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REVIEW ARTICLE

A short guided tour through functional and structural features of saposin-like proteins

Heike BRUHN[1]
Research Center for Infectious Diseases, Röntgenring 11, D-97070 Würzburg, Germany

SAPLIPs (saposin-like proteins) are a diverse family of lipid-interacting proteins that have various and only partly understood, but nevertheless essential, cellular functions. Their existence is conserved in phylogenetically most distant organisms, such as primitive protozoa and mammals. Owing to their remarkable sequence variability, a common mechanism for their actions is not known. Some shared principles beyond their diversity have become evident by analysis of known three-dimensional structures. Whereas lipid interaction is the basis for their functions, the special cellular tasks are often defined by interaction partners other than lipids. Based on recent findings, this review summarizes phylogenetic relations, function and structural features of the members of this family.

Key words: antimicrobial activity, innate immunity, lipid metabolism, lipid-binding, membrane interaction, saposin-like protein.

INTRODUCTION

The primary goal of genome sequencing projects is the assignment of a functional context to mere sequence data. Now, right in the middle of the genomics era, it becomes more and more evident that the former one-gene-one-protein-one-function assumption cannot satisfy the complexity of life. On the one hand, different splicing and post-translational modifications increase the number of protein species; on the other hand, more and more proteins are found to act in different functions dependent on their specific situation and environment. Concerning the family of SAPLIPs (saposin-like proteins), which are membrane-interacting proteins of different size and activities, a number of recent articles (references cited below) elucidate novel functions for even the oldest members. Thus this was the starting point to sum up and survey the knowledge and mysteries about this essential protein family.

PHYLOGENY – WHERE TO LOOK FOR SAPLIPs

The family of SAPLIPs is named according to saposins, four small proteins derived from a large precursor protein identified as cofactors in sphingolipid catabolism. Subsequently, 235 different proteins have been designated as SAPLIPs in relevant databases. They are found in one of the most primitive levels of eukaryotes, namely amoebozoans, as well as in mammals [1]. Prokaryotic genomes are devoid of these genes. The only three bacterial sequences assigned to this family in the InterPro database (http://www.ebi.ac.uk/interpro) lack the typical pattern of cysteine residues (accession number Q9FBA5 from *Borrelia hermsii*, accession number Q5XZA4 from *Borrelia garinii* and accession number Q5X256 from *Legionella pneumophila*). Moreover, BLAST searches in the NCBI database of microbial genomes (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi) do not result in any hits. Climbing up the phylogenetic tree, according to the finished or unfinished genomes that are accessible by search tools in the NCBI, Superfamily (http://supfam.mrc-lmb.cam.ac.uk/SUPERFAMILY/) or InterPro databases, nearly the only genes found are in amoebozoa (*Entamoeba histolytica*, *Entamoeba dispar* and *Dictyostelium discoideum*), in the heteroloboseid amoeba, *Naegleria fowleri*, and then next in fungi and metazoa; i.e. no candidates are found in the genomes of diplomonadida, apicomplexa or kinetoplastida that have by now been analysed just as well. Interestingly, the only exception is a GPI (glycosylphosphatidylinositol) inositol deacylase with a saposin-like domain which has been reported for *Trypanosoma brucei*, although the finished genome project does not contain the gene. Further up the bikont branch of the tree, plants possess a class of aspartic proteases, which contain a recognizable SAPLIP domain, but in a permutated manner, leading to the name 'swaposin'. Regarding ophistokonts, only three out of the seven fully sequenced fungal genomes, namely *Candida albicans*, *Debariomyces hansenii* and *Yarrowia lipolytica* bear SAPLIP genes, all coding for metalloesterases. All other known SAPLIP sequences are found in metazoa. Thus phylogenic data reveal the SAPLIP domain as an ancestral molecule. Internal gene duplication, as well as successive mutational diversification during evolution, result in the sequence variety and functional versatility that is observed today.

PHYSIOLOGICAL FUNCTION VERSUS *IN VITRO* ACTIVITIES

The human genome bears seven different genes coding for precursors of 11 functionally different proteins with a SAPLIP domain. The various activities may be clustered roughly into the three major, but intertwining, groups that are depicted in Figure 1. Namely, these are: (i) mere membrane binding accompanied by only local disordering of the lipid structures for membrane targeting. These activities are suggested for the multidomain enzymes acid sphingomyelinase [2] and acyloxy acylase [3] and the hypothetical metallophosphoesterase, identified by the Human Genome Project (accession number Q81UN0); (ii) membrane perturbation, but without permeabilization, e.g. lipid extraction and presentation for enzymatic activity; and (iii) permeabilization as a killing principle – the essential activity of defence proteins.

