review

Saposin-like proteins (SAPLIP) carry out diverse functions on a common backbone structure

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A polypeptide motif characterized by the locations of six cysteines and several hydrophobic residues is found in saposins A–D, sulfated glycoprotein-1, acid sphingomyelinase (ASM), acyloxyacyl hydrolase (AOAH), surfactant protein B (SPB), and *Entamoeba histolytica* pore-forming peptides (amoebapores) (1–4). The same motif is present, in an altered form, in plant aspartyl proteases (5). Each of the animal proteins binds to, or interacts with, one or more lipids, yet their properties and presumed functions in vivo are otherwise quite varied. In this article we review information about the members of this family and propose, as suggested in part by others (4), that three intradomain disulfide linkages create a common structural framework upon which amino acids in four amphipathic alpha helices can carry out diverse functions.

The amino acid sequences of the saposin-like domains are compared in **Fig. 1.**

Known members of the SAPosin-LIke Protein (SAPLIP) family

Saposins are water-soluble cofactors for specific sphingolipid glycohydrolases (see reviews in references 6 and 7). (The term saposin was recently introduced to simplify a complex nomenclature (summarized in reference 8); saposins B and C are also known as sphingolipid activator proteins (SAPs) -1 and -2, respectively, and many authors prefer this terminology. Saposin is used here to emphasize the amino acid sequence similarity and common precursor of four of the sphingolipid activator proteins.) Saposins A–D ($M_r \simeq 10{,}000$) are derived by proteolytic cleavage from a 554 amino acid precursor, prosaposin. They are found principally in lysosomes, where they facilitate the catabolism of glycosphingolipids that have short hydrophilic head groups (9). Deficiencies of prosaposin (10, 11), saposin C (12, 13), and saposin B (14–16) have been associated with lipid storage disorders in humans.

Saposins A and C facilitate the action of β-glucosylceramidase and β-galactosylceramidase by binding directly to these enzymes. Although the saposin is required for enzymatic activity in vitro (17, 18), full activity of the enzymes also requires the presence of a negatively charged lipid such as phosphatidylserine (17). Studies using monoclonal antibodies to different epitopes on β-glucosylceramidase suggested that saposins A and C bind to separate sites on the enzyme (19), and more recent studies by the same group indicate that saposin C binds the enzyme with higher affinity (20) and has greater cofactor activity (18). The ability of mutations in saposin C to produce a Gaucher-like phenotype in humans (12, 13) also casts doubt on the physiological importance of saposin A in normal glucosylceramide catabolism.

In vitro, saposin C can also augment the action of galactosylceramidase on lactosylceramide (21), induce destabilization and fusion of phosphatidylserine-containing vesicles at low pH (22), and promote glucosylceramidase binding to vesicle bilayers (23). Recent reports indicate that saposin D, the most abundant saposin in normal tissues (24), stimulates acid ceramidase activity in vitro, probably by binding to the enzyme (25, 26). Although isolated saposin D deficiency has not been reported, prosaposin deficiency has been associated with striking elevations in tissue ceramide levels (11), a finding that now could be explained by deficiency of prosaposin-derived saposin D.

In contrast to the other saposins, in vitro saposin B binds numerous lipid substrates, rather than the enzymes themselves. Its broad substrate specificity in vitro

Abbreviations: SAPLIP, saposin-like protein; ASM, acid sphingomyelinase; AOAH, acyloxyacyl hydrolase; SPB, surfactant protein B; SAP, sphingolipid activator protein; sap, saposin; SPG-1, sulfated glycoprotein-1; LPS, lipopolysaccharide; BHK, baby hamster kidney.

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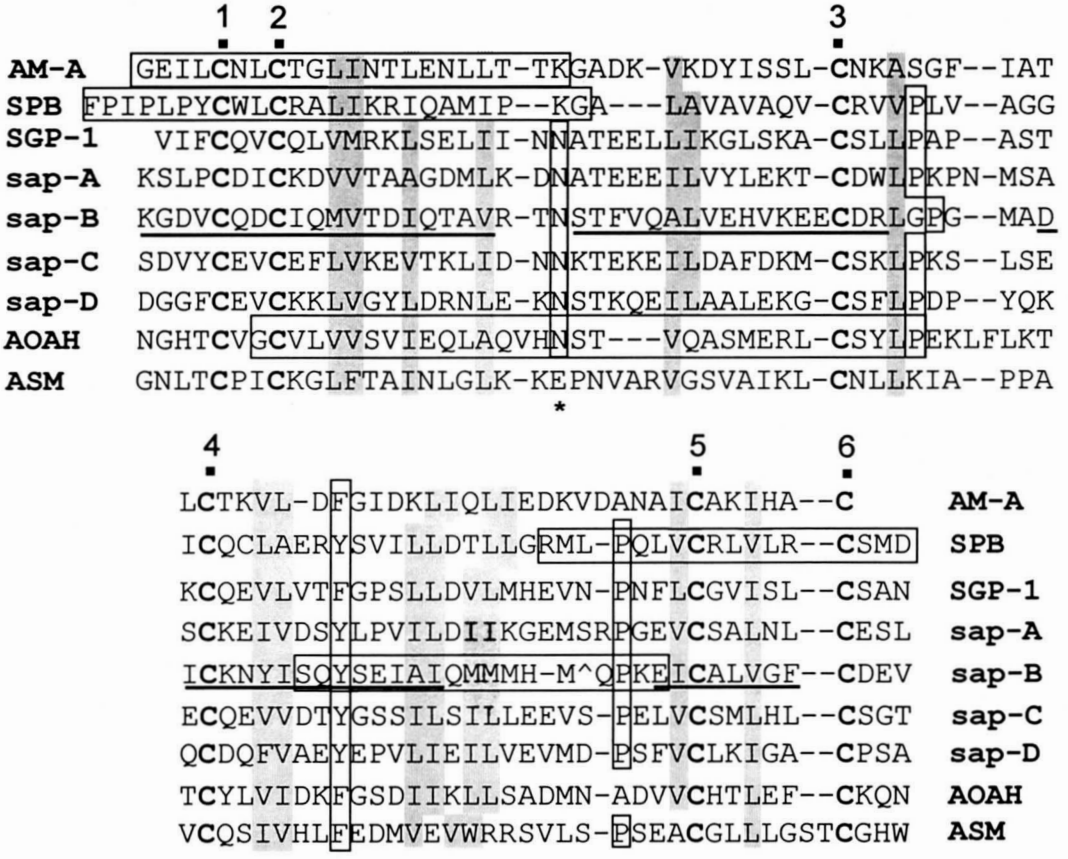

