American Journal of Pathology, Vol. 145, No. 6, December 1994

Developmental and Tissue-Specific Expression of Prosaposin mRNA in Murine Tissues

Ying Sun,* David P. Witte,† and Gregory A. Grabowski*

From the Divisions of Human Genetics and Pathology,† Department of Pediatrics, Children's Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, Ohio*

Prosaposin is a multifunctional locus in humans and mice that encodes in tandem and in the same reading frame four glycoprotein activators, or saposins, of lysosomal hydrolases. These ubiquitously expressed glycoproteins and the precursor, prosaposin, have been proposed to function in glycosphingolipid catabolic pathways and glycolipid transport. To characterize the temporal and spatial expression of the prosaposin locus, prenatal and postnatal mouse tissues were screened by in situ hybridization with a mouse antisense riboprobe for prosaposin. Prenatally, prosaposin mRNA was expressed differentially in the placenta and prominently in the decidua basalis and capsularis where expression was gestational age dependent. No other region of high-level expression was detectable in the prenatal mouse. In comparison, high-level differential expression of prosaposin was clearly evident postnatally in a variety of organs, including secretory epithelial cells of the choroid plexus, ependymal lining, upper trachea, esophagus, cortical tubules of the kidney, sertoli cells of the testes and epididymis. Discrete localization of prosaposin mRNA expression was also found in neurons of the cerebral cortex, cerebellar cortex, and lateral columns of the spinal cord as well as in hepatocytes of the mature liver. Very high levels of expression were found in specialized tissues including the Harderian glands and macrophages of lymph nodes, lungs, splenic tissue, and thymus. These studies indicate that the expression of the prosaposin locus, a presumed "housekeeping" gene, is under tissue- and cell-specific differential control. The spatial organization of expression suggests a role for this locus in the expression of glycosphingolipid-storage diseases. (Am J Pathol 1994, 145:1390–1398)

Prosaposin, an intriguing multifunctional protein, is the precursor of four different glycoprotein activators of lysosomal hydrolases.[1–3] In the rat, prosaposin has been termed SGP-1, sulfated glycoprotein 1, since it is a major sulfated glycoprotein of sertoli cells.[4] The human prosaposin gene also produces an mRNA (~3 kb) that encodes in-frame four highly homologous saposins that are proteolytically released from the precursor (Figure 1).[2,3] In humans, rats, and mice the precursor protein, prosaposin, encodes in tandem the highly homologous saposins, A, B, C, and D, that participate in the sequential degradation of glycosphingolipids to sphingosine and fatty acids.[2–5] Although the exact sequence of proteolytic steps is not known, final processing occurs in the lysosome after being targeted via the mannose-6-phosphate receptor system.[6,7] Specific deficiencies of saposin B lead to clinical syndromes that are indistinguishable from the deficiency of the cognate lysosomal hydrolase, arylsulphatase A.[8–10] By comparison, saposin C deficiency leads to a Gaucher-like disease with accumulation of glucosylceramide due to the inability of the normal acid β-glucosidase in achieving maximal hydrolytic activity.[11–13] Although specific deficiencies of saposin A and saposin D have not been described, it is anticipated that deficiencies of these saposins will help clarify their physiological roles. A complete deficiency of prosaposin due to an initiator codon mutation results in the storage of multiple glycosphin-

Supported by grants from the National Institutes of Health (GAG; DK 36729) and the National Gaucher Foundation (NGF grant 19). Additional support was from the Children's Hospital Research Foundation and the Markey Center for Pediatric Molecular Genetics at the Children's Hospital Medical Center, Cincinnati, OH.

Accepted for publication August 25, 1994.

Address reprint requests to Gregory A. Grabowski, M.D., Director, Division of Human Genetics, Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229–3039.