Abbreviations used: GPI, glycosylphosphatidylinositol; LPS, lipopolysaccharide; MIR, myosin-regulatory-light-chain-interacting protein; MSAP, MIR-interacting saposin-like protein; SAPLIP, saposin-like protein; SP-B, surfactant protein B.

[1] email heike.bruhn@mail.uni-wuerzburg.de

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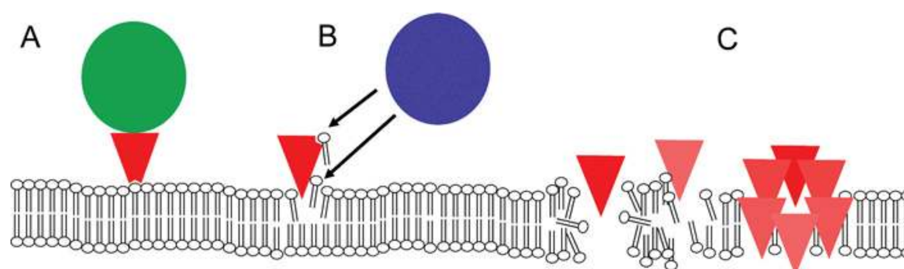

Figure 1 Schematic depiction of the three main activities of SAPLIPs

(**A**) Membrane targeting by the SAPLIP domain. (**B**) Presentation of lipids as substrate for an independent enzyme, either by extraction from the membrane or by disturbance of the well-packed lipid order. (**C**) Membrane permeabilization by perturbation owing to single molecules or by pore-formation of oligomeric proteins. Red triangle, SAPLIP domain; green circle, enzymatic domain; blue circle, independent enzyme acting on lipids (arrows).

Table 1 Different functions of SAPLIPs and their sources

(a) Autonomous domains

	Function	Organism
Lipid catabolism/transfer		
Saposins A, B, C, D	Sphingolipid catabolism and antigen presentation	*Homo sapiens*, *Equus caballus*, *Bos taurus*, *Mus musculus*, *Oryctolagus cuniculus*, *Rattus norvegicus*, *Xenopus laevis*
Surfactant stabilization		
Surfactant protein B	Surfactant stabilization	*Homo sapiens*, *Canis familiaris*, *Mus musculus*, *Oryctolagus cuniculus*, *Ovis aries*, *Rattus norvegicus*
Defence proteins		
Granulysin	Antimicrobial and cytolytic activity	*Homo sapiens*
NK-lysin		*Sus scrofa*
NK-lysin orthologues		*Equus caballus*, *Bos taurus*
Amoebapores		*Entamoeba histolytica*
Amoebapore orthologues		*Entamoeba dispar*, *Entamoeba invadens*
Amoebapore-like protein		*Fasciola hepatica*
Naegleriapores		*Naegleria fowleri*
Clornorin		*Clonorchis sinensis*
Cell proliferation regulation		
Prosaposin	Lipid transfer, neurite outgrowth and apoptosis regulation	*Homo sapiens*, *Equus caballus*, *Bos taurus*, *Mus musculus*, *Oryctolagus cuniculus*, *Rattus norvegicus*, *Xenopus laevis*
MSAP	Neurite outgrowth	*Homo sapiens*

(b) Multidomain proteins

	Function	Organism
Lipases		
Metallophosphoesterases (e.g. acid sphingomyelinase)	Sphingomyelin hydrolysis	*Homo sapiens*, *Caenorhabditis elegans*, *Ciona intestinalis*, *Anopheles*, *Drosophila*, *Mus musculus*, *Rattus norvegicus*
GDSL (Gly-Asp-Ser-Leu) lipase (e.g. acyloxy hydrolase)	LPS deacetylation	*Homo sapiens, Rattus norvegicus*
Stage regulation		
Countin	Cell number counting	*Dictyostelium discoideum*
Crystallin		
J3-crystallin	Crystallin	*Tripedalia cystophora*
Proteases		
Plant aspartic proteases	Proteolytic degradation defence	Viridiplantae

Table 1 presents a summary of known SAPLIPs and their various functions in different species.