Fig. 1. The region of sequence similarity is shown for *E. histolytica* amoebapore A (AM-A), surfactant protein B (2nd repeat) (SPB), the rat sulfated glycoprotein 1 3rd repeat (SGP-1), saposins A–D (sap-A, etc.), AOAH, and ASM. The conserved Cys residues (numbered sequentially from the N-terminus) are in bold type. The common site for N-linked glycosylation is shown in a vertical box with an underlying asterisk. Common pro (P) or tyr (Y)/phe (F) residues are shown in vertical boxes and hydrophobic sequences are shaded. Horizontal boxes show the region of amoebapore A that is similar to mellitin, the regions of SPB corresponding to the B1 peptide of Waring et al. (61) and the p64-80 peptide of Revak et al. (78), the region of saposin B corresponding to a ganglioside-binding peptide (36), and the region of AOAH that is absent in a partially active, alternatively spliced mutant (55). ^ indicates the site of a three amino acid insertion (QDQ) between Cys_4 and Cys_5 in the saposin B sequence (14). The proposed helices in saposin B are underlined.

suggested that it might be a "physiological detergent" (6, 27). The importance of these activities in vivo is uncertain, however. Patients reported to have isolated saposin B ("sulfatide activator") deficiency (28, 29) have phenotypically resembled patients with metachromatic leukodystrophy, with compatible nerve pathology (29), metachromatic staining of intraleukocytic deposits, and accumulation of sulfatide in tissues (28); their fibroblasts have shown in vitro abnormalities in sulfatide metabolism (28, 29). The limited nature of these abnormalities casts some doubt on the physiological relevance of the broad in vitro binding/activation properties of saposin B.

Prosaposin is found in a membrane-bound form as well as extracellularly; its expression during development is under tissue- and cell-specific control (30). In vitro, prosaposin has most of the catalysis-enhancing activities of the four individual saposins (7). Prosaposin and all of the saposins bind in vitro to gangliosides (31), cerebrosides (32), sulfatides (32), and ceramide (26) and can facilitate glycolipid insertion into erythrocyte ghosts (31) or brain microsomes (32), suggesting a potential role in membrane biogenesis. When the binding and transfer abilities of the saposins were compared, those that bound ganglioside G_{M1} with high affinity (prosaposin, sap-A, and sap-B) were less effective promoters of ganglioside transfer to erythrocyte membranes than those that bound with low affinity (sap-C and sap-D) (31). In addition to these putative roles in intracellular lipid movement, prosaposin can evidently associate with a lysosomal protease, cathepsin D, from the rough endoplasmic reticulum through the Golgi apparatus to lysosomes, where the proteins dissociate (33). This recent observation raises the possibility that prosaposin may target proteins to lysosomes (33) (see below). Prosaposin was also recently reported to stimulate neurite outgrowth and protein phosphorylation in neuroblastoma cells (34); these neurotrophic activities were

shared by saposin C but not by the other saposins. (Although high- and low-affinity saposin C binding sites on neuroblastoma cells were found, the nature of the cell-surface receptor has not been described.) The mouse prosaposin gene has recently been disrupted by gene targeting (K. Suzuki, personal communication), and studies on prosaposin "knock-out" homozygotes should be available soon.

Alternative splicing of prosaposin mRNA, initially reported by Holtschmidt et al. (16), results in the inclusion or exclusion of a 3-amino acid insertion (Gln-Asp-Gln) in the saposin B sequence (see Fig. 1). Lamontagne and Potier (35) found differential expression of the two forms of prosaposin mRNA in various tissues; the insertion-containing form dominated in skin fibroblasts, brain, and pituitary gland, while the mRNA lacking exon 8 was the dominant form in liver and lymphoblasts (35). The same authors reported that a synthetic peptide derived from residues 52 to 69 of saposin B (see boxed sequence in Fig. 1) binds G_{M1} ganglioside (36), and that including the 3-amino acid insertion in this peptide abolished binding to G_{M1} ganglioside while increasing binding to sulfatide. They suggested that, in different tissues, alternative splicing of prosaposin mRNA may provide saposin B with different binding specificities, possibly an adaptation to differing tissue sphingolipid compositions (35). This interesting hypothesis would be supported by direct detection of the two forms of saposin B protein in tissue, as well as by demonstration that the insertion has the expected impact on saposin B-lipid binding.

