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Invited Review

Molecular role of sulfated glycoprotein-1 (SGP-1/Prosaposin) in Sertoli cells

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Summary. Sulfated Glycoprotein-1 (SGP-1) is a major polypeptide secreted by rat Sertoli cells. Sequence analysis revealed a 70% sequence similarity with human prosaposin and a 80% similarity with mouse prosaposin. Both human and mouse prosaposin are 65-70 kDa proteins cleaved in the lysosomes into four 10-15 kDa proteins designated saposins A, B, C and D. Lysosomal saposins function as enzymatic activators that promote the hydrolysis of certain glycolipids. SGP-1 (70 kDa) was first considered as being exclusively secreted to the extracellular space. However, recent immunocytochemical studies using an anti SGP-1 antibody demonstrated the presence of this protein in Sertoli cell lysosomes. In addition Sertoli cell lysosomes isolated by cellular fractionation were found to contain a 65 kDa form of SGP-1 or lysosomal prosaposin, as well as, the 15 kDa saposins. Morphological and immunocytochemical evidences also indicated that both prosaposin and saposins may reach Sertoli cell phagosomes by lysosomal fusion. These phagosomes contain cytoplasmic residual bodies detached from spermatids during spermiation. Thus, prosaposin and their derived saposins must play a role in the hydrolysis of membrane glycolipids present in phagocytosed residual bodies. On the other hand, the function of the secreted form of SGP-1 is still unclear. However, SGP-1 was seen to interact with the plasma membrane of developing spermatids. Due to its capacity to bind certain types of gangliosides, SGP-1 appears to act as glycolipid transfer between Sertoli cells and the developing spermatids.

Key words: Sertoli cell, Sulfated glycoprotein-1, Prosaposin, Saposins

Introduction

The main interest of Sertoli cell for cellular and molecular biologists resides in its multiple functions. One of them, is its supportive role to maintain in place clones of differentiating germinal cells forming the seminiferous epithelium (Sertoli, 1865).

Another function assigned to this cell is the phagocytosis of residual cytoplasmic bodies that detach from late spermatids at the time of spermiation and also of degenerating germinal cells (Morales et al., 1985, 1986). Recent data demonstrated that phagocytosis is one facet of the endocytic function of Sertoli cells. Indeed active fluid-phase and absorptive endocytosis take place at the apex of Sertoli cells. This endocytosis results in the formation of lysosomes which eventually fuse with phagosomes containing the residual bodies and contribute to their elimination from the seminiferous epithelium (Fig. 1). Thus, phagocytosis and endocytosis are well integrated processes in the Sertoli cell (Clermont et al., 1987).

An additional function has been disclosed by electron microscopists and physiologists and that is the formation of a barrier by means of tight junctions between adjacent Sertoli cells. Fawcett and his collaborators demonstrated that these tight junctions formed an effective barrier that prevented the penetration of macromolecules from the interstitial space to the lumen of the seminiferous tubules (Dym and Fawcett, 1970). The Sertoli cell tight junctions also demarcate distinct compartments of the seminiferous epithelium, that is, a basal compartment which faces the interstitial space and houses the proliferating spermatogonia and preleptotene spermatocytes, and an adluminal compartment facing the lumen which houses the spermatocytes and the spermatids (Dym and Fawcett, 1970).

In physiological terms the blood-testis barrier has a more complex function that the simple exclusion of different types of macromolecules. It is known that Sertoli cells are capable of secreting fluids, proteins and ions, and that the composition of the seminiferous tubular fluid is different from that of the plasma and testicular lymph. Thus, one of the main function of the blood-testis barrier is to allow the creation of a special environment within the seminiferous tubules in which germinal cells undergo meiosis and develop into

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spermatozoa (Setchell, 1980). The barrier also regulates the concentration of substances of endocrinological importance such as androgen binding protein (ABP) which is highly concentrated in the seminiferous tubular fluid (Setchell, 1980). Another possible function of the blood-testis barrier is to prevent that proteins produced