The SAPLIP domain may exist for itself independently as a functional unit or as a part of a multidomain protein, but even the autonomous domains fulfil a variety of different cellular functions in humans, e.g. as co-factors of lipid-degrading enzymes, such as the name-giving saposins [4,5], as regulators of surfactant tension, i.e. SP-B (surfactant protein B) [6], or as antimicrobial or cytolytic effector proteins, e.g. granulysin [7]. Our knowledge of the physiological function of most of the mammalian SAPLIPs could unequivocally be derived only from the molecular analysis of associated diseases. A complete deficiency of prosaposin, the precursor protein of the mature saposins A, B, C and D, results in the storage of multiple glycosphingolipids resembling a combined lysosomal hydrolase deficiency [8]. The selective deficiencies of saposin B or C cause an atypical form of metachromatic leukodystrophy [9–11] or a variant of Gaucher's disease [12,13] respectively, and the mutation of SP-B leads to alveolar proteinoses [14–16]. These stories told by mutants reveal not only the biological impact of SAPLIPs, but also the difficulty in elucidating their function which is, up to now, not obvious from sequence data. *In vitro* studies specify the mentioned activities

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(for a recent review, see [17]). Saposin B serves as stimulator of sulphatide degradation by arylsulphatase A [18], saposin C assists in glucosylceramide degradation by glucosylceramide-β-glucosidase [19], as well as in galactosylceramide and sphingomyelin catabolism [20,21], saposin A helps to degrade galactosylceramide and glucosylceramide [22,23], and saposin D stimulates acid ceramidase and sphingomyelinase [24,25]. The physiological relevance of these activities was verified by complementation of precursor-deficient fibroblasts in cell culture with the single saposins [26]. Moreover, these *in vitro* studies revealed more general capabilities relying only on the lipid substrate. The proteins were able to assert vesicle modifications, such as permeabilization, aggregation and fusion, which were analysed in comprehensive detail. These activities are highly dependent on lipid composition, the ratio of lipid/protein, as well as pH and buffer conditions. Interestingly, all of the saposins show markedly increased activities at acidic pH. They are all known to bind especially to vesicles composed of galactosylceramide, glucosylceramide, sulphatides [27] or phosphatidylserine [28] under acidic conditions, whereas only saposin A also binds sphingomyelin [27]. Even their precursor, prosaposin, binds and transports gangliosides [29]. A more specialized activity is the observed vesicle fusion by saposin C [30,31]. The underlying membrane-restructuring effects were recently observed directly by atomic force microscopy [32], which clearly showed the influence of lipid composition: whereas saposin C induces destabilization of pure phospholipid bilayers, domain formation without impairment of membrane integrity was observed in the presence of cholesterol and sphingomyelin. In contrast, saposin D provokes vesicle clearance, presumably due to lipid extraction [33,34].

Essentially similar *in vitro* activities are reported for the surfactant tension regulating SP-B. With an increasing protein/phospholipid ratio, SP-B binds and aggregates on the membrane, leading, dose-dependently, to membrane destabilization, which ultimately results in membrane fusion. Negatively charged phospholipids, such as phosphatidylglycerol, provoke an enhancing effect [35–37]. In spite of these parallels to saposins, SP-B is water-insoluble and, moreover, is the only covalently connected homodimer, using a seventh cysteine residue for bridging.

Human granulysin [38] and its porcine counterpart, NK-lysin [39] are effector proteins of the innate immune system found in the granules of T-lymphocytes and NK (natural killer) cells, co-localizing here with perforin. They possess antimicrobial activity against a whole list of micro-organisms, including bacteria, fungi and protozoa [40–44]. Most notably, both are also active against intracellular mycobacteria [43,45]. Here, perforin appears to be necessary to pave the way for the entry of granulysin into infected cells to allow lysis of the intracellular microbes. For granulysin, induced apoptosis has been reported to be the major mechanism of cell death [46–48]. Different from most other SAPLIPs, both proteins act apparently pH-independently. Recently, homologues of NK-lysin have been identified in bovine and horse T-lymphocytes [49] (GenBank® accession number AAN10122) with sequence identities of 59 % for the bovine protein, bo-lysin, and 69 % for the horse NK-lysin-like protein respectively. These findings enable the establishment of an animal model for the activity of these effector proteins – since the usually exploited model organism, mouse, does not express an orthologue.