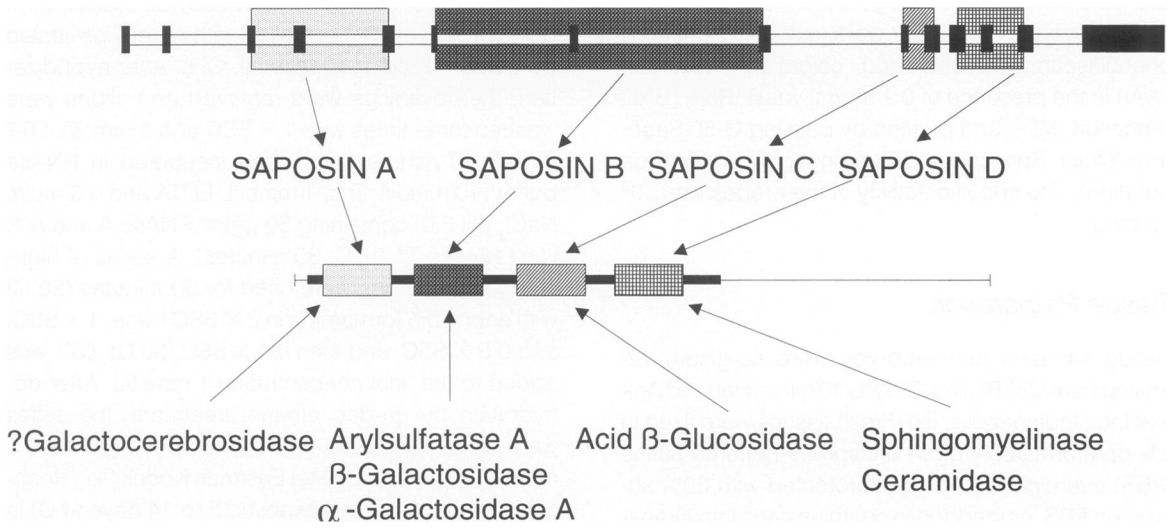


Figure 1. *Schematic of the prosaposin cDNA. The organization represents a composite of that from the human, rat, and mouse. The major difference between human and murine is the presence of a 90-bp insertion between the saposin C and D regions of the precursor. Below the cDNA are indicated the lysosomal hydrolases that are known to be activated by each saposin in vitro.*

golipid substrates resembling a multilysosomal hydrolase deficiency.[14] To date, none of these selective or general deficiencies of saposins is compatible with longterm survival.

Because lysosomes exist in all tissues, the genes for proteins of this organelle have been thought of as "housekeeping" without a high degree of regulation. Reiner et al[15,16] suggested that acid β-glucosidase may be expressed at different levels in different tissues and could be up-regulated in Gaucher disease. In addition, several lysosomal enzymes are known to vary in their specific activities in given tissues, but none of these have been studied systematically at the mRNA level. Prosaposin provides an intriguing locus to evaluate for temporal and spatial expression, since it occupies a central role in the regulation of multiple enzymes in the lysosomal catabolic cascade.[17] Collard et al[4] suggested small variations in total mRNA by Northern analysis in rat tissues during development. However, this variation was quite small, and differential expression in various cell types was not explored. Similarly, Sprecher-Levy et al[18] detected hindbrain and corpus luteum expression of prosaposin in whole-embryo sections.

In this communication, we report the cloning and characterization of a full-length clone encoding murine prosaposin and its tissue localization by *in situ* hybridization. These studies indicate that mRNA levels are both spatially and temporally expressed at very different levels in specific cell types of various organs throughout the body. Our findings suggest an important role in the regulation of the prosaposin locus for the expression of various lysosomal storage diseases at critical times in development.

Materials and Methods

Riboprobe Preparation

Using the human cDNA, a full-length mouse prosaposin cDNA (2.5 kb) was cloned from a λZap mouse liver library and completely sequenced.[19] This clone was substantially similar to that previously reported[18] and has the organization shown in Figure 1. An 885-bp fragment (60% of the coding region (base pairs 162–1047)) was subcloned into Bluescript vector at the *Eco*RI and *Sst*I sites. For prosaposin riboprobes, the subclone was linearized with either *Sty*II or *Eco*RI. The linearized templates were treated with proteinase K (0.1 mg/ml) and sodium dodecyl sulfate (0.1%) at 37 C for 30 minutes and then purified by phenol and chloroform extraction. The radiolabeled sense (*Sty*II) and antisense (*Eco*RI) single-stranded RNA probes were synthesized using 1 μg of linearized template and 50 units T7 or T3 polymerase (Stratagene transcriptional kit; La Jolla, CA), respectively, in the presence of 1× reaction buffer (0.4 mmol/L ATP, GTP, and TTP; 0.01 mmol/L uridine triphosphate (UTP) and 30 mmol/L dithiothreitol (DTT)) and 40 units RNAsin (Promega Biotec, Madison, WI), and 200 μCi ^{35}S-UTP (800 Ci/nmol; Amersham Corp., Arlington Heights, IL) were included in each reaction. The *in vitro* transcription mix was allowed to react at 37 C for 3 hours. Then, the mixture was treated with 1 unit

DNAse (RNAse-free, Stratagene) for 10 minutes at 37 C. The reaction products were extracted once by phenol/isoamyl alcohol and chloroform (25:1:24, v/v/v) in the presence of 0.1 mg/ml yeast tRNA (BRL, Bethesda, MD), and purified by passing G-50 Sephadex Quick-Spin column (Boehringer Mannheim Corporation). The specific activity of the probes was 10^8 cpm/μg.

Tissue Preparation

Tissue samples and embryos were surgically removed from C57BL/6 or C57BL/10 mice (Harland Animal Inc., Indianapolis, IN). Fresh tissues were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS) overnight at 4 C, cryoprotected with 30% sucrose in PBS, embedded in ornithine carbamoyltransferase compound (Miles, Inc., Charlotte, NC), and snap-frozen in liquid nitrogen. Embedded tissue blocks were stored at −80 C until use.