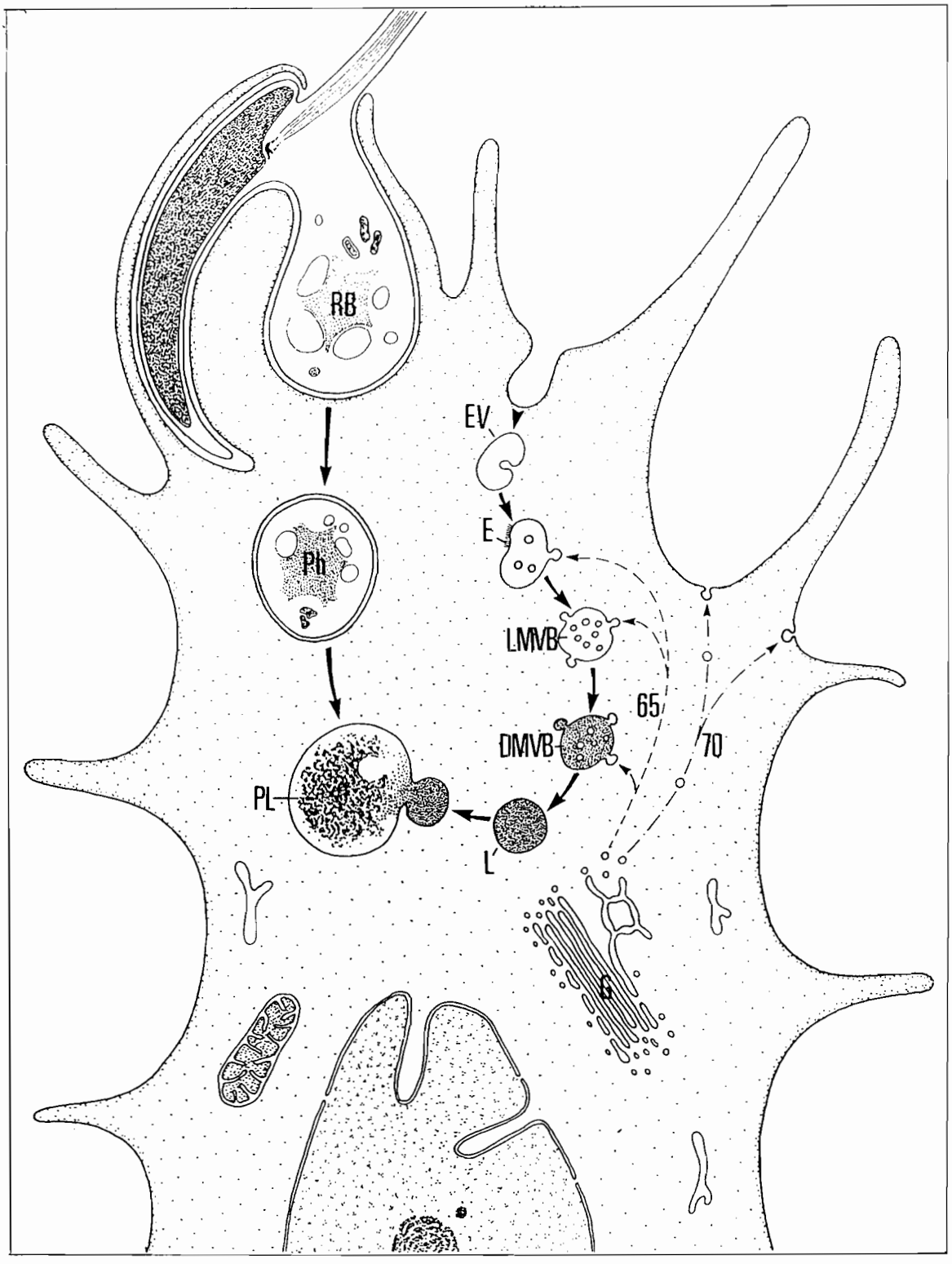

Fig. 1. Schematic drawing illustrating the endocytic and phagocytic activities taking place at the apex of a Sertoli cell. Endocytic vesicles (EV) internalize fluids and membranes and subsequently transform to endosomes (E), light multivesicular bodies (LMVB), dense multivesicular bodies (DMVB) and mature lysosomes (L). Spermatid's residual bodies (RB) are internalized into phagosomes (Ph) presumably via receptor-mediated phagocytosis. Lysosomes fuse to the phagosomes and the resulting phagolysosomes (PL) digest the residual bodies. The drawing also illustrates the lysosomal (65 kD) and secretory (70 kD) pathways of the two forms of SGP-1. G: Golgi apparatus.

by the haploid spermatids and spermatozoa enter in contact with the immunological system of the body (Setchell, 1980).

Another function of Sertoli cell is related to its secretory activity. Sertoli cells secrete proteins that falls in four main categories: 1) *Transport and bioprotecting proteins* such as transferrin, sulfated glycoprotein-1 (prosaposin), sulfated glycoprotein-2 (clusterin), etc; 2) *Proteases and protease inhibitors* such as plasminogen activators, cyclic protein-2, cystatin, α-2 macroglobulin, cathepsin L, etc; 3) *Basement membrane* products such as collagen IV, laminin, heparan sulphate, etc; 4) *Hormones and growth factors* such as müllerian inhibiting substance (MIS), inhibin, TGF-α, TGF-ß, etc (for review see Griswold, 1993).

Link between sulfated glycoprotein-1 and prosaposin

Sulfated glycoprotein-1 (SGP-1) is a major secretory product of rat's Sertoli cells in culture and in vivo (Sylvester et al., 1989; Igdoura and Morales, 1995a). Sequence comparison revealed a 76% homology between the cDNAs of rat SGP-1 and human prosaposin (Collard et al., 1988). Human prosaposin is a 70 kDa protein which is proteolytically cleaved, within lysosomes, into four 10-15 kDa polypeptides termed saposins A, B, C and D (O'Brien and Kishimoto, 1991). In the lysosomes the saposins activate the hydrolysis of sphingolipids by specific hydrolases (Ho and O'Brien, 1971; Li and Li, 1976; Kretz et al., 1990). Saposins A and C stimulate the hydrolysis of glucosylceramide by ß-glucosylceramidase and galactosylceramide by ß-galactosylceramidase (Kleinschmidt et al., 1987; Morimoto et al., 1988, 1989). Saposin B is the activator of arylsulfatase A, α-galactosidase and ß-galactosidase (Fig. 2) (Gärtner et al., 1983; O'Brien et al., 1988). Saposin D is a sphingomyelinase activator protein (Morimoto et al., 1988). The deficiency of saposin B has been linked to a variant form of the lysosomal storage disease metachromatic leukodystrophy (Stevens et al., 1981) and the deficiency of saposin C has been linked to a variant form of Gaucher's disease (Christomanou et al., 1986). No specific deficiency of saposin A and D have yet been reported.

While human prosaposin was known to be targeted to the lysosomes, rat testicular SGP-1 was first thought to be secreted to the lumen of seminiferous tubules without being processed into the 15 kDa saposins (O'Brien et al., 1988). A proline rich segment composed of 31 amino acids present in rat SGP-1 but absent in human prosaposin was implicated in the secretory routing of SGP-1 to the extracellular space. However, recent immunocytochemical studies performed in Sertoli cells and epithelial cells of the efferent ducts of the rat have demonstrated that a 65 kDa form of SGP-1 may be targeted to the lysosomes and/or be post-translationally modified to a 70 kDa mature protein which is routed to the extracellular space (Igdoura et al., 1993; Igdoura and Morales, 1995a). In fact, immunoprecipitation and immunoblotting of lysosomal proteins obtained from lysosomes isolated from rat Sertoli cells with an antibody against rat SGP-1 identified a 65 kDa form and 15 kDa polypeptides that corresponded to saposins (Igdoura and Morales, 1995a). Furthermore, analysis of rat Sertoli cell saposins, by reverse phase HPLC, revealed the presence of four peaks which correlated well with the corresponding HPLC profile obtained for the human saposins (Morimoto et al., 1988). Thus, the four peaks corresponded seemingly to the four lysosomal activator proteins or saposins (Igdoura and Morales, 1995a).