However, even SAPLIPs which fulfil similar functions, in this case antimicrobial and cytolytic activity, do not show pronounced sequence identities or conserved motifs which could give hints to their mechanism. The amoebapores of the pathogenic parasite *E. histolytica* [50,51] are evidently the protozoan counterparts of NK-lysin and granulysin [52] regarding their activities [50,53,54], but the sequence identities between them are below 20 % as shown in Figure 4(A). Owing to their cytolytic activity, the amoebapores are the major pathogenicity factors of the parasite [55,56], even though their main function *in vivo* is presumably of bacteriolytic manner, as they have been shown to interact with phagocytosed bacteria [57]. In contrast with the mammalian defence proteins, the amoebapores form channel-like pores of defined size rather than perturbing the membrane order by electrostatic processes [58]. Some other SAPLIPs identified in bacteria-feeding organisms presumably fulfil a similar antimicrobial function, e.g. the NK-lysin-like protein from *Fasciola hepatica* [59,60], clonorin from *Clonorchis siniensis* [61], the naegleriapores of *Naegleria fowleri* [62,63], several putative proteins detected in *Caenorhabditis elegans* [64] or the recently described prosaposin-like protein of *Trichinella spiralis* [65]; however, definite evidence for their function *in vivo* is lacking, even if *in vitro* characterization of most of these proteins suggest an antimicrobial function.

Interestingly, the circular permuted SAPLIP domain of aspartic plant proteases also potently exerts membrane permeabilizing activity by itself. As this domain is cleaved from the catalytic domain, and accordingly is absent in the mature protein, the hypothesis arose that it might also act independently as a part of the plant defence mechanism against pathogens [66]. Like other SAPLIPs, it prefers negatively charged lipids and low pH. Another possible and perhaps additional function suggested for this domain is the targeting of the protease to vacuoles by membrane interaction [67], which may also include the exit from the endoplasmic reticulum [68].

Switching generally from the autonomous SAPLIPs to the multidomain proteins, in which the SAPLIP domain is just one player in the team work of domains, much less is known of their single activities. This is true, for example, for the lipid interaction of the SAPLIP domain of human acyloxy acylase, a lipase acting on LPS (lipopolysaccharide) and several glycosphingolipids. After proteolytic processing of the precursor, the SAPLIP domain and the large catalytic domain appear to be connected by a disulphide bond. According to data obtained from recombinantly expressed variants, the SAPLIP domain contributes to LPS recognition, as well as intracellular targeting and catalytic function [69]. Interestingly, the aforementioned GPI inositol deacylase characterized in *T. brucei* shares the domain organization with the acyloxy acylase and possesses 35 % identity with the human enzyme [70]. Another human multidomain enzyme, acid sphingomyelinase, is not only stimulated by saposins, but also contains a homologous domain itself [2]. Mutational analysis suggests an activator-like effect of this domain and perhaps additionally an influence on enzyme stability [71].

The rapidly increasing mass of sequence data of growing genome projects reveal an unanticipated number of SAPLIP sequences in various organisms. Since the definite function of a SAPLIP is not deducible from mere sequence data, most of the novel sequences are of unknown or only assumed function. Even in the primitive amoebozoon *E. histolytica*, 16 SAPLIP sequences in addition to the well-characterized amoebapores have been identified, partly inherent in putative proteins up to 1000 amino acids with unknown function ([72], and J. Winkelmann, M. Leippe and H. Bruhn, unpublished work). In the slime mould *D. discoideum*, the genome of which also bears 11 genes encoding SAPLIP-sequences, a protein complex called cell-counting factor has been characterized, which is involved in the regulation of the number of cells flocking together to become the fruiting body. One of its components termed countin also contains a SAPLIP domain [73]. This protein is able to bind to cells and to potentiate the pulses of the aggregation trigger cAMP [74].