A larger insertion at the same site in saposin B, resulting in the addition of 11 amino acids rather than 2 or 3, had previously been found in a 22-year-old patient with metachromatic leukodystrophy (14). This insertion occurred when a single base change in the middle of a 4-kb intron created a new 3′ splice junction that led to alternative splicing (37). The resulting protein was unstable during intracellular processing.

In addition to the six conserved cysteines, the four saposins have a conserved site for N-linked glycosylation. The importance of this glycose chain is suggested by the occurrence of metachromatic leukodystrophy-like abnormalities in an infant whose saposin B gene was mutated so that its glycosylation consensus sequence was absent (15, 38). Subsequent studies showed that enzymatic removal of the glycose chain does not alter the cofactor function or protease resistance of saposin B in vitro (39). A role in protein folding was suggested (39). Functional nonglycosylated saposin C has been produced in *E. coli* (18) and by chemical synthesis (40), however.

All of the saposins are heat-stable and resistant to most proteases. They may exist as homodimers (7). Their circular dichroism spectra indicate that each saposin has a highly ordered secondary structure in aqueous solution; O'Brien and Kishimoto (8) suggested that each should contain a hydrophobic pocket and bind lipid. Identification of the disulfide linkages in porcine saposin B (cerebroside sulfate activator) led Stevens et al. (41) also to predict a structure that contains four amphipathic helical domains with a relatively hydrophobic central core.

Rat *sulfated glycoprotein-1* (SGP-1) is homologous to human prosaposin (70% identity) (42). It is synthesized by testicular Sertoli cells, where it is found in secondary lysosomes (43), and it is present in high concentrations in the luminal fluid of seminiferous tubules and epididymis. It is thought to be involved in degrading lipids in residual bodies and/or in the modification of membrane lipids during sperm maturation (43), although the specific lipids with which it interacts are unknown. SGP-1 (M_r = 70,000) contains four repeats of the saposin-like domain and is probably a form of prosaposin. It may be processed within efferent duct cells to 15 kDa peptides, consistent with the formation of saposins (44).

Acid sphingomyelinase (ASM) is a lysosomal enzyme that hydrolyzes sphingomyelin to ceramide and phosphocholine. It may participate in intracellular signalling triggered by tumor necrosis factor-α (45). The enzyme also has phospholipase C activities toward 1,2-diacylglycerophosphocholine and 1,2-diacylglycerophosphoglycerol (for a review, see ref. 46). The human ASM cDNA sequence (47) reveals a signal peptide, a 42-residue amino-terminal region that contains a potential N-linked glycosylation site, a 76-residue saposin-like region (3), and a 504-residue catalytic domain. The mature form of ASM is a single polypeptide that, with attached glycose chains, has an apparent M_r of approximately 70,000 (61,000 peptide) (48). ASM lacks the potential glycosylation site located between Cys residues 2 and 3 in the saposins, sulfated glycoprotein-1, and AOAH. Inherited deficiency of ASM results in lysosomal sphingomyelin storage disorders (Niemann-Pick disease types A and B) (46). Although none of the reported disease-associated mutations has involved the SAPLIP motif of ASM, changing Cys_5 to Arg produced an inactive enzyme in vitro (49).

Saposins can augment the activity of acid sphingomyelinase in vitro (6), although they are not required for its activity; saposin D is most stimulatory (50). These stimulatory activities vary considerably in the presence and absence of detergent (51), and their relevance in vivo is controversial. Tissues from patients with prosaposin deficiency, for example, have had nearly normal sphingomyelin levels and fibroblasts cultured from such patients have shown normal sphingomyelin turnover,

suggesting that none of the saposins is essential for sphingomyelinase activity in vivo (11).

Acyloxyacyl hydrolase (AOAH) is a lipase, found in phagocytic cells, that cleaves fatty acyl chains from bacterial lipopolysaccharides (LPSs) and many glycerolipids (52). LPSs are bacterial cell-surface molecules that, like eucaryotic glycosphingolipids, have both hydrophilic (polysaccharide) and hydrophobic (fatty acyl) domains. AOAH treatment greatly reduces the ability of LPSs to elicit endotoxic responses in animals, consistent with a role in LPS detoxification (53). The smaller of the two AOAH peptide subunits contains the SAPLIP motif, while the larger subunit (48 kDa) contains the sequence Gly-X-Ser-X-Gly that, as in many other lipases, forms part of the active site (1). The two subunits are encoded on the same mRNA. Unlike ASM, which has a single polypeptide chain, production of mature AOAH involves disulfide bonding to link the subunits followed by proteolytic cleavage to form two chains (1, 54). In addition to the saposin-like motif, the predicted amino acid sequence of the small subunit includes a Cys residue at position 123 that is expected to form part of the subunit-linking disulfide bond (1). There is no evidence that AOAH activity is enhanced by cellular cofactors.