Mechanism of targeting of SGP-1

An interesting question concerning the origin of the lysosomal form (65 kD) of rat SGP-1 in Sertoli cells is how this protein reach the lysosomal compartment? Two different mechanism can be postulated: 1) The 65 kD form of rat SGP-1 may be delivered to the lysosomal compartment directly from the Golgi apparatus; 2) The 70 kDa secreted form of rat SGP-1 may reach the

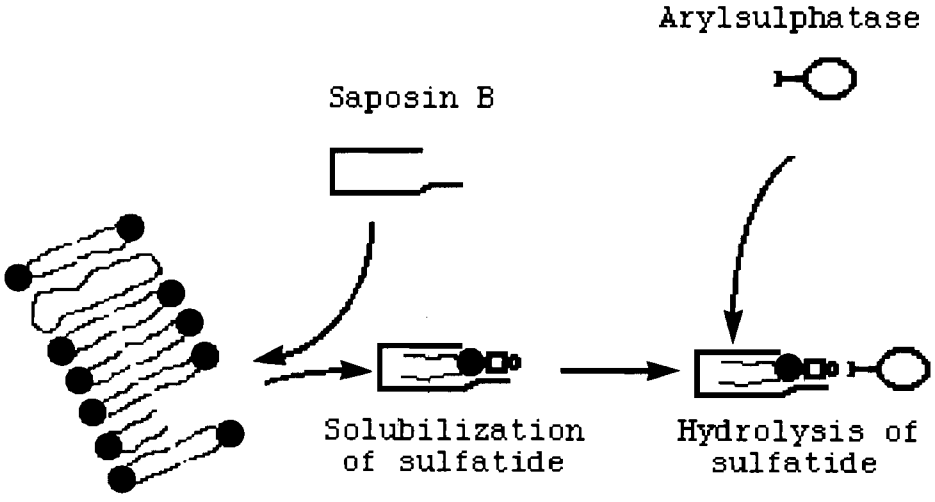

Fig. 2. General model for the action of saposins as examplified with Saposin B. In a first step, saposin B solubilizes a glycolipid (sulfatide) from the lysosomal membrane by a still unknown mechanism and accomodates it into a hydrophobic pocket. In a second step the specific hydrolase (in this case arylsulphatase A) locates the substrate into its catalytic site and cleaves the ester sulfate. Other hydrolases possesses a recognition site which interacts with the saposin molecule.

lysosomes from the lumen of the seminiferous tubules via fluid-phase or absorptive endocytosis. We have obtained evidence substantiating the first mechanism. Firstly, subcellular fractionation and immunochemical analyses revealed the absence of the 70 kDa form of SGP-1 in the lysosomes indicating that secreted SGP-1 is not present in the lysosomal compartment (Igdoura and Morales, 1995a). Secondly, metabolically labeling of SGP-1 with ^{35}S methionine demonstrated that although the 65 kDa protein is the precursor of both the 70 kDa secreted SGP-1 and the 15 kDa lysosomal saposins, only the 65 kDa polypeptide reach the lysosomes (Igdoura et al., 1993). Thirdly, immunogold labeling of Sertoli cells with antibody rinsed against rat SGP-1 demonstrated the absence of this protein within endocytic vesicles and endosomes indicating that the secreted form of SGP-1 does not enter the endocytic compartment (Igdoura and Morales, 1995a).

More recently we have investigated the lysosomal targeting of the 65 kDa protein from the Golgi apparatus. When subcellular Golgi fractions obtained from Sertoli cells of adult rat testis, were permeabilized with saponin, the 65 kDa protein was not released, suggesting that this form of SGP-1 was associated to the Golgi membrane. When the permeabilized Golgi fraction were further challenged with free mannose-6-phosphate, again the treatment did not result in the displacement of the 65 kDa protein which remained attached to the Golgi membranes. Finally, the N-glycosylation inhibitor tunicamycin did not block the transport of SGP-1 to the lysosomes. Taken together these data suggested that the 65 kDa form of SGP-1 was routed to the lysosomes in a mannose-6-phosphate independent manner (Igdoura and Morales, 1995b).