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To complicate further the puzzle of SAPLIP function, recent data indicate that some parts fit into several different spaces. The saposins, known to be co-factors in sphingolipid catabolism as already mentioned, were shown to represent a hitherto missing link in antigen representation of lipids. They are able to extract several different lipids from membranes and load them on to the antigen-presenting molecules CD1d and CD1b [75–77]. In the case of digalactosylceramide, the saposins are also required for processing before the molecule may serve as an antigen. Moreover, their precursor, prosaposin, plays a role in the field of apoptosis regulation. First, it was shown that it possesses neurite outgrowth activity [78], presumably by triggering a PI3K (phosphoinositide 3-kinase)-dependent signal cascade after binding to a specific receptor [79,80]. The responsible entity was mapped to the N-terminal portion of saposin C, and peptides derived from it are known as prosaptides [81] Recently, Misasi et al. [82] discovered the extension of the area of prosaposin operation to cells of non-neurological origin. The protein prevents TNFα (tumour necrosis factor α)-induced cell death by stimulating signal cascades in which signal-regulated protein kinases are involved. In a similar way, saposin C itself was shown to prevent apoptosis in prostate cancer cells [83]. Interestingly, neurite outgrowth activity has also been identified for a larger human SAPLIP variant called MSAP [MIR (myosin-regulatory-light-chain-interacting protein)-interacting saposin-like protein] [84], formerly described as integral membrane protein TMEM4 (transmembrane protein 4) with unknown function [85]. MSAP inhibits MIR, a protein which stimulates the ubiquitination of the myosin light chain regulatory protein by direct interaction. The resulting enhanced concentration of the regulatory protein stimulates neurite outgrowth.

The most striking features of these novel SAPLIP activities are that they are apparently independent of lipid interactions. Thus an exciting question arising is whether the SAPLIP fold itself is not only ideally suited for lipid binding, but whether it is also a versatile scaffold for protein–protein interactions. Regarding MSAP, the binding to its partner might be influenced by the two insertions of 30 and 40 amino acid residues respectively into the assumed SAPLIP fold. Thus an alternative explanation would mean that these novel activities might rely mainly on extensions of the SAPLIP fold, e.g. the interdomain regions in prosaposin. Since no interacting surfaces are mapped so far, this remains an open question. Yet another example of the versatility of prosaposin is its complex formation with the aspartic protease procathespsin D, as shown recently, which enhances autoactivation of procathepsin D, as well as cleavage of prosaposin into the single domains [86]. The physiological function of the complex is yet not clear, but a role in mannose 6-phosphate-independent lysosomal targeting was suggested. Notably, the lysosomal trafficking of saposins has been reported to be dependent on the transmembrane Golgi protein sortilin, rather than on the mannose 6-phosphate receptor [87,88].

A well-known example for proteins acting in different functional contexts are crystallins of the eye lens, which have often been recruited from pre-existing proteins. And unsurprisingly, SAPLIP domains participate here also. In the jellyfish *Tripedalia cystophora*, the J3-crystallin consists of two adjacent saposin domains [89].

Apparently, the better and more rapid the methods become to assign function to a novel protein, the more complex are the resulting networks where several cellular reactions and pathways are connected by the action of multifunctional proteins.

STRUCTURE AND MECHANISTICS

Beginning on the lowest structural level, the protein sequence, SAPLIPs are highly diverse, mostly with identities below the so-called threshold zone of 25–30 % identity usually taken to define homology. The unrooted tree of representatives of known proteins (of human origin, whenever sequences of several species are available) in Figure 2(A) illustrates the degree of sequence variability. Most of the sequences spring up nearly directly at the centre and, except for true isoforms or orthologues, hardly any branch with several sequences is detectable. Nevertheless, from the corresponding sequence logo (Figure 2B), the conserved features become apparent, namely the distribution of hydrophobic amino acids which build the protein core and the six invariable cysteine residues that form the disulphide bridges which are responsible for the extraordinary stability of the domains. Combined with the common position of the predicted secondary structure of four to five α-helices, these are sufficient features to result in a common fold, as expected for a family of homologous proteins.

Even if knowledge of the three-dimensional structure will mostly not reveal unknown physiological functions, at least the mechanism of known activities can be elucidated by structural analysis. At the present time, six structures of SAPLIPs are solved, beginning with NK-lysin in 1997 [90]. All show the same α-helical fold of five helices connected by a conserved pattern of three disulphide bonds, with granulysin as the only exception, bearing only five cysteine residues (and only four in the mature form) which are accordingly involved in no more than two bridges. The structures of NK-lysin, granulysin [91], saposin C [92] and even the permuted SAPLIP domain of the plant protease phytepsin [67] are well superimposable and are nearly identical, showing the five helices packed in two leaves (Figure 3). The first leaf comprises the short helices 4 and 5 packed nearly perpendicularly against helix 1; the other one consists of helix 2 and 3. Interestingly, the disulphide bonds connect cysteine residues exclusively within one leaf, not between the leaves. The angle of helices 1 and 2 towards each other is somewhat twisted in amoebapore A [93], but only saposin B shows a surprisingly different assembly: it crystallizes as a dimer in which the compact monomers seen in the other structures are opened towards each other in a V shape. In this way, they leave a large cavity that is ready to bind lipids [94]. For full lipid extraction, as necessary for stimulation of the cerebroside sulphate hydrolysis reaction by arylsulphatase A, the tips of the monomers, which constrict the cleft, are flexibly opened.