When expressed in baby hamster kidney (BHK) cells, the AOAH large subunit lacked enzymatic activity, was relatively unstable, and did not concentrate in lysosomes (55). The saposin-like small subunit thus appears to govern the intracellular localization and stability of AOAH in these cells. Although the small subunit is required for catalysis, abolition of enzymatic activity by mutations involving the active-site serine in the large subunit indicates that this subunit is also essential (55). An alternatively spliced form of AOAH that lacks 32 amino acids within the small subunit, including Cys residues 2 and 3 (Fig. 1), was less enzymatically active and much less stable than the major form of the enzyme. Moreover, proteolytic cleavage to form the two AOAH chains, which increased the enzyme's activity toward LPS by 10- to 20-fold, did not alter its activity toward phosphatidylcholine (55), suggesting that the orientation of the two chains is important for substrate recognition and/or catalysis.

The AOAH SAPLIP domain contains a site for N-linked glycosylation, and, like the glycan found in all of the saposins, its function has eluded definition. Removing this chain by site-specific mutagenesis had no impact on the intracellular localization, secretion, or stability of recombinant AOAH in BHK cells (55). The mutated enzyme had approximately 3-fold greater activity toward LPS, however.

Surfactant protein B (SPB) is a 9 kDa hydrophobic protein, produced in alveolar type II cells, that enhances the rate of spread of surfactant along the water–air interface in the pulmonary alveolus. Deficiency of surfactant protein B has been found in infants with congenital alveolar proteinosis (56, 57); a frameshift mutation at codon 121 of the SPB cDNA has been implicated in cases from several unrelated families (57). The mature SPB protein is produced by proteolytic cleavage of a preproprotein (approximate M_r = 42,000 (58)) by a cathepsin D-like protease (59). The surfactant potency of the protein can be mimicked by peptides that contain alternating hydrophobic and hydrophilic domains (60, 61), suggesting that the other features of SPB structure play supporting roles insofar as its surfactant action is concerned (62). The disulfide linkages in porcine SPB are identical to those in saposin B (see below) (63). A seventh Cys residue, located two residues after Cys_4, may be linked to its counterpart in a second molecule of SPB, explaining the occurrence of native dimers (63, 64). Unlike the other proteins considered here, SPB is soluble in organic solvents (e.g., chloroform/methanol). It preferentially binds phosphatidylglycerol, a lipid that is enriched in surfactant (65).

Amoebapores. The pathogenic protozoan *Entamoeba histolytica* produces pore-forming proteins (amoebapores) that may account for the ability of this parasite to destroy human cells. cDNAs encoding three distinct proteins, termed amoebapores A–C, have been sequenced. Each amoebapore is 77 amino acids in length (66). CD spectroscopic studies of amoebapore A are consistent with an all alpha-helical molecule (4). The N-terminal 24 amino acid domain of the amoebapore A and B sequences is similar to that of melittin, a membrane-disrupting peptide found in bee venom. In these amoebapores, seven of the first twelve residues are identical to those found in the melittin alpha helix (4). Amoebapore C, which lacks the melittin similarity, is equally potent as a pore-former, however. As pointed out by Leippe et al. (4), the amoebapores have a "membrane criterion profile" (hydrophobic and hydrophilic regions within the peptide sequence) that is remarkably like that of saposin C, which also has membrane-perturbing (22) activity and can initiate signal transduction (34). In the region between Cys_4 and Cys_5, the amino acid sequences of the amoebapores and saposin C are quite similar (ref. 66 and Fig. 1), suggesting that this domain in the third alpha helix (4) may contribute to the membrane-perturbing ability of these proteins.

Plant aspartic proteases, such as the barley aspartic protease, contain a sequence insertion of 104 amino acids not found in similar aspartic proteases, such as mammalian cathepsin D (67). The insertion resembles a portion of prosaposin and is partly removed during processing of the heterodimeric enzyme from a 48 kDa (32 + 16 kDa) to a 40 kDa (29 + 11 kDa) form (5, 67). Modeling suggests that the insertion may fold as a

structurally autonomous domain within a surface loop of the protease (5). The unusual feature of this member of the SAPLIP family is the fact that residues corresponding to Cys_4, Cys_5, and Cys_6 (Fig. 1) are upstream of those that correspond to Cys_1, Cys_2, and Cys_3, so that helices 3 and 4 are amino-terminal to helices 1 and 2. This arrangement can be accommodated by surprisingly minor adjustments to the structural model (see below).

The closest known homologues to the plant aspartyl proteases are an insect lysosomal protease (68) and human cathepsin D (69). These animal enzymes do not contain the SAPLIP insert, but it has recently been reported that human procathepsin D is non-covalently associated with prosaposin in subcellular compartments from the endoplasmic reticulum and Golgi to the lysosome, where the proteins dissociate (33). This observation led Guruprasad et al. (5) to propose that the plant aspartic protease saposin-like domain targets this enzyme to vacuoles, a notion that is also consistent with the ability of the AOAH small subunit SAPLIP to target AOAH to fibroblast lysosomes (or to prevent degradation before or after arrival in lysosomes). It seems likely that SAPLIPs will be found to function similarly for targeting other lysosomal hydrolases, including the insect lysosomal protease. These are examples of inter-molecular and intra-molecular SAPLIP interactions that may carry out analogous functions.