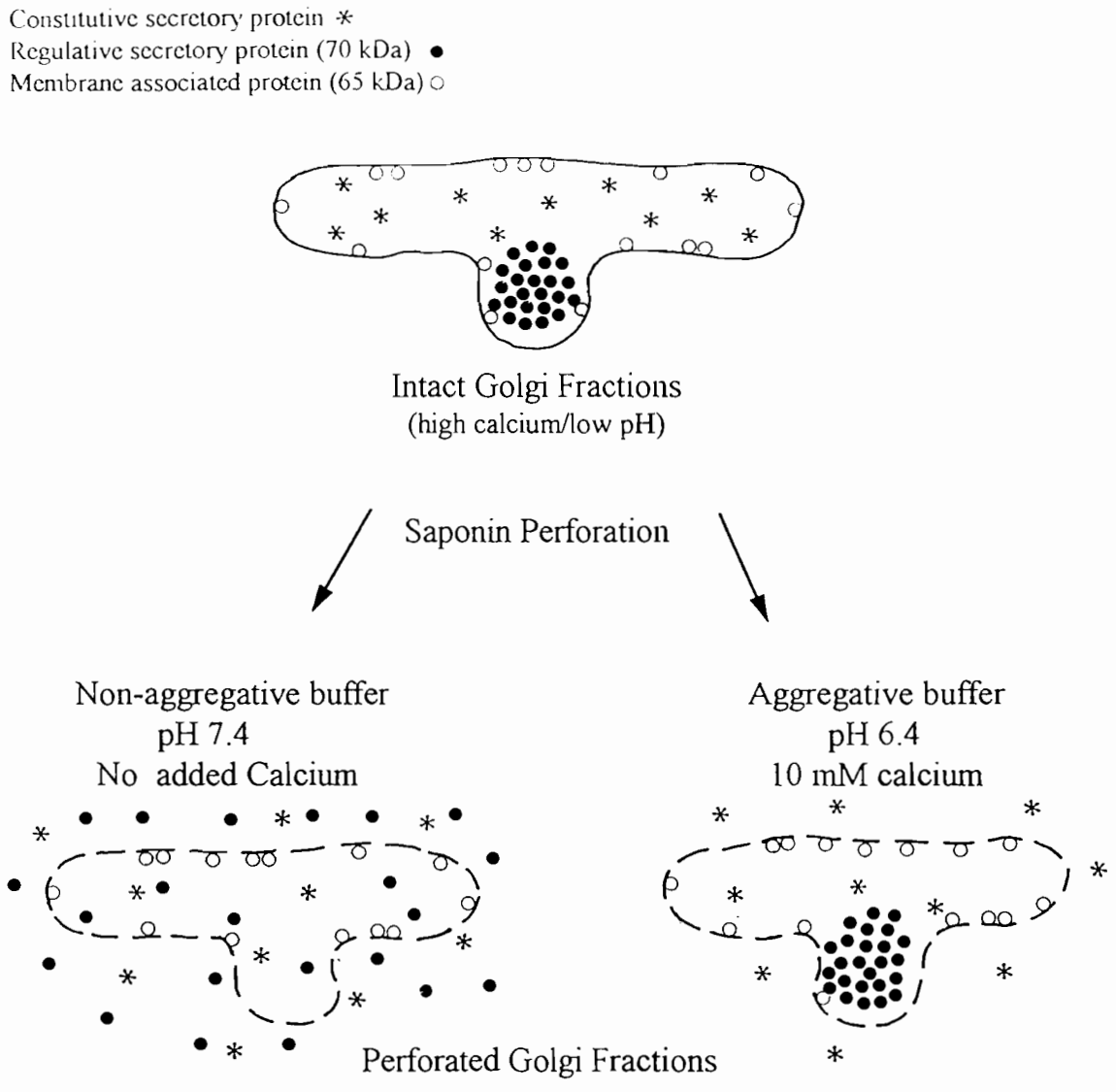

Fig. 3. Diagram illustrating the retention or release of the 70 kDa form of SGP-1 in intact and perforated Sertoli cell Golgi fractions after incubation in aggregative and non-aggregative buffers. The 70 kDa form aggregates at pH 6.4 and in the presence of calcium.

The 70 kDa secreted form of SGP-1 was also present in the Golgi fractions. Saponin treatment released this protein at pH 7.4 but retained it at pH 6.4 and high calcium. These findings suggest that the 70 kDa form of SGP-1 may aggregate into secretory granules in the trans aspect of the Golgi apparatus and then secreted into the lumen of the seminiferous tubule, perhaps in a regulated manner (Fig. 3) (Tooze et al., 1993; Igdoura and Morales, 1995b). The selective aggregation of regulated secretory proteins in the *trans*-Golgi network (TGN) is thought to be a key step in their sorting to secretory granules (Tooze et al., 1993). However, it is unclear whether aggregation alone is sufficient for sorting or simply one step during sorting. Selective aggregation of soluble secretory proteins apparently requires their association to integral membrane proteins or to homologous molecules anchored to the TGN membrane, serving as nucleation center (Tooze et al., 1993) (Fig. 3). We have proposed that some of the 65 kDa membrane associated SGP-1 may act as nucleation sites in the TGN to facilitate the aggregation of the 70 kDa protein (Igdoura and Morales, 1995b).

In conclusion, two different forms of SGP-1 exist in the male reproductive system. The first one in a 70 kDa protein that is secreted into the lumen of the seminiferous epithelium where it binds to the plasma membrane of the sperm (Sylvester et al., 1989). Although the function of this protein is still unknown it has been suggested that it may play a role in the modification of the plasma membrane of spermatozoa either as glycolipid transfer protein or as a promoter of lipid hydrolysis (Collard et al., 1988). The second one is a 65 kDa protein, which is considered to be a *bona fide* prosaposin. It is targeted to the lysosomes, in a mannose-6-phosphate independent manner, where it is further processed to the 15 kDa saposins (Igdoura and Morales, 1995a).

Molecular cloning and sequencing of mouse SGP-1

Recently, we used two synthetic oligonucleotide primers prepared according to two known sequences of the 5' and 3' untranslated regions of the prosaposin mRNA, to screen and amplify rat testicular Zap II and a mouse testicular Uni-Zap XR, cDNA libraries. Positive PCR products of 1.7 kb were isolated, subcloned into the pGEM plasmid and sequenced.

An NCBI database search, employing the BLAST network service (Altschul et al., 1990) showed a near identical nucleotide and amino acid sequence homology between our rat cDNA and the rat SAGP-1 cDNA cloned by Collard et al. (1988). Similarly, our mouse SGP-1 cDNA was almost identical to two mouse prosaposin cDNAs (Tsuda et al., 1992; Sprecher-Levy et al., 1994). Interspecies similarity between rat and mouse SGP-1 cDNAs was 88% and between rat SGP-1 and human prosaposin cDNAs was 70% homology. Nucleotide and amino acid sequence analysis demonstrated the presence of a proline rich sequence of 31 amino acids present in the mouse and rat cDNAs but absent in the human prosaposin cDNA (Figs. 4, 5).