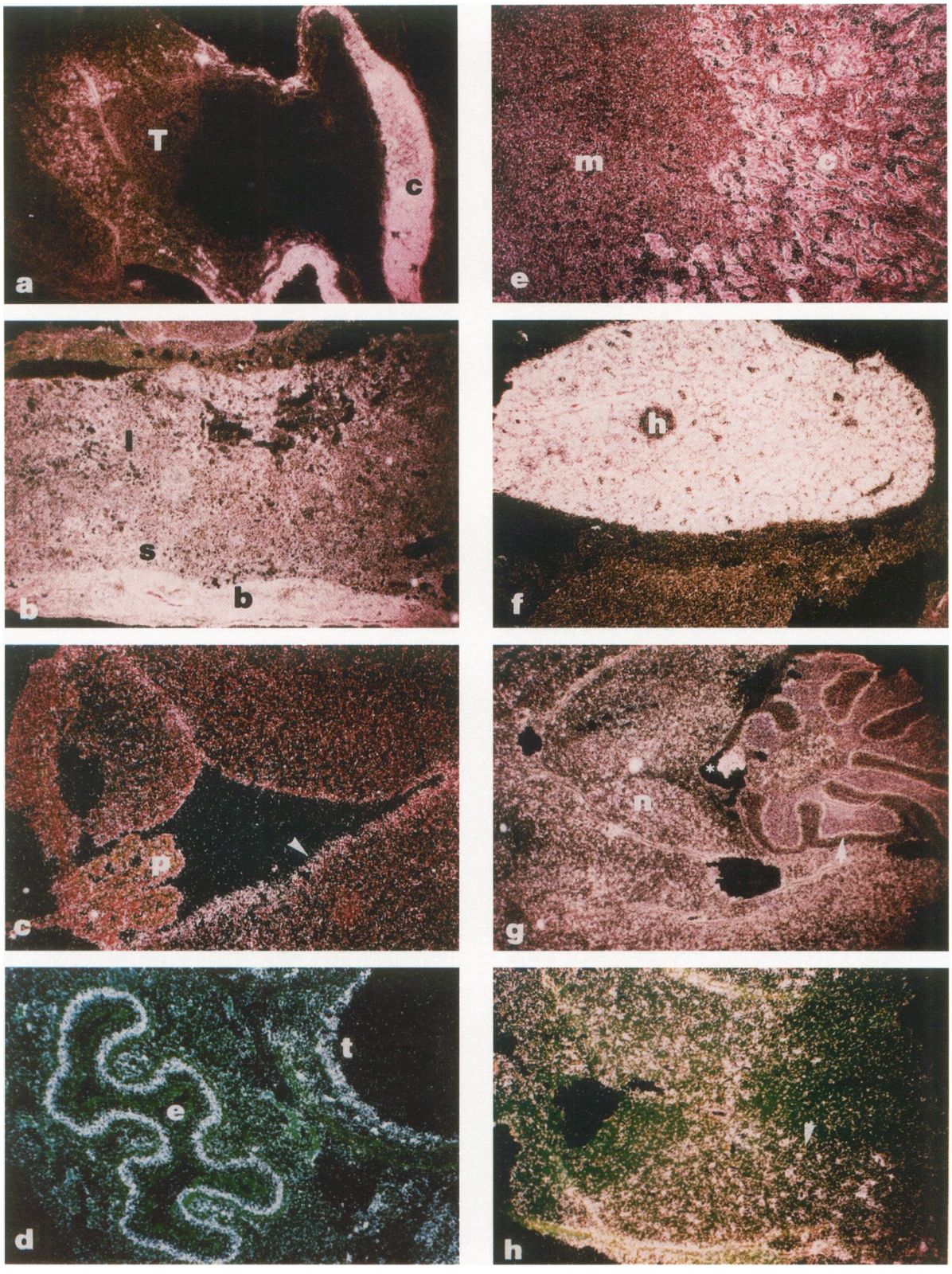
In-Situ Hybridization

The frozen sections (7 μ) on silane-coated slides were air dried and postfixed in 4% paraformaldehyde for 1 hour.[20] The tissue sections were treated sequentially with 70% ethanol (10 minutes) and 2 × SSC (10 minutes). The pretreatment of tissues was at room temperature. The sections then were treated with 20 μg/ml proteinase K (5 minutes) and incubated in 0.13 mol/L Tris and 0.1 mol/L glycine, pH 7.0, to inhibit the enzyme. The slides were rinsed in 2 × SSC for 10 minutes. To reduce nonspecific probe binding, sections were treated with 0.25% acetic anhydride in 0.1 mol/L triethanolamine, pH 8.0, (10 minutes), washed in 2 × SSC (10 minutes), and dehydrated with graded ethanol washes (70, 90, and 100%). Prehybridization was carried out at 42 C for 15 minutes in a hybridization cocktail (2 × SSC, 50% formamide, 10% dextran sulfate, 0.75 mg/ml yeast tRNA, 0.75 mg/ml herring sperm DNA, 5 mmol/L DTT, 0.1 mg/ml bovine serum albumin, 1 × Denhardt's solution and 0.1 mmol/L thio-uridine monophosphate (thio-UMP)). The sections were hybridized with 4 × 10^7 cpm/ml of either the antisense or sense probes in the cocktail without thio-UMP at 42 C overnight with coverslips sealed by Cemen (Kindler, Cincinnati, OH). After hybridization, the coverslips were removed and slides were washed three times with 1 × SSC and 1 mmol/L DTT (50 C, 10 minutes) and then incubated in RNAse buffer (10 mmol/L Tris, 1 mmol/L EDTA and 0.5 mol/L NaCl, pH 8.0) containing 50 μg/ml RNAse A and 500 U/ml RNAse T1 37 C, 30 minutes). A series of high-stringency washes were used for 30 minutes (50 C) with each 50% formamide in 2 × SSC twice, 1 × SSC, and 0.5 × SSC, and then 0.1 × SSC (55 C). DTT was added to the final concentration 1 mmol/L. After dehydration by graded ethanol treatment, the slides were dipped in Kodak NTB2 emulsion (1 g/ml in 0.5 mol/L ammonium acetate; Eastman Kodak Co., Rochester, NY) at 42 C and exposed 5 to 14 days (4 C) in the light-tight box. The slides were developed in Kodak D19 developer for 5 minutes, rinsed in water 1 minute, immersed 5 minutes in Kodak fixer, and rinsed again in water. All slides were counterstained with hematoxylin and eosin. The section labeled with sense probe served as negative control, while the antisense probe detected the positive signals in the samples. The silver grains were visualized under dark-field microscopy and appear as white in the photographs.[20,21] The low level of expression observed in all tissues is referred to as "basal."

Results

In the placenta the expression pattern of prosaposin was gestational-age dependent. In the day 8 embryo, intense signal in a radial distribution was present in the inner-zone decidual cells that surrounded the recently implanted embryo. Outside of this zone the decidual cells showed little signal. The developing embryo and early invasive trophectodermal tissue had no signal. By day 10, the most intense signal was restricted to the decidua capsularis. As the trophectodermal tissue, which was still negative, invaded deeper into the uterine lining, only the decidual cells at this interface showed strong expression (Figure 2a). By day 15 the decidua basalis showed the most