Secondary structure and disulfide bonds

The disulfide bonding pattern has been determined for two members of the family. Johansson, Curstedt, and Jörnvall (63) found the pattern for porcine surfactant protein B to be Cys_1-Cys_6, Cys_2-Cys_5, and Cys_3-Cys_4. Knowledge-based protein structure modeling approaches (70) predict that the same pattern would be found in the other proteins with saposin-like motifs. Support for this prediction was provided by Stevens et al. (41) and Sano et al. (71), who described disulfide linkages in porcine and bovine saposins C, respectively. The importance of the disulfide linkages in maintaining the structure of the domain is underscored by the ability of mutations that disrupt one of these bonds, by changing a Cys to another amino acid, to interfere with function (**Table 1**).

The three disulfide bonds impart an unusual degree of stability to the motif (7). The CD spectra of the saposins (8) and amoebapores (4) are also consistent with a high degree of alpha helical structure. Computer-assisted secondary structure prediction techniques applied to a number of saposin-like domain family members (4, 41, 72), coupled with the known disulfide bridge pattern, led to the prediction of a four-helix bundle model for saposin B (41). The model provides compact, stable domain structures that are compatible with observations regarding the protease resistance and heat stability of the proteins (see Kishimoto, Hiraiwa, and O'Brien (7) for review) and with their anomalous migration in SDS polyacrylamide gels (4, 55, 73) and in gel filtration columns (74).

A preliminary model

A computer-generated picture of a potentially typical saposin-like domain is shown in **Fig. 2.** This preliminary model is a depiction of saposin B as it would look as a four alpha-helical bundle as proposed by Stevens et al. (41), constrained by the experimentally determined disulfide-bonding pattern. This representation suggests a core orientation that is intermediate between the proposed models of Stevens et al. (41) and O'Brien and Kishimoto (8). In addition to the helix predictions of Stevens et al. (41), gaps in multiple alignments of SAPLIP sequences (e.g., Fig. 1), including alternatively spliced forms (35), were used to predict helix boundaries for saposin B (underlined in Fig. 1). Based on helix length, number, inter-helix loop length, and disulfide connectivity, compatibility of a SAPLIP sequence with a structure basically similar to that of hemerythrin (75) was surmised. The crystal structure of hemerythrin was then used as a template for modeling saposin B (Fig. 2). Ponting and Russell (personal communication) recently

TABLE 1. Mutations that disrupt disulfide linkages

Protein	Mutation	Functional Consequence	Reference
Saposin B	$Cys_4 \rightarrow$ Ser	Metachromatic leukodystrophy-like	Holtschmidt (16)
Saposin C	$Cys_5 \rightarrow$Phe	Gaucher-like	Schnabel (12)
	$Cys_5 \rightarrow$Gly	Gaucher-like	Rafi (13)
Saposin C (recombinant)	$Cys_5 \rightarrow$Phe or Gly	binding, no activation of acid β-glucosidase	Qi (18)
AOAH	32 amino acid deletion, including Cys_2 and Cys_3	reduced enzymatic, activity, stability, abnormal intracellular localization	Staab (55)
ASM	$Cys_5 \rightarrow$ Arg	inactive enzyme	Ida (49)

The numbering of the Cys residues is shown in Figure 1.