A close analysis of the nucleotide and amino acid sequences of the isolated prosaposin cDNAs revealed several differences. The mouse prosaposin amino acid sequence reported by Sprecher-Levy et al. (1994) showed a three amino acid insertion (phenylalanine-phenylalanine-serine = DDQ) which was not found in another mouse prosaposin cDNA isolated by Tsuda et al. (1992). this insertion was also absent in our rat and mouse cDNAs and in the human sequence (Fig. 4). The alignment of the rat SGP-1, with that of the mouse and human prosaposin amino acid sequences, deduced for their respective cDNAs, revealed the presence of an extra histidine in the human sequence which was absent in the rat and mouse cDNAs.

Interestingly, the proline rich segment of 31 amino acids, initially implicated in the secretory routing of SGP-1 to the extracellular space, is not a unique feature of the rat protein and the existing evidence suggest that this segment is not involved in the different trafficking patterns of testicular SGP-1. Furthermore, human prosaposin, which lacks the proline rich segment, may also be secreted in various tissues such as mammary glands and platelets, and exist abundantly in various other secretory fluids (Kondoh et al., 1991).

Some other heterogeneities have been reported among human prosaposin, mouse prosaposin and rat SGP-1, the most notorious one being a nine base pairs insertion (CAG GAT CAG) coding for three amino acids (QDQ) initially reported by Zhang et al., (1990) and Holtschmidt et al. (1991). Recently, Lamontagne and Potier (1994) demonstrated the presence of small amounts of alternatively spliced human prosaposin mRNAs, as determined by PCR, and suggested the existence of a small exon coding for these aminoacid residues. According to Lamontagne and Potier (1994) the QDQ insertion is present in saposin B of the brain and pituitary glands but absent from the liver. The QDQ insertion was also implicated in preventing the binding of saposin B to G_{M1}-ganglioside and in the increasing of its affinity to sulfatide and sphingomyelin (Lamontagne and Potier, 1994).

The demonstration of an equivalent form of testicular SGP-1 in the mouse testis underlines the importance of this protein in the male reproductive system, particularly the lysosomal form of SGP-1 which plays a role in the hydrolysis of glycolipids and sphingomyelin. Interestingly, glycolipids are found in the plasma membrane and in membranes delimiting the endocytic and lysosomal compartments. Glycolipids are integral components of the outer leaflet facing the extracellular domain or the lumen of intracellular organelles. Furthermore, the degradation of glycolipids within the lysosomes occurs through the sequential hydrolysis of their linked oligosaccharides by specific lysosomal hydrolases that required the concerted action of activator proteins such as saposins (Fig. 3) (Li and Li, 1976; O'Brien and Kishimoto, 1991).

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Rat
Mouse
Human
1                                                                   54
ATTGCAGCCTGCGGAGTGAAGCGCGCC      ATG TAT GCT CTC GCT CTC CTC GCC AGC
ATTGCAGCCTGCGGAGTGAAGCGCGCC      +++ ++C ++C +T+ ++C C++ T++ +++ +++
----------------------CGCGCT      +++ ++C ++C +T+ TTC +++ ++G +++ +++

55                                                                 105
CTT CTG GTC ACC GCT CTG ACC AGC CCT GTC CAG GAC CCG AAG ATA TGC TCT
+++ +++ +C+ +++ +++ +++ +++ +++ +++ +++ ++A +++ +++ +++ +C+ +++ +++
++C +++ +G+ G+G +++ ++A G++ GG+ ++G +++ +TT +GA +T+ ++A GA+ +++ A+C
        .....e1 e2.....
106                                                                156
GGG GGC TCA GCA GTA GTG TGC AGA GAT GTG AAG ACG GCG GTG GAC TGT AGG
+++ +++ +++ +++ ++G +++ +++ +++ +++ +++ +++ +++ +++ ++G +++ +++ G++
A++ +++ ++G +++ ++G TG+ +++ CAG A++ +++ +++ +++ +++ TCC +++ ++C G++

157                                                                207
GCT GTG AAG CAC TGC CAG CAG ATG GTC TGG AGC AAG CCC ACA GCA AAA TCC
++C +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ ++G +++ +++
++A +++ +++ +++ +++ +T+ +++ +CC ++T +++ +A+ +++ ++A +++ +TG +++ +++
                                                  .....e2 e3.....
208                                                                258
CTT CCT TGT GAC ATA TGC AAA ACG GTT GTC ACC GAA GCT GGG AAC TTG CTG
+++ +++ ++C +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++
+++ ++C ++C +++ +++ +++ +++ GAC +++ +++ +++ +C+ +++ ++T G+T A++ +++

259                                                                309
AAA GAT AAT GCT ACT GAG GAG GAG ATC CTC CAT TAC CTG GAG AAG ACC TGT
+++ +++ +++ +++ ++C +++ +++ +++ +++ ++T +++ +++ +++ +++ +++ +++ +++
++G ++C +++ ++C +++ +++ +++ +++ +++ ++T GT+ +++ T++ +++ +++ +++ +++
                .....e3 e4.....
310                                                                360
GCG TGG ATT CAT GAC TCC AGC CTG TCA GCC TCT TGC AAG GAG GTG GTT GAC
+A+ +++ +++ +++ +++ +++ +++ +++ ++G +++ ++G +++ +++ +++ +++ +++ +++
+AC +++ C++ +CG A+A C+G +A+ A++ ++T ++T ++A +++ +++ +++ A+A ++G +++