Figure 2. *Localization of mouse prosaposin mRNA expression by in situ* hybridization using a mouse antisense riboprobe. (**a**) *In the day 10 placenta, prosaposin mRNA signal localized to the decidua capsularis* (*c*). *Basal signal was present in the invading trophoblastic* (*T*) *tissue.* (**b**) *In the day 15 placenta intense signals were present in the decidua basalis* (*b*), *and weaker signals were in the spongiotrophoblastic layer* (*s*) *and labyrinthine placenta* (*l*). *Basal signal was detected in the overlying yolk sac membrane.* (**c**) *In the day 14 embryo, prosaposin mRNA was detected in the primitive ependymal layer* (*arrowhead*) *and at lower levels in the choroid plexus epithelium* (*p*). *The remaining CNS tissues showed basal expression levels similar to those in many other tissues.* (**d**) *Strong, but highly restricted, signals for prosaposin mRNA expression were present in the esophagus* (*e*). *This signal was limited to the squamous mucosa. Equally strong signals were present in the mucosal epithelial cells* (*t*) *of the trachea.* (**e**) *In the kidney, the signal was localized to the cortical tubular epithelial cells* (*c*). *Only basal signals were present in the cells of the medulla* (*m*). (**f**) *High levels of prosaposin mRNA were expressed in the Harderian gland* (*h*) *cuboidal cells.* (**g**) *In the day 9 postnatal brain, the prosaposin mRNA signal was intense in the Purkinje cell layer* (*arrowhead*) *of cerebellum, neurons of the cerebellar stalk and the cerebrum* (*n*), *and epithelial cells of the choroid plexus* (*). (**h**) *In the postnatal spinal cord, neurons of the lateral horns were positive* (*arrowhead*) *as were ependymal cells of the central canal*


T
c
a
m
e
l
s
b
b
h
f
p
c
n
g
e
t
d
h

intense signal and the labyrinthine placenta also now was positive but not as intense as the decidual cells. The spongiotrophoblastic layer at the interface between the labyrinthine placenta and the decidua basalis also was strongly positive (Figure 2b).

There was no signal in the day 10 embryo. By day 14 most of the embryonic tissue showed diffuse low-level expression that was just slightly above the basal level. At this time the brain showed areas of expression above the general background level of intensity. This signal was mostly restricted to the choroid plexus, ependymal lining, and immediate subependymal layer (Figure 2c). The deeper layers showed low-level expression. The spinal cord also showed low-level expression, but there was increased expression in the spinal ganglia. Although the expression in these components of the central nervous system (CNS) was increased above most embryonic tissue, it was not of the high intensity present in the placental tissues. These results are summarized in Table 1.