Dimerization is a common scheme realized in many SAPLIPs, but with different strategies. The water-insoluble SP-B, as aforementioned, utilizes a seventh cysteine residue for intermolecular bonding. Also the pore-forming amoebapore A is a dimer in its active state. Here, the arrangement is stabilized by electrostatic interactions involving a sole histidine residue, which is conserved between the amoebapore isoforms. One face of the dimer is exclusively hydrophobic, allowing the insertion into the membrane. Upon lipid interaction, the oligomerization is extended to ring-like pores with channel characteristics [58]. According to an experimentally supported model, the inner face of the pore is lined by polar residues, whereas the outer surface interacts with the hydrophobic lipids. In contrast, the functionally related NK-lysin permeabilizes membranes in a monomeric state by an electrostatic process called molecular electroporation [95], essentially similar to its human homologue granulysin. In both proteins, there are no hydrophobic regions on the surface ready to enter the membrane, but there are a lot of positively charged amino acids. In NK-lysin, mainly those of helix 3 induce an electric field strong enough to perturb the order of the lipid packing, leading to permeabilization. Granulysin is an even more positively charged protein with a net charge of $+11$, with mainly arginine residues clustering, especially at the tip of helices 2 and 3. These charges presumably form the initial contact with the membrane. However, in order to enable interaction with the hydrophobic core of the

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Figure 2 Diversity and conserved features of SAPLIP sequences

(**A**) Unrooted tree of the experimentally characterized SAPLIPs mentioned in the text. The early divergence of the single sequences reflects their diversity. AP-like, amoebapore-like. (**B**) The corresponding sequence logo reveals conserved features. The invariable cysteine residues are displayed in yellow, hydrophobic amino acid residues are coloured green, polar residues are purple, negatively charged ones are red, positively charged ones are blue and others are grey.

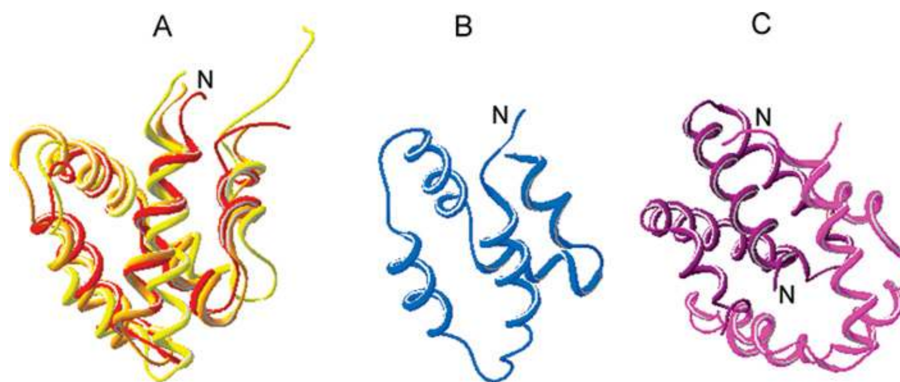

Figure 3 Structures of SAPLIPs

(**A**) Superposition of NK-lysin (red; Protein Data Bank code 1NKL), granulysin (orange; Protein Data Bank code 1L9L) and saposin C (yellow; Protein Data Bank code 1M12). (**B**) Structure of amoebapore A (Protein Data Bank code 1OF9), the orientations of helices 1 and 5 are twisted compared with (**A**). (**C**) Structure of a saposin B dimer (Protein Data Bank code 1N69) showing the open conformation.

bilayer at all, a rotation of the two leaves of the protein towards each other, called ‘scissoring’, is postulated which generates hydrophobic patches on the protein surface [91]. Altogether, it appears that the ultimate function of these defence proteins, namely membrane permeabilization, is achieved by different mechanisms. A fact which is reflected by their diverse sequences, as apparent in an alignment of known SAPLIPs with evidently antimicrobial or cytolytic activity (Figure 4A). No common scheme regarding the