361                                                                411
TCT TAC CTG CTT GTC ATC CTG GAC ATG ATT AAG GGG GAG ATG AGC AAC CCC
+++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ ++C +++ +++ +++ +++ ++T
++C +++ ++C +++ +++ +++ +++ +++ ++C +++ ++A ++A ++A +++ +++ CGT ++T
                                              .....e4 e5.....
412                                                                462
GGG GAA GTG TGC TCT GCG CTC AAC CTC TGC CAG TCC CTT CAG GAG TAC TTG
+++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++
+++ ++G +++ +++ +++ ++T +++ +++ +++ +++ +++ ++T ++C +++ A++ C++ C+A
                                                          ^
463                                                                513
GCC GAG CAA AAC CAG AGA CAG CTG GAG TCC AAC AAG ATC CCG GAG GTG GAC
+++ +++ +++ +++ +++ +A+ +++ +++ +++ +++ +++ +++ +++ +++ +T+ +++ +++
++A +++ +TG ++T +++ +AG +++ +++ +++ +++ ++T +++ +++ ++A +++ C++ +++
```

Fig. 4

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514                                                                 564
CTG GCC CGC GTG GTT GCC CCC TTC ATG TCC AAC ATC CCT CTC CTG CTG TAC
A++ +++ ++T +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++
A++ A+T GAG +++ ++G +++ +++ +++ +++ G++ +++ +++ +++ +++ ++C ++C +++

565                                                                 615
CCT CAG GAT CGG CCT CGC AGC CAG CCG CAG CCC AAG GCT AAC GAG GAC GTC
+++ +++ +++ +AC ++C +++ +++ +++ ++C ++A ++T +++ +++ +++ +++ +++ +++
+++ +++ ++C G+C ++C +++ +++ A++ ++C +++ ++A +++ +A+ ++T +G+ +++ ++T
                                          .....e5 e6.....
616                                                                 666
TGC CAG GAC TGT ATG AAG TTG GTG ACT GAC ATC CAG ACT GCT GTG AGG ACC
+++ +++ +++ +++ +++ +++ C++ +++ T++ ++T G++ +++ +++ +++ +++ +A+ +++
+++ +++ +++ ++C ++T C++ A++ +++ +++ +++ +++ +++ +++ +++ ++A C++ +++

667                                                                 717
AAC TCC AGC TTT GTC CAG GGC TTG GTG GAC CAC GTC AAG GAG GAT TGT GAC
+++ +++ +++ +++ A++ +++ +++ +C+ +++ +++ +++ ++G +++ +++ +++ +++ +++
+++ +++ +C+ +++ +++ +++ +C+ +++ T++ +TG G+T +++ +++ +++ ++G +++ +++

718                                                                 768
CGC CTC GGG CCA GGC GTG TCT GAC ATA TGC AAG AAC TAT GTT GAC CAG TAT
+++ +TG +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ ++C C+G +++ +++ +++
+++ +++ ++C ++T +++ A++ G+C +++ +++ +++ +++ +++ +++ A+C AG+ +++ +++
                            .....e6 e7.....
769                                                                 819
TCT GAG GTC GCC GTC CAG ATG ATG ATG CAC ATG CAA CCC AAG GAA ATC TGT
++C +++ +++ TGT +++ +++ +++ TG+ +++ +++ +++ +++ +++ +++ +++ +++ +++
+++ ++A A+T ++T A++ +++ +++ +++ +++ +++ +++ +++ +++ +++ ++G +++ +++
                                      .....e7^e9.....
820                                                                 870
GTG ATG GTT GGC TTC TGT GAT GAG GTC AAG AGA GTG CCA ATG AGG ACT CTG
+++ C++ +C+ +++ +++ +++ A++ +++ +++ +++ +++ +++ +++ +++ +A+ +++ +++
+C+ C++ +++ ++G +++ +++ +++ +++ ++G ++A GAG A++ ++C +++ CA+ +++ +++

871                                                                 921
GTC CCC GCC ACT GAG GCC ATC AAG AAT ATC CTC CCT GCC CTG GAG CTG ACG
+++ ++T +++ ++C +++ A++ ++T +++ ++C +++ +++ +++ +++ +++ +++ A++ +T+
+++ +++ +++ +AA +T+ +++ TC+ +++ +++ G++ A++ +++ +++ +++ ++A +++ GT+

922                                                                 972
GAG CCC TAT GAG CAG GAT GTG ATC CAG GCC CAA AAT GTG ATT TTC TGC CAA
+++ +++ +++ +++ +++ +++ C++ G++ +++ +++ ++C +++ +++ +++ ++A +++ ++G
+++ +++ AT+ A++ A++ C+C GA+ G++ ++A ++A A+G TC+ +AT G++ +A+ ++T G+G
        .....e9 e10.....
973                                                                1023
GTT TGT CAG CTT GTG ATG CGC AAG TTG TCT GAA CTG ATT ATC AAC AAT GCC
ACC +++ +++ +++ +++ +++ AAT +++ ++T +++ ++C +++ +++ G++ ++T +++ +++
++G +++ G+A T+C C++ G++ AAG G++ G++ A+C A+G +++ +++ GA+ +++ ++C AAG
```

Fig. 4

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1024                                                                1074
ACT GAG GAA CTT CTA ATT AAA GGT TTG AGT AAA GCC TGC TCC CTG CTC CCT
+++ +++ ++G ++C +++ G++ +++ +++ +++ ++C ++C ++A +++ G+A +++ +++ ++C
+++ +++ A+A GAA A++ C+C G+C +C+ ++T GAC +++ ATG +++ ++G AA+ ++G ++G
....e10 e11.....

1075                                                                1125
GCT CCT GCT TCA ACC AAG TGC CAG GAA GTG CTG GTA ACA TTT GGC CCC TCC
A+A +++ ++C AG+ +++ ++G +++ +++ +++ +++ G++ +G+ +++ +++ +++ +++ +++
AAG T+C CTG ++G GAA G++ +++ +++ ++G +++ G++ +AC ++G +AC +++ AG+ +++

1126                                                                1176
CTG TTG GAC GTC CTC ATG CAT GAG GTG AAC CCG AAC TTT CTG TGC GGT GTG
+A+ +++ GAC A++ T+T ++C +++ +++ +++ +++ ++C +G+ +C+ +++ +++ +++ +++
A+C C++ TC+ A++ C+G C++ G+G +++ ++C +G+ ++T G+G C+G G++ +++ A+C A++

1177                                                                1227
ATC AGC CTC TGT TCT GCC AAC CCG AAT TTG GTG GGG ACC CTT GAA CAA CCT
+++ C++ +++ +++ G++ +++ CG+ +++ G+G +++ +++ +A+ G+A +++ ++G ++G +++
C+G CA+ +++ ++C +++ +G+ +CG ----------------------------------------