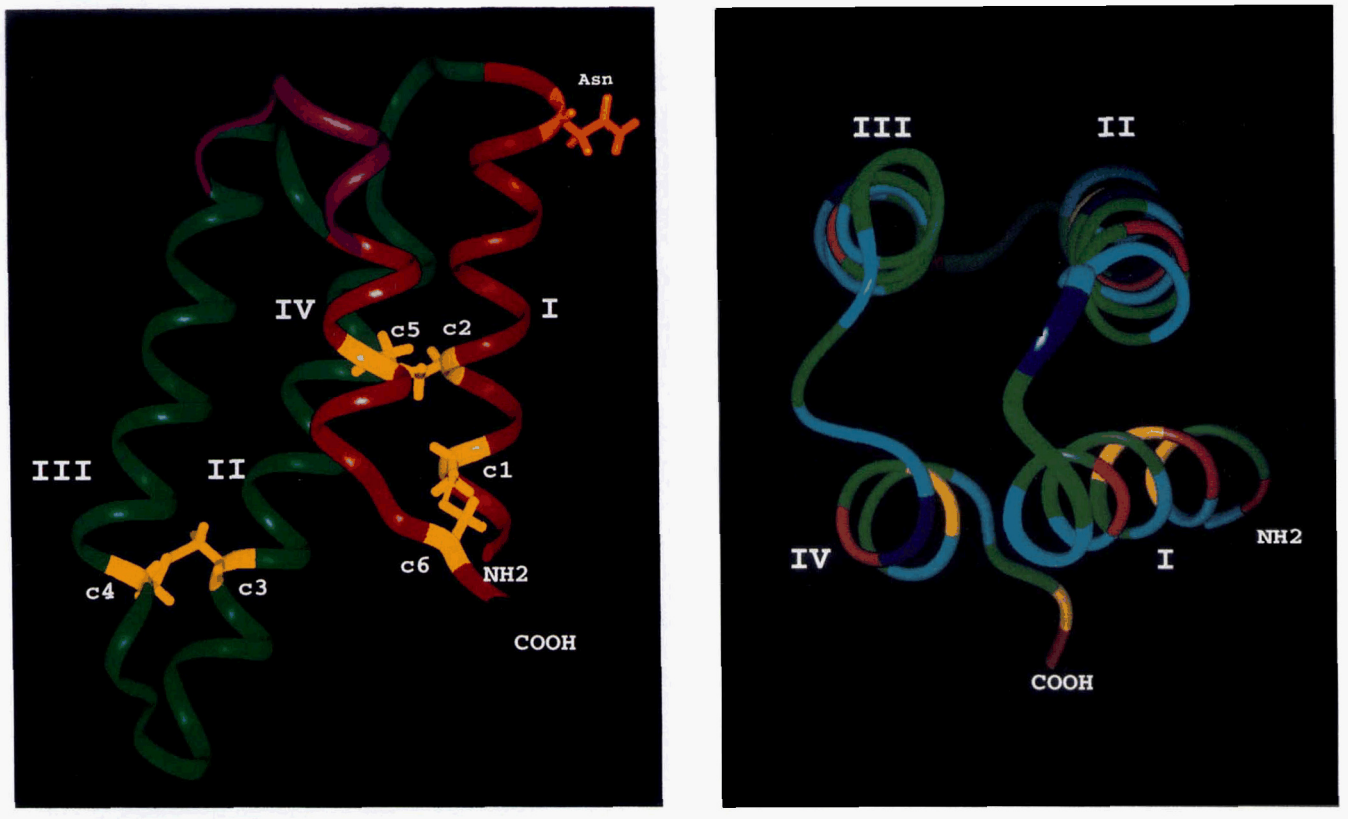

Fig. 2. Left panel: A preliminary molecular model of saposin B represented as an alpha carbon backbone ribbon. Alpha helices are shown in red and green and numbered I–IV. Cystines are shown in yellow and half-cystines are numbered c1–c6. The amino (NH2) and carboxyl (COOH) termini are indicated, as is the asparagine involved in N-linked glycosylation (orange). An alternatively spliced form of Saposin B contains the insertion Gln-Asp-Gln; its position is shown by the additional ribbon (magenta) between helices III and IV. The model was built from a hemerythrin template using Biosym (San Diego, CA) homology modeling software. Right panel: An end-on view of the model. Ribbon coloring indirectly indicates the amphipathic nature of the helices. Colors correspond to amino acid groups: green: Ala, Ile, Leu, Met, Phe, Pro, Trp, Val; light blue: Asn, Gln, Gly, His, Ser, Thr, Tyr; dark blue: Arg, Lys; Red: Asp, Glu; yellow: Cys.

constructed a saposin model structure based on the crystal structure of uteroglobin (76). While the two models are similar in a number of ways, the angle between helical pairs (I + IV) and (II + III) is wider in the uteroglobin-based model than in the hemerythrin-based version. It is conceivable that this angle varies with the hydrophobicity of the environment; association with membranes or binding lipid may be facilitated by a greater splay between the helices, which exposes more hydrophobic residues in saposin B.

The model may be used to speculate about the structure–function relationships of several of the proteins.

a) A saposin B-derived peptide that binds G_{M1} ganglioside (36) and sulfatide (35) was derived from much of helix III and helix IV (see horizontal boxed sequence in Fig. 1). A 3-amino acid (QDQ) insertion in the peptide abolished ganglioside binding while increasing binding to sulfatide (35). The location of this insertion in the saposin B model is shown in magenta in the left panel of Fig. 2. If similar binding behavior occurs with the native form(s) of saposin B (see above), the 3-amino acid insert might affect binding indirectly (e.g., by imposing inter-helical motion constraints or alterations) or directly (e.g., the insertion of the aspartate might influence substrate binding).

b) In surfactant protein B, helices I and IV contain basic/hydrophobic sequences that can mimic the surfactant activity of the protein (61, 77, 78); in the folded structure, these helices (shown in red in the left panel of Fig. 2) are adjacent to one another with the basic residues oriented along the solvent face, suggesting a likely domain for binding negatively charged phosphatidylglycerol. A seventh Cys residue, postulated to account for dimerization of native surfactant protein B, is located in helix III, quite distant from the proposed site of phospholipid interaction.

c) In recombinant saposin C, mutations that altered Cys_5 to Phe or Gly, which should distort (at a minimum) the positions of helices I and IV, had little effect on the ability of the molecule to bind acid β-glucosidase, yet

activation of the enzyme did not occur (18). The saposin C-induced conformational change in the enzyme was suggested to involve highly specific interactions between the two proteins.

d) The location of the consensus site for asparagine-linked glycosylation (found in the saposins, sulfated glycoprotein-1, and AOAH) is relatively removed from the domains that seem to carry out known functions. Perhaps this helps explain its dispensibility for all functions tested in vitro (see saposins and acyloxyacyl hydrolase, above). To explain the occurrence of saposin B deficiency in an infant with a mutation that prevented glycosylation at this site, a role of the glycose chain in protein folding during biosynthesis was suggested (39).

e) An AOAH variant that lacks both Cys_2 and Cys_3 (see Fig. 1) retains substantial (40% of native) enzymatic activity yet it is unstable and does not concentrate in lysosomes, the site of AOAH processing in BHK cells (55). Speculating from the model in Fig. 2, this variant would lack helices I and II, but helices III and IV might assume alpha helical conformations. As lysosomal localization of AOAH does not require glycosylation of the SAPLIP-containing subunit (see above), the failure of this mutant to concentrate in lysosomes suggests that helices I–II could contain a (mannose-6-phosphate independent) lysosomal targeting sequence. This notion is consistent with the lysosome-targeting function of certain SAPLIPs (prosaposin and plant aspartyl protease) proposed by others (5, 33). Alternatively, the variant AOAH SAPLIP domain might simply allow degradation of the enzyme before or after its arrival in lysosomes.