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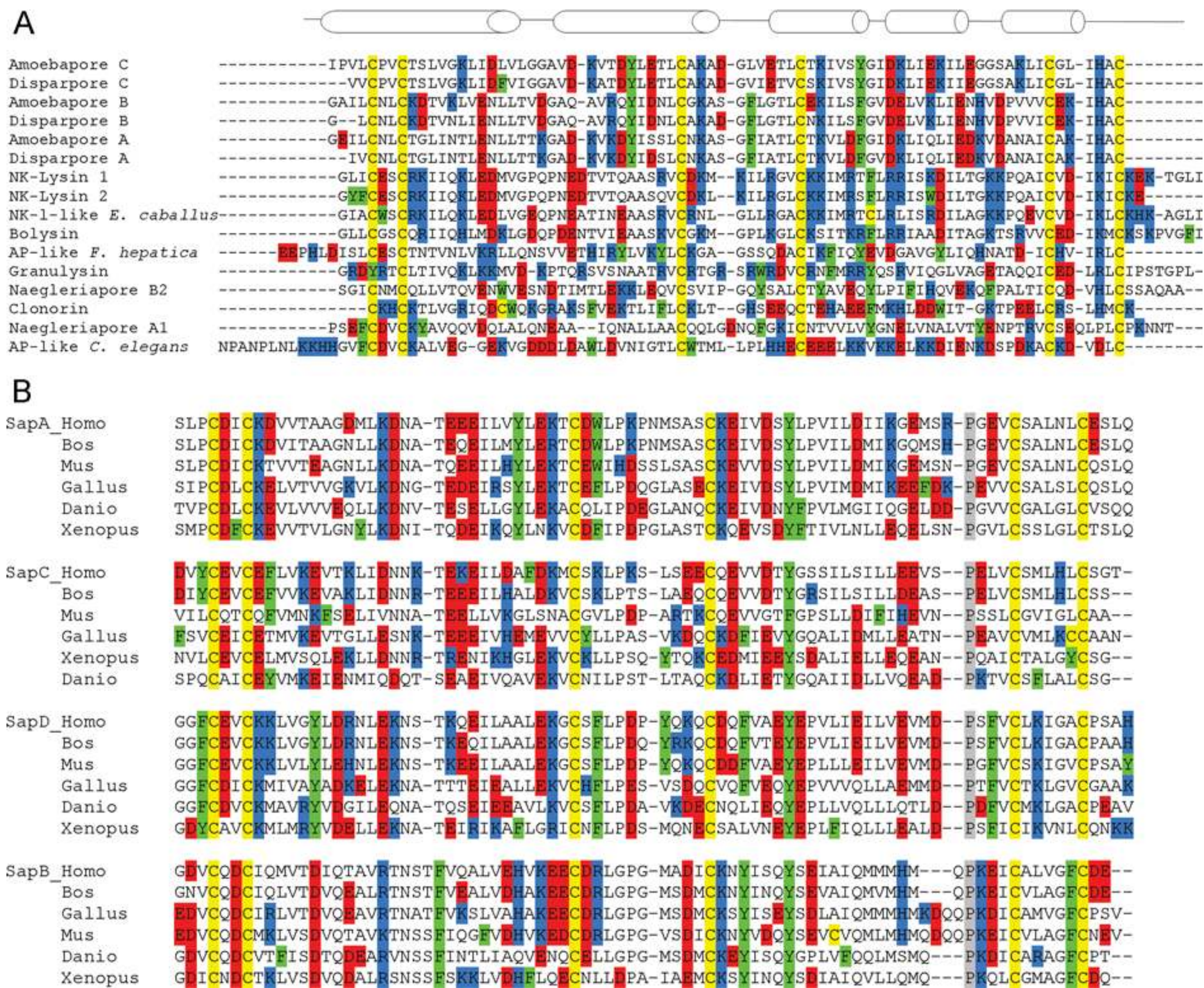

Figure 4 Sequence alignment of selected SAPLIPs

Negatively charged amino acid residues are coloured red, positively charged ones are blue and aromatic residues are displayed in green. (**A**) Proteins with antimicrobial activity. The position of the α-helices of NK-lysin is shown at the top. AP-like, amoebapore-like; *E. caballus*, *Equus caballus*. (**B**) Saposin isoforms from different species: *Homo*, *Homo sapiens*; *Bos*, *Bos taurus*; *Mus*, *Mus musculus*; *Gallus*, *Gallus gallus*; *Xenopus*, *Xenopus laevis*; *Danio*, *Danio rerio*.