1228                                                                1278
GCA GCA GCC ATT GTA TCT GCA CTG CCC AAA GAG CCT GCA CCG CCA AAA CAG
++G C++ CG+ +++ +++ +++ +++ +++ +T+ +++ +++ ++C A++ +++ +++ ++G +++
------------------------------------------------------------------

1279                                                                1329
CCT GAA GAG CCC AAG CAG TCT GCA TTG CGC GCC CAT GTG CCT CCT CAG AAG
++C +C+ C++ +++ +++ +++ ++G +++ +++ +++ +++ +++ +++ +++ +++ +++ +++
--------------- CG+ +T+ C++ +++ C++ AC+ +TT ++C +++ A++ +AG +CA +++
                                    .....e11 e12.....
1330                                                                1380
AAC GGG GGG TTC TGT GAG GTG TGC AAG AAG CTG GTC ATC TAT TTG GAA CAT
++T ++T +++ +++ +++ +++ +++ +++ +++ ++A +++ +++ C++ +++ +++ +++ +++
G++ ++T ++C +++ ++C ++A +++ +++ +++ +++ +++ ++G GGT +++ +++ ++T +GC

1381                                                                1431
AAC CTG GAG AAA AAC AGC ACC AAG GAG GAG ATC CTG GCT GCA CTT GAG AAG
+++ +++ +++ +++ +++ +++ +++ +++ +++ ++A +++ +++ ++C +++ +++ +++ +++
+++ +++ +++ +++ +++ +++ +++ +++ C++ +++ +++ +++ +++ ++T +++ +++ ++A

1432                                                                1482
GGC TGC AGC TTC CTG CCA GAC CCT TAC CAG AAG CAG TGT GAT GAA TTT GTG
+++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ ++C +++ ++C +++ +++
+++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ C+G +++ +++
                                        .....e12 e13.....
1483                                                                1533
GCT GAG TAT GAG CCC TTA CTG CTG GAA ATC CTT GTG GAG GTG ATG GAT CCT
+++ +++ +++ +++ +++ ++G ++A T++ ++G +++ ++C +++ ++A +++ +++ +++ +++
++A +++ ++C +++ +++ G+G +++ A+C ++G +++ ++G +++ +++ +++ +++ +++ +++
```

Fig. 4

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1536                                                                1584
TCC TTT GTG TGC TCG AAA ATT GGA GTC TGC CCT TCT GCC TAT AAG CTG CTG
GGA +++ +++ +++ +++ +++ +++ +++ ++T +++ +++ +++ +++ +++ +++ +++ +++
+++ ++C +++ +++ +T+ +++ +++ +++ +C+ +++ ++C ++G +++ C++ +++ +CC T++
          .....e13 e14.....
1585                                                                1635
CTG GGA ACC GAG AAG TGT GTC TGG GGC CCA GGC TAC TGG TGT CAG AAC AGT
+++ +++ +++ +++ +++ +++ +++ +++ +++ ++T A++ +++ +++ +++ +++ +++ +TG
T++ +++ ++T +++ +++ +++ A+A +++ +++ +++ A++ +++ +++ ++C +++ +++ +CA

1636                                                                1686
GAG ACT GCT GCC CGA TGC AAT GCT GTC GAT CAT TGC AAA CGC CAT GTG TGG
+++ +++ ++C +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++ +++
+++ ++A ++A +++ +AG +++ +++ +++ +++ ++G +++ +++ +++ +++ +++ +++ +++
                  .....e14 e15.....
1687
AAC TAG
+++ +++
+++ +++
```

Fig. 4. Comparison of the nucleotide sequence of rat SGP-1 (top) with mouse (middle) and human (bottom) prosaposin respectively. Nucleotide numbering are placed in the margins. Identical nucleotide bases are represented with plus signs (+). In the case of the human sequence the exon boundaries are indicated with the letter (e) and the numbering identifies the exon numbers. Note that in the human it was found an extra codon CAC (^) between bases 453-454. Also in the mouse and human sequences it has been reported the presence of 9-bases (CAG GAT CAG) or 6-bases (GAT CAG) insertions between bases 801-802 (^). The 3' untranslated region has not been included.

```
Rat
Mouse
Human

1                                                                     34
M Y A L A L L A S L L V T A L T S P V Q D P K I C S G G S A V V C R
+ + + + + + F + + + + A + + + + + + + + + + T + + + + + + + L + +
+ + + + F + + + + + + G A + + A G + + L G L + E + T R + + + + W + Q

35                                                                    68
D V K T A V D C R A V K H C Q Q M V W S K P T A K S L P C D I C K T
+ + + + + + + + G + + + + + + + + + + + + + + + + + + + + + + + + +
+ + + + + S + + G + + + + + L + T + + N + + + V + + + + + + + + + D

69                                                                   102
V V T E A G N L L K D N A T E E E I L H Y L E K T C A W I H D S S L
+ + + + + + + + + + + + + + + + + + + + + + + + + + + E + + + + + + +
+ + + A + + D M + + + + + + + + + + + V + + + + + + + D + L P K P N M

103                                                                  136
S A S C K E V V D S Y L P V I L D M I K G E M S N P G E V C S A L N
+ + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + + +
+ + + + + + + + + + + + + + + + + + I + + + + + + R + + + + + + + + +

137                                                                  170
L C Q S L Q E Y L A E Q N Q R Q L E S N K I P E V D L A R V V A P F
+ + + + + + + + + + + + + + + K + + + + + + + + + + + + M + + + + + + +
+ + E + + + K H + + + L + +#K + + + + + + + + + + L + M T E + + + + +
```