Analyses of postnatal tissues showed differential expression of prosaposin mRNA in various cell types within tissues of several organs (Table 2). Although low-level expression was present in many postnatal tissues, high levels of prosaposin mRNA expression were observed in epithelial cells of the esophagus and upper trachea (Figure 2d). The mucosal epithelial cells of the stomach, duodenum, jejunum, ilium, and colon had only background levels. The lamina propria of villi of the jejunal mucosa showed moderate intensity of staining for prosaposin mRNA (Table 2). Other organs of the gastrointestinal system showed variable levels of prosaposin expression. In the liver the signal distribution was diffuse and localized to hepatocytes and bile duct epithelial cells. The level of expression in hepatic stromal cells and Kupffer cells was similar to basal levels in many other tissues and provided a highlighting of the background for the high levels of expression in hepatocytes. The pancreatic acinar and the islet cells showed basal levels of expression, and moderate expression was noted in the pancreatic ductal epithelial cells. Salivary glands including the submaxillary glands had only basal expression.

In the kidney prosaposin mRNA expression was confined to the tubular epithelial cells in the cortex (Figure 2e). The high levels of expression in these epithelial cells contrasted with the low-level expression in the tubular epithelial cells in the medulla or glomerular cells. The epithelial cells of the calyces, pelvis, ureter, and bladder showed low levels of expression. The developing fetal kidney showed low levels of expression in all cells, although the 18-day embryo had slightly increased levels in the epithelial cells of the maturing cortical tubules. The outermost nephrogenic zone of the cortex did have minimal expression in the primitive developing tubules. Two other secretory organs had high levels of prosaposin mRNA expression. The Harderian gland (Figure 2f), a secretory gland in the posterior aspect of the orbit, demonstrated intense uniform expression in cuboidal cells lining the glands. These cells were starkly outlined by their positive staining that contrasted with the background levels in connective tissue cells. By comparison, the lactating mammary gland had different levels of expression that was dependent on the lactation activity. Nonlactating glands showed only low levels of expression in contrast to actively lactating glands that showed high expression levels (Table 2).

In lymphoid and hematopoietic tissue, differential expression was noted in various cell types. In lymph nodes, the central medullary areas and the sinusoids had intense positivity localized to macrophages. The follicles and intrafollicular tissue in lymph nodes and spleen only contained occasional positive cells that likely represent macrophages. In lung, high levels of expression were noted in the alveolar macrophages. In the thymus, individual positive cells were located mostly in the medulla with fewer in the cortex. In the bone marrow, megakaryocytes and immature myeloid and erythroid cells were negative. Most mature neutrophils also were negative, but an occasional neutrophil was positive. Mononuclear cells that resembled monocytes were strongly positive.

Dramatic differential expression of prosaposin mRNA was found in the CNS and the male and female reproductive tracts. In the CNS of the postnatal mouse, high-level prosaposin expression was localized to neurons, epithelial cells of the choroid plexus, and the ependymal lining of the ventricular system. As shown in Figure 2g, an intense and selective positivity was noted in the cerebellum Purkinje cells and in the neurons of deep cerebellar nuclei. The non-neuronal cells including astrocytes and oligodendrocytes showed low-level expression. The epithelial cells of the choroid plexus were intensely positive for prosa-

Table 1. *Prenatal Expression of Prosaposin mRNA*

Tissue	Cell-specific expression	Degree of expression
Yolk sac		+
Placenta	Decidua capsularis	++++
Dorsal root ganglia	Neurons	+++
Choroid plexus	Epithelial cells	++
Ventricles (CNS)	Ependymal cells	++

Positivity graded from cross sections and tangential sections were avoided. + = low-level basal expression → ++++ = high-level expression.

Table 2. *Postnatal Expression of Prosaposin mRNA*

Tissue	Specific Expression	Degree of Expression
Central nervous system		
Cerebrum (cortex and nuclei)	Neurons	++++
Cerebellum (cortex)	Purkinje cells	++++
Cerebellum (stalk)	Neurons	++++
Spinal cord	Lateral column neurons	++++
Spinal cord (central canal)	Ependymal cells	+++
Choroid plexus	Epithelial cells	++++
Ventricles	Ependymal lining cells	++++
Eye	Retina/Ciliary body	++/++
Visceral tissues		
Lungs	Alveolar macrophages	++
Liver	Hepatocytes	+++
	Bile duct epithelium	++
Spleen	Macrophages	++
Lymph nodes/thymus	Medullary macrophage	+++
Esophagus	Epithelial cells	+++
Pancreas	Ductal epithelial cells	++
Duodenum, jujunum, ileum, colon	Mucosal epithelial cells	+
Breast	Mammary gland epithelial cells (lactating)	+++
Testes	Sertoli cells	++++
Ovaries	Corpus luteum	++++
Kidney	Cortical tubular epithelial cells	++++

+ = low-level basal expression → ++++ = high-level expression.

posin mRNA (Figure 2g). The ependymal lining cells of the ventricles and central canal of the spinal cord and non-neuronal cells in the white matter showed moderate and low-level expression, respectively (Figure 2, g and h). By comparison, neurons in the lateral horns of the spinal cord were intensely positive (Figure 2h).