Not all potential structural models would be consistent with the occurrence of flipped SAPLIP subdomains, such as those found in plant aspartic proteases, in which Cys_4, Cys_5, and Cys_6 are amino-terminal to Cys_1, Cys_2, and Cys_3. A bisymmetric four helix model (Fig. 2, (41)) can accommodate the flip simply by connecting helices I and IV, rather than II and III, as shown schematically in **Fig. 3.** In the flipped SAPLIP, the disulfide bonds would again link Cys_{1-6}, Cys_{2-5}, and Cys_{3-4}. Ponting and Russell (79) suggest a similar flip accommodation for the aspartic protease domain, which they refer to as a "swaposin".

Evolutionary considerations

The availability of partial genomic sequence for human prosaposin (80) and rice aspartic protease (Asakura, T., et al., GenBank accession number D32165) allows comparison of intron placement in the two genes (**Fig. 4**). In the rice gene, exon VIII codes for Cys_4, exon IX codes for Cys_5 and Cys_6, exon X includes Cys_1 and Cys_2 and exon XI codes for Cys_3. This pattern is similar to that seen in the human prosaposin gene for exon VI (Cys_4 of saposin B), exon VII (Cys_5 and Cys_6 of saposin B), exon VIII (Cys_1 and Cys_2 of saposin C) and exon IX (Cys_3 of saposin C). While the general pattern is similar, the exact intron locations with respect to the sequences are different in the two cases. Intron placement is probably not a good indicator that any particular current saposin region is the closest relative to the plant domain (81). In a comparison of the plant domain and prosaposin, the highest sequence similarity is actually in a

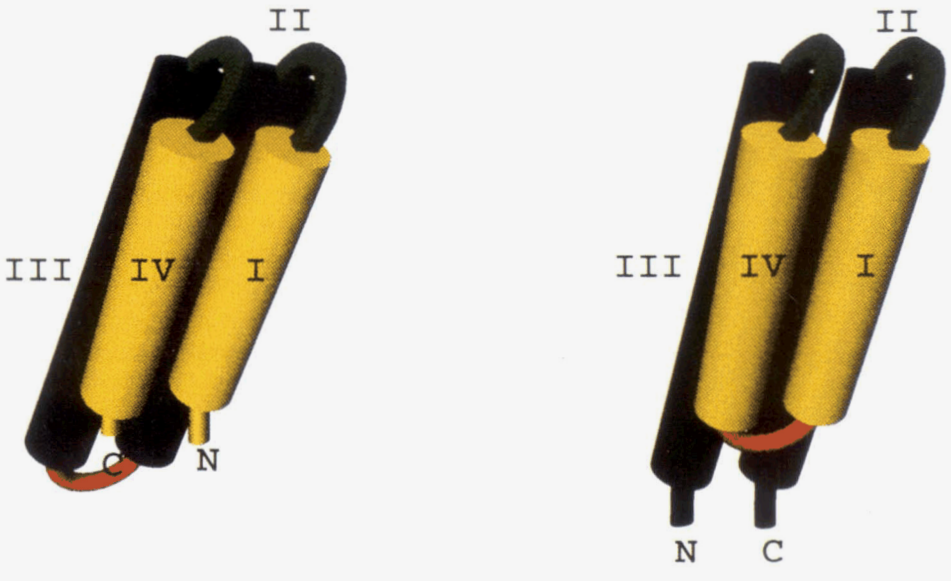

Fig. 3. The plant aspartic protease SAPLIP domain may fold similarly to other SAPLIP domains. The flipped plant SAPLIP subdomains can be accommodated by the model shown in Fig. 2 by a change in inter-helix connectivity. Left: Proposed saposin B helix orientation. Right: Proposed rice aspartic protease SAPLIP domain helix orientation.