Fig. 5

```
171                                                                 204
M S N I P L L L Y P Q D R P R S Q P Q P K A N E D V C Q D C M K L V
+ + + + + + + + + + + H + + + + + + + + + + + + + + + + + + + + +
+ A + + + + + + + + + + G + + + K + + + + D + G + + + + + + I Q M +

205                                                                 238
T D I Q T A V R T N S S F V Q G L V D H V K E D C D R L G P G V S D
S + V + + + + K + + + + + + I + + F + + + + + + + + + + + + + + + +
+ + + + + + + + + + + T + + + A + + E + + + + E + + + + + + + M A +

239                                                                 272
I C K N Y V D Q Y S E V A V Q M M M H M Q P K E I C V M V G F C D E
+ + + + + + L + + + + + + + C + + + W + + + + +*+ + + + + + + L A + + + N +
+ + + + + + I S + + + + + I + I + + + + + + + + + + + + + + A L + + + + + +

273                                                                 306
V K R V P M R T L V P A T E A I K N I L P A L E L T D P Y E Q D V I
+ + + + + + + K + + + + + + + + T + + + + + + + + + + M M + + + + + + + L V
+ + E M + + Q + + + + + + K V + S + + V I + + + + + + V E + I K K H E V

307                                                                 340
Q A Q N V I F C Q V C Q L V M R K L S E L I I N N A T E E L L I K G
+ + H + + + L + + T + + + + + + N + F + + + + V + + + + + + + + V + +
P + K S D V I + E + + E F L V K E V T K + + D + + K + + K E I L D A

341                                                                 374
L S K A C S L L P A P A S T K C Q E V L V T F G P S L L D V L M H E
+ + N + + A + + + D + + R + + + + + + + V G + + + + + Q + + I F I + +
F D + M + + K + + K S L + E E + + + + V D + Y + S + I + S I + L E +

375                                                                 408
V N P N F L C G V I S L C S A N P N L V G T L E Q P A A A I V S A L
+ + + S S + + + + + + G + + A + R + E + + E A + + + + + + P R + + + + + +
+ + + E L V + S M L H + + + G T R - - - - - - - - - - - - - - - - - -

409                                                                 442
P K E P A P P K Q P E E P K Q S A L R A H V P P Q K N G G F C E V C
L + + + T + + + + + + A Q + + + + + + + + + + + + + + + + + + + + + + + +
- - - - - - - - - - - - - - L P + + T V + + T Q P + D + + + + + + + +

443                                                                 476
K K L V I Y L E H N L E K N S T K E E I L A A L E K G C S F L P D P
+ + + + L + + + + + + + + + + + C + + + + + + + + + + + + + + + + + + + +
+ + + + G + + D R + + + + + + + + + Q + + + + + + + + + + + + + + + + + +

477                                                                 510
Y Q K Q C D E F V A E Y E P L L L E I L V E V M D P S F V C S K I G
+ + + + + + D + + + + + + + + + + + + + + + + + + + + + + + G + + + + + + +
+ + + + + + Q + + + + + + + + V + I + + + + + + + + + + + + + + + L + + +
```

Fig. 5

```
511                                                                 544
V C P S A Y K L L L G T E K C V W G P G Y W C Q N S E T A A R C N A
+ + + + + + + + + + + + + + + + + + S + + + + + M + + + + + + + +
A + + + + H + P + + + + + + + + I + + + S + + + + + T + + + + Q + + +

545                 554
V D H C K R H V W N
+ + + + + + + + + +

+ E + + + + + + + +
```

Fig. 5. The complete deduced aminoacid sequence (top) of rat SGP-1 cDNA is compared with the deduced homologous sequences of mouse (middle) and human (bottom) prosaposin cDNAs. Plus signs (+) represents identical aminoacids. The different aminoacids are indicated by their respective letters. Asterisk (*) between aminoacids 259 and 260 indicates a three aminoacid insertion (aspartic acid - aspartic acid - glutamine = DDQ) reported in the mouse (Tsuda et al., 1992; Sprecher-Levy et al., 1994). A histidine (H) insertion that may be present or absent in the human sequence is also indicated (#) between aminoacids 150 and 151. Dashed line indicates a stretch of aminoacids absent in human prosaposin.

Role in residual bodies degradation

Some evidence indicated that the 65 kDa and 15 kDa SGP-1 proteins, present in rat Sertoli cell lysosomes, are delivered to the phagosomes containing internalized residual bodies. The residual bodies are pieces of cytoplasm that are eliminated from late spermatids at the time of spermiation. Such cytoplasmic bodies remain attach to the surface of Sertoli cells and soon after, they are phagocytosed. Immunogold labeling with SGP-1 antibody demonstrated that phagosomes located in the supranuclear region of these cells are unlabeled (Igdoura and Morales, 1995a). Subsequently, the phagosomes migrate toward the base of the Sertoli cells and fuse with lysosomes transforming into large phagolysomes (Morales et al., 1985). The membranous components of the residual bodies undergo rapid lysis in the phagolysosomes and coincidently immunogold labeling with SGP-1 antibody strongly labeled these structures. Thus, it appears that the lysosomal forms of SGP-1 reach the Sertoli cell phagosomes as a result of the fusion with existing lysosomes and thus play a role in the rapid hydrolysis of membrane glycolipids found in phagocytosed residual bodies (Igdoura and Morales, 1995a).

Future directions

The function of the secreted form of SGP-1 is still unknown. However, prosaposin as well as SGP-1 could act as glycolipid transfer proteins (Hiraiwa et al., 1992). Since glycolipids were shown to mediate heterologous cell contacts, including the preferential adhesion of different cell types (Marchase, 1977) it is possible that glycolipid modification of Sertoli cell and/or germinal cell membranes play a role in certain processes such as the induction of spermiation. Nevertheless, further investigations are required to clarify the function(s) of secreted SGP-1 in testis.

The mechanisms of lysosomal and secretory targeting of SGP-1/prosaposin are also unclarified issues. The availability of specific DNA probes and the generation of mutations in specific domains of the prosaposin gene may help to determine whether specific sequences are responsible for the sorting and differential transport of the 65 and 70 kDa forms of SGP-1.

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