In the male and female reproductive systems, differential expression of prosaposin mRNA was localized to epithelial cells of the epididymis, seminal vesicles, and sertoli cells of the testes (data not shown). In the epididymis, a similar pattern of expression was seen in the epithelial lining cells of all the tubules, but low or background expression was noted in the ducts of the caudal epididymis, in the vas deferens, and in the prostate. In the female reproductive system only low levels of expression were noted in the uterus; the endometrial lining surfaces and endometrial glands; epithelial cells of the fallopian tubes; and endocervical, cervical, and vaginal mucosa; but strong expression was present in the cells of the corpus luteum.

A survey of a variety of other tissues showed only basal levels of activity. These included a variety of cell types within the heart, lung, and thyroid.

Discussion

The present results show the temporal and spatial distribution of prosaposin expression in the mouse. The level of expression of prosaposin is developmentally regulated and highly dependent on the cell type and maturation. Despite the ubiquitous role of lysosomal hydrolases in all tissue types, prosaposin exhibits high-level expression in only a subset of specialized cell types. This distribution includes a number of highly differentiated cell types with a diverse range of functions. Many of these cells are epithelial cells associated with secretory activity. Examples of this class of prosaposin-expressing cells include lactating breast epithelium, lipid-secreting Harderian gland epithelium, and the Sertoli cells in the testes. In the ovary high levels of prosaposin expression are restricted to the corpus luteum, which has secretory function and contains complex lipids. The keratinizing squamous epithelium lining of the esophagus showed high expression in the basal and spinous layers. Squamous epithelial cells also contain complex sulfated lipids that require metabolic processing. Macrophages are the prototype of lysosomal processing for a wide range of complex cellular components and showed high expression of prosaposin. In comparison, Kupffer cells did not show high expression, which suggests additional differentiation of these macrophage lineage cells *in situ*.

The development regulation is best illustrated in the CNS. During the preparation of the present studies, Sprecher-Levy et al[18] examined mouse embryo sections and suggested that the hindbrain structures may have increased expression in embryos. Our studies confirm this in the choroid plexus epithelial lining and the ependymal lining of the ventricles. Outside of these restricted areas during the early-to-middle stages of gestational development there was

generally only low-level expression in the primitive neuroglial tissue. The expression level in the embryonic CNS in general remained low until the last few days of gestation, when the developing cortical layers in both the cerebrum and cerebellum began to show increasing levels of expression. This level of expression continued to increase to the postnatal state when high-level expression was present but only in well-differentiated neurons, choroid plexus, and ependymal lining cells.

Similarly the expression of prosaposin was also highly regulated in the placenta. Following implantation the distribution of prosaposin expression was continually modified as the placenta grew. The expression began in decidual cells in a circumferential pattern immediately around the implanted embryo, but as the ectoplacental cone began to invade the decidualized uterine mucosa prosaposin expression became preferentially localized to the decidual cells along this invasion front. As the trophoblastic tissue invaded the uterine stroma there was extensive tissue remodeling involving cell death in the decidual cell population. Similarly, prosaposin expression was also up-regulated in the decidua capsularis, where decidual cells were also undergoing cell degeneration as this layer involuted.

This degree of cellular specificity in both temporal and spatial distribution has not been recognized previously for any lysosomal protein and suggests a regulation of the multifunctional gene for prosaposin independent of other lysosomal proteins. Different cellular and spatial distributions of mRNA expression were observed for acid β-glucosidase and acid lipase (Grabowski, unpublished observations). In the brain the change in level of prosaposin expression may correlate with a switch toward myelination and the different needs for GSLs in the CNS. The high levels of expression in hepatocytes and epithelial cells have no known functions or relationship to development aspects of GSL metabolism. These findings suggest additional roles for the saposins and/or prosaposins other than those involved with lysosomal catabolism of lipids.[17,22–25]