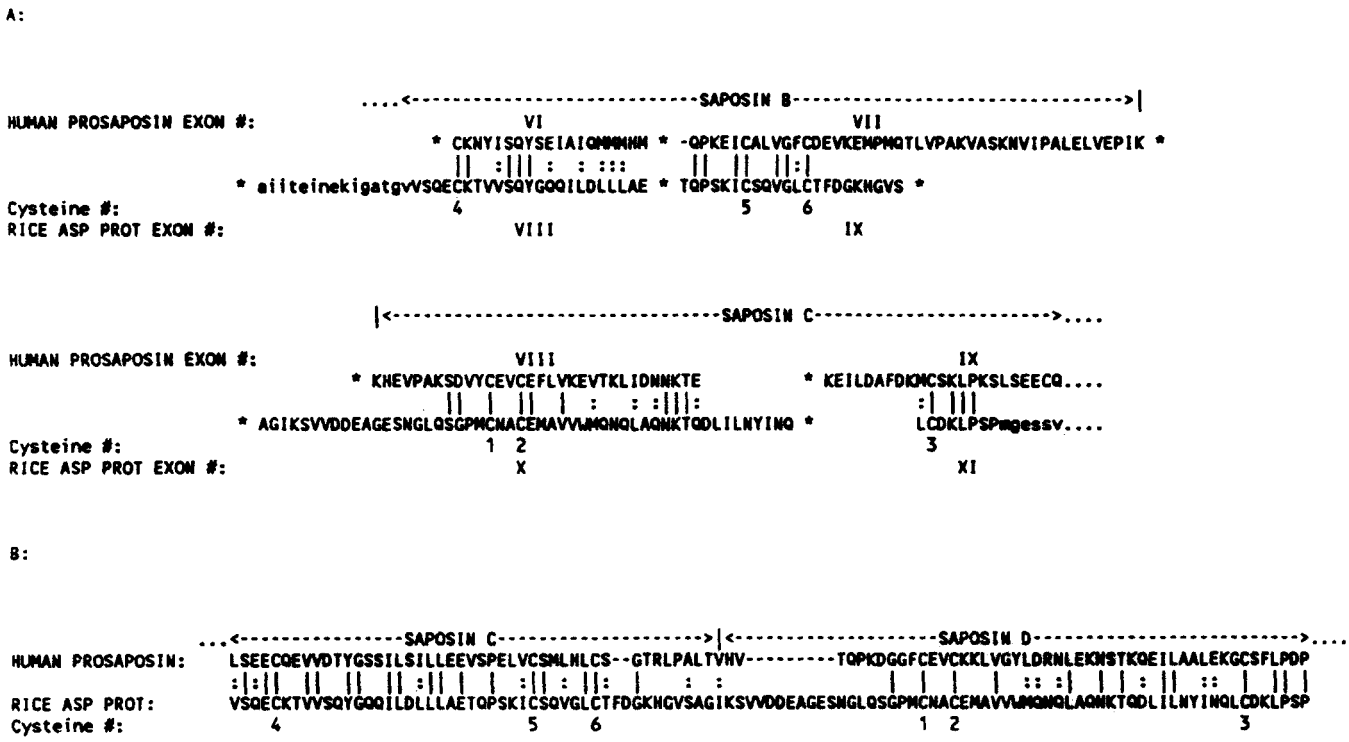

Fig. 4. The rice aspartic protease SAPLIP domain exhibits a genomic structure similar to prosaposin exons VI–IX, but the amino acid sequence is most similar to parts of saposins C and D, coded for by prosaposin exons IX and X. A: An alignment of exons VI, VII, VIII and part of IX of the human prosaposin gene (80) with exons VIII, IX, X and part of XI that code for the SAPLIP domain in the rice aspartic protease gene (Asakura et al., Genbank Accession Number D32165). Non-SAPLIP sequences in the rice protease are shown in lower case. Human exons VI–IX code for parts of saposins B and C as indicated. Intron positions are indicated by asterisks. B: An alignment of the rice aspartic protease SAPLIP domain with portions of human saposins C and D. In both A and B, Cysteine numbering is as in Fig. 1 and amino acid identities are indicated by bars, conservative substitutions by colons.

region with different patterns of introns and exons. The similarity is highest between the plant domain and a region commencing midway through saposin C and including Cys_4, Cys_5, and Cys_6 of saposin C, continuing through the saposin C–saposin D junction, and terminating after Cys_3 of saposin D (Fig. 4, panel B). Rorman, Scheinker, and Grabowski (80) proposed a model for the evolution of the human prosaposin gene that involved duplication events and at least one rearrangement involving a double crossover. It appears that the plant domain diverged from a SAPLIP ancestor at an intermediate stage of such duplication and rearrangement events, leading to the observed sequence and intron placement patterns.

Overall characteristics

Based on the information reviewed here, we suggest that the saposin-like proteins (SAPLIPs) share a motif that consists of four disulfide-linked amphipathic helical bundles. A similar conclusion was reached for various members of the family by Leippe et al. (4), O'Brien and Kishimoto (8), and Ponting (3). The known adaptations of this core structure may interact with lipids in a number of ways, acting as enzyme cofactors (saposins), transporting lipids within and between membranes (prosaposin, saposins A–D), interacting with phospholipid bilayers (saposins, surfactant protein B, amoebapores), carrying out enzymatic reactions (ASM, AOAH), reducing surface tension at lipid–air interfaces (surfactant protein B), and acting as transmembrane pore-forming toxins (amoebapores), fusogenic peptides (saposin C), and neurotrophic factors (prosaposin, saposin C). In addition, they may function in intracellular protein targeting (prosaposin, AOAH, barley grain aspartic protease) and in protein–protein interactions, including dimerization (saposins, surfactant protein B). The SAPLIP motif may be compared to other multifunctional protein domains that contain disulfide bonds, including kringles, EGF repeats, and sushi repeats (82, 83). Such structures are structurally similar but functionally very versatile. For example, sushi repeats have been found in over 30 different types of proteins with functions as diverse as coagulation factors and cytokine receptors (82). The sushi structure apparently forms a stable scaffold that can accommodate many combinations of amino acids. Likewise, the SAPLIP motif of

disulfide-bonded alpha helices appears to support a variety of different functions (4). Elucidating the structural basis for these functions will be a challenging and fascinating problem for future research.

Note added in proof: Since completion of this review, another SAPLIP has been described (M. Andersson et al. 1995. *EMBO J.* **14:** 1615–1625; M. Andersson et al. 1995. *FEBS Lett.* **362:** 328–332). It is NK-lysin, an antimicrobial and tumorolytic peptide isolated from porcine small intestine and thought to be derived from lymphocytes (NK cells). It has the expected SAPLIP disulfide bonding pattern. The neurotrophic sequence of saposin C has also been reported (O'Brien et al. 1995. *FASEB J.* **9:** 681–685).

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