characteristics of the protein surface, such as charge distribution or conserved positions of amino acids with functional groups or an aromatic side chain, is detectable. In contrast, the saposins, which extract and present lipids from various membranes for enzymatic processing or antigen presentation, are all mainly negatively charged proteins without pronounced electropositive patches on their surface (Figure 4B). Nevertheless, only two amino acid residues with functional groups are strictly conserved between the saposin isoforms, namely a tyrosine residue in the loop between helix 3 and 4, and a proline residue at the beginning of helix 5. Whereas the proline residue might be of structural benefit at this position, since many SAPLIPs possess a proline residue at the end of that loop (position 86 in the sequence logo of Figure 2), both of the known saposin structures can explain a pivotal functional role of the tyrosine residue. In saposin B, it is part of the seal of the cavity in a closed conformation of the monomers. Saposin C, on the other hand, possesses three clusters of glutamate residues with elevated pK_a values, which are supposed to be involved in membrane binding [92]. The tyrosine residue is adjacent to one of the clusters and might, as an aromatic residue, interact either with the hydrophobic part of the lipids or with their sugar headgroups. The negatively charged surface of the saposins accounts also for their pH-dependence. In saposin C, a number of the acidic amino acid residues have to be protonated before a lipid interaction can take place. The similar feature, the markedly increased activity with decreasing pH, is caused by a different mechanism in the amoebapores: the sole histidine residue functions as a pH-dependent activity switch, allowing the electrostatic interactions that are crucial for dimerization only at low pH [93,96].

Most notable is the structure of a circular bacterial protein, bacteriocin AS-48, which possess the same fold, but without any disulphide bridge, and which is also able to bind lipids and to display antimicrobial activity [97,98]. This case might be an example of convergent evolution, since the sequence does in no way resemble SAPLIPs, but nevertheless accentuates the ability of the SAPLIP fold itself to be a sufficient prerequisite for proteins to interact with lipids. This fact matches in turn the diversity of SAPLIP sequences, leading to the variety of defined functions on the basis of lipid interaction. Interestingly, bacteriocin AS-48 is also postulated to act in a dimeric state, in which the hydrophobically stabilized dimers in solution change the orientation of the monomers to expose the hydrophobic face upon lipid binding [99]. Dimerization of paralogous proteins is commonly

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used as a means to increase the variety of interacting molecules and, particularly for antimicrobial effectors, to increase thereby the number of possible specificities [100]. Hence, this process might be a reason also for the variety of novel SAPLIPs found in genomes of bacteria-consuming organisms like *D. discoideum* or *N. fowleri*.

Another structural feature of the proteins not to be underestimated is glycosylation. Several mature SAPLIPs are glycosylated, i.e. all saposins, the domains of acyloxyacyl hydrolase and phytepsin, but this modification could not be related to protein activity for any of them. An interesting assumption arose from the finding that proteins which are released from the identical precursor were detected in different amounts in *N. fowleri* [62]. Strikingly, they differ in the glycosylation state. Hence, glycosylation may define the lifetime or stability of the protein in the cell rather than determine its activity. Another assumption regarding the stability of SAPLIPs is their protection by complex formation with lipids or proteins, as suggested for the different resistance against proteolytic degradation for saposins [86].

A TRIAL OF ORDER

The family of saposin-like proteins is a highly diverse gathering of evolutionary related loners, not only with regard to their cellular function, but also concerning the molecular mechanisms used to create these activities. Their common feature, the interaction with lipids, builds the framework for a wide field of possible and surprising activities, revealing the creativity of Nature and forming a playground for the creativity of scientists, as the saposins revealed. Some common strategies, such as dimerization, glycosylation or pH-sensitivity, as well as unique lipid specificities, are the basis for special tasks. But the ultimately defined cellular functions are often driven by interaction partners other than lipids, from lipid-degrading enzymes through lipid-presenting molecules to entirely lipid-independent binding events. The consideration of these interactions is still in its infancy and might reveal even more exciting extensions of their operational areas. The development of bioinformatic methods to predict the observed activities could assist to pinpoint functions. Hopefully, the identification and characterization of novel family members as well as of the older-known members by biochemical and bioinformatic means will lead a step further to a better understanding of the versatile functions of this protein family.

As this review cannot be a complete summary of all serious publications in the field, I apologize to all those authors whose results are not mentioned. Special thanks to M. Leippe and J. Winkelmann for fruitful discussions. H.B. is a recipient of a HWP fellowship of the University of Würzburg.

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