To date, the only other lysosome-related protein with demonstrated temporal and spatial regulation is the mannose-6-phosphate receptor/insulin-like growth factor receptor II (M6P/IGF II). The expression of M6P/IGF II was shown by both *in situ* hybridization and by quantitative receptor analysis to be very high in a variety of embryonic and fetal tissues.[26–28] As development proceeds, the levels of M6P/IGF II mRNA and receptor diminish, potentially in relationship to differentiation of more primitive cell types to maturity. This was particularly the case in hepatectomized animals whose levels of M6P/IGF II were maintained at a high level until the hepatocytes were fully differentiated when the levels decreased.[29,30] In comparison, our results suggest that the expression of prosaposin mRNA has the reverse pattern. Both the level and spatial distribution of mRNA for prosaposin increases and becomes more discrete, respectively, as differentiation proceeds. Thus, in the developing CNS, immature neurons have low levels of prosaposin mRNA expression similar to other CNS cell types. The same pattern was present in all other tissues. Only shortly before birth, and potentially under hormonal control, did the high-level expression of prosaposin mRNA become clear in specific CNS cell types. It is important to recognize that prosaposin mRNA is expressed in all tissues on a continuous basis throughout development as a necessary part of the activity of several GSL hydrolases. However, the localization of positive signals to certain cell types requires at least a several fold level of prosaposin mRNA compared with the surrounding tissues. The fact that other studied lysosomal hydrolases (acid β-glucosidase and lysosomal acid lipase) do not show the degree of temporal and spatial expression as prosaposin indicates an independent function and regulation for prosaposin during development and throughout life.

The temporal and spatial distribution of prosaposin mRNA expression also has implications for the control of GSL metabolism and the phenotypical expression of a variety of lysosomal storage diseases. Prosaposin and its derived saposins A, B, C, and D, are present primarily in the extracellular milieu or within lysosomes, respectively.[24] Their respective functions also appear to differ with prosaposin being a transport protein for lipids and the saposins enhancing the activity of lysosomal hydrolases.[17,23] Some lysosomal hydrolases in GSL catabolism have little variation around a set-point level of specific activity and/or mRNA (eg, acid β-glucosidase) in particular tissues. These data suggest that in particular tissues or cell types prosaposin expression could be the major modulator of the flux of GSLs through this pathway. This is supported by our studies comparing the pattern of prosaposin mRNA expression with that obtained using the Sudan B black (SBB) stain for complex fats in the developing brain. In the day 14 embryo, weak SBB staining was mostly confined to the choroid plexus epithelium, ependymal cells, and immediately beneath the ependymal layer. As the brain developed, SBB staining increased in maturing neuronal layers and was most intense in mature postnatal mouse neurons (data not shown). These were nearly the same patterns of prosaposin mRNA expression. Similarly, disorders involving acid

β-glucosidase, arylsulfatase A, β-galactosidase, α-galactosidase A, and, potentially, sphingomyelinase could have their phenotypical manifestations modulated due to the interaction of varying levels of prosaposin during development due to normal shifts in lipid metabolism during development. For example, a mutation that results in the decreased affinity of acid β-glucosidase for saposin C might be expected to result in more severe fetal damage, where the level of saposin C is low. A less progressive nature of the resultant disease would be expected after birth, since the levels of saposin C were up-regulated and a threshold of activity for disease progression could be exceeded by shifting the equilibrium to the formation of enzyme/saposin C complexes. Such modulations of the interactions of the saposins with their lysosomal hydrolases or with the amount of saposin available to bind certain toxic GSLs clearly could affect the developing brain more than the postnatal brain. In addition, the temporal and spatial distribution of prosaposin expression may impact approaches to the therapy of lysosomal hydrolase deficiencies that require the participation of the saposins for full activity.

