter gene expression were seen in a variety of cell cultures for many elements contained in the 2400 bp 5' to the major transcription start site [20]. The present transgenic mouse analyses show that the preferential CNS or visceral tissue promoter elements were localized to the proximal 310 bp and more distal 5'-flanking regions respectively. Cellular localization of the transgenes with the luciferase mRNA also provides strong evidence for the presence of selective CNS promoter regions within the 310 bp region immediately 5' to the major transcription start site.

Increasing the length of the promoter region from −310 to −2400 bp was accompanied by the appearance of broad-based expression throughout the viscera. There was substantial variation in the levels of luciferase activity and RNA detected across different transgenic mouse lines. The positional effects of transgenes randomly integrated into the genome might account for such variation [28]. However, the patterns of activity expression within the various tissues were consistent. Computer searches across this region did not identify any major transcription-factor-binding sites that are known to be tissue-specific or to promote particular patterns of expression. The majority of predicted sites have activator protein 1 (AP-1), chicken homeobox gene of the caudal type (CdxA) and Oct-1 consensus sequences. These are general transcription factors that are not specific to the CNS or viscera. DNA footprinting in vitro did not reveal DNase I protection of these sites within the 310 bp segment with several mouse cell types from visceral sources, nor with NS20Y neuroblastoma cells [25]. The corresponding region from the human promoter contained AP-1 and Oct-1 sites that were protected by nuclear extracts from H441 cells, a human lung papillary adenocarcinoma cell line [30].

The deletion of RORE and the Sp1 cluster decreased the expression level of luciferase activity in CNS tissues. However, there remained a general lower level of expression that supports the existence of additional modulator sequences for CNS expression in this extended region (−310 to −2400 bp). Computer searches across the region from −742 to −2400 bp revealed the presence of several transcription-factor-binding sites, including Brn-2 and Oct-6 (Tst-10), which function as facilitators in the CNS (Figure 1). Oct-6 is involved in peripheral nervous system and CNS myelination [31]. Brn-2 is important to the development of the hypothalamus/posterior pituitary axis [32]. Another explanation for the remaining luciferase activity in CNS with the deletion construct −2400 bp is the possibility of RORE and/or the Sp1 cluster controlling expression in specific cell types in the CNS that would not be obvious from tissue homogenates.

These results implicate the presence of a tissue-preferential modulator(s) sequence in the region from −43 to −2400 bp. A schematic representation (Figure 1) is provided for the promoter of the mouse prosaposin gene. The data from transgenic mice are consistent with a necessary region of 310 bp of the immediate 5'-flanking sequence for preferential expression within the CNS. Extending more 5' to this CNS region, additional CNS regulatory elements are present and visceral activity is promoted. Finally, a general facilitator element is present within the 1700 bp 5' to the −742 LUC construct. This region is populated with a variety of sequences, none of which are known to have specific regulatory effects on lysosomal enzymes or prosaposin. Continuing studies of this promoter region should facilitate the delineation of prosaposin expression and function by preferential rescue approaches.

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**REFERENCES**


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