References

1. O'Brien JS, Kretz KA, Dewji N, Wenger DA, Esch F, Fluharty AL: Coding of two sphingolipid activator proteins (SAP-1 and SAP-2) by same genetic locus. Science 1988, 241:1098–1101
2. Nakano T, Sandhoff K, Stumper J, Christomanou H, Suzuki K: Structure of full-length cDNA coding of sulfatide activator, a co-β-glucosidase and two other homologous proteins: two alternate forms of the sulfatide activator. J Biochem 1989, 105:152–154
3. Gavrieli-Rorman E, Grabowski GA: Molecular cloning of a human co-β-glucosidase cDNA: evidence the four sphingolipid hydrolase activator proteins are encoded by single genes in humans and rats. Genomics 1989, 5:486–492
4. Collard MW, Sylvester SR, Tsuruta JK, Griswold MD: Biosynthesis and molecular cloning of sulfated glycoprotein 1 secreted by rat sertoli cells: sequence similarity with the 70-kilodalton precursor to sulfatide/GM1 activator. Biochemistry 1988, 27:4557–4564
5. Reiner O, Dagan O, Horowitz M: Human sphingolipid activator protein-1 and sphingolipid activator protein-2 are encoded by the same gene. J Mol Neurosci 1989, 1:225–233
6. Fujibayashi S, Wenger DA: Biosynthesis of the sulfatide/G_{M1} activator protein in control and mutant cultured skin fibroblasts. Biochim Biophys Acta 1986, 875:554–562
7. Fujibayashi S, Wenger DA: Synthesis and processing of sphingolipid activator protein-2 in cultured human fibroblasts. J Biol Chem 1986, 261:15339–15343
8. Zhang X-L, Rafi MA, DeGala G, Wenger DA: The mechanism for a 33-nucleotide insertion in mRNA causing sphingolipid activator protein (SAP-1) deficient metachromatic leukodystrophy. Hum Genet 1991, 87:211–215
9. Schmid B, Paton BC, Sandhoff K, Harzer K: Metabolism of GM1 ganglioside in cultured skin fibroblasts: anomalies in gangliosidoses, sialidoses, and sphingolipid activator protein (SAP, saposin) 1 and prosaposin deficient disorders. Hum Genet 1992, 89:513–518
10. Holtschmidt H, Sandhoff K, Kwon HY, Harzer K, Nakano T, Suzuki K: Sulfatide activator protein: alternative splicing that generates three mRNAs and a newly found mutation responsible for a clinical disease. J Biol Chem 1991, 266:7556–7560
11. Christomanou H, Chabäs A, Pämpols T, Guardiola A: Activator protein deficient Gaucher's disease. A second patient with the newly identified lipid storage disorder. Klin Wochenschr 1989, 67:999–1003
12. Schnabel D, Schröder M, Sandhoff K: Mutation in the sphingolipid activator protein 2 in a patient with a variant of Gaucher disease. FEBS Lett 1991, 284:57–59
13. Rafi MA, de Gala G, Zhang XL, Wenger DA: Mutational analysis in a patient with a variant form of Gaucher disease caused by SAP-2 deficiency. Somat Cell Mol Genet 1993, 19:1–7
14. Schnabel D, Schreoder M, Furst W, Klein A, Hurwitz R, Zenk T, Weber J, Harzer K, Paton BC, Poulos A, Suzuki K, Sandhoff K: Simultaneous deficiency of sphingolipid activator proteins 1 and 2 is caused by a mutation in the initiation codon of their common gene. J Biol Chem 1992, 267:3312–3315
15. Reiner O, Horowitz M: Differential expression of the human glucocerebrosidase-coding gene. Gene 1988, 73:469–478
16. Reiner O, Wigderson M, Horowitz M: Structural analysis of the human glucocerebrosidase gene. DNA 1988, 7:107
17. van Echten G, Sandhoff K: Ganglioside metabolism: enzymology, topology and regulation. J Biol Chem 1993, 268:5341–5344
18. Sprecher-Levy H, Orr-Urtreger A, Lonai P, Horowitz M: Murine prosaposin: expression in the reproductive system of a gene implicated in human genetic disease. Cell Mol Biol 1993, 39:287–299
19. Sanger F, Nicklen S, Coulson AR: Determination of nucleotide sequences in DNA. Science 1981, 214: 1205–1208
20. Witte DP, Welch TR, Beischel LS: Detection and cellular localization of human C4 gene expression in the renal tubular epithelial cells and other extrahepatic epithelial sources. Am J Pathol 1991, 139:717–724
21. Aronow BJ, Lund SD, Brown TL, Harmony JA, Witte DP: Apolipoprotein J expression at fluid-phase interfaces: potential role in barrier cytoprotection. Proc Natl Acad Sci USA 1993, 90:725–729

22. Morimoto S, Yamamoto Y, O'Brien JS, Kishimoto Y: Distribution of saposin proteins (sphingolipid activator proteins) in lysosomal storage and other diseases. Proc Natl Acad Sci USA 1990, 87:3493–3497
23. Hiraiwa M, Soeda S, Kishimoto Y, O'Brien JS: Binding and transport of gangliosides by prosaposin. Proc Natl Acad Sci USA 1992, 89:11254–11258
24. Kishimoto Y, Hiraiwa M, O'Brien JS: Saposins: structure, function, distribution and molecular genetics. J Lipid Res 1992, 33:1255–1267
25. Furst W, Sandhoff K: Activator proteins and topology of lysosomal sphingolipid catabolism. Biochim Biophys Acta 1992, 1126:1–16
26. Senior PV, Byrne S, Brammar WJ, Beck F: Expression of the IGF-II/ mannose-6-phosphate receptor mRNA and protein in the developing rat. Development 1990, 109:67–73
27. DeChiara TM, Robertson EJ, Efstratiadis A: Parental imprinting of the mouse insulin-like growth factor II gene. Cell 1991, 64:849–859
28. Alexandrides T, Moses AC, Smith AJ: Developmental expression of receptors for insulin, insulin-like growth factor (IGF-I), and IGF-II in rat skeletal muscle. Endocrinology 1989, 124:1064–1076
29. Sklar MM, Thomas CL, Municchi G, Roberts CT: Developmental expression of rat insulin-like growth factor-II/mannose 6-phosphate receptor messenger ribonucleic acid. Endocrinology 1992, 130:3484–3491
30. DeChiara TM, Efstratiadis A, Robertson EJ: A growth-deficiency phenotype in heterozygous mice carrying an insulin-like growth factor II gene disrupted by targeting. Nature 1990 345:78–80