REVIEW

# PLP-dependent enzymes as entry and exit gates of sphingolipid metabolism

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**Abstract: Sphingolipids are membrane constituents as well as signaling molecules involved in many essential cellular processes. Serine palmitoyltransferase (SPT) and sphingosine-1-phosphate lyase (SPL), both PLP (pyridoxal 5′-phosphate)-dependent enzymes, function as entry and exit gates of the sphingolipid metabolism. SPT catalyzes the condensation of serine and a fatty acid into 3-keto-dihydrosphingosine, whereas SPL degrades sphingosine-1-phosphate (S1P) into phosphoethanolamine and a long-chain aldehyde. The recently solved X-ray structures of prokaryotic homologs of SPT and SPL combined with functional studies provide insight into the structure–function relationship of the two enzymes. Despite carrying out different reactions, the two enzymes reveal striking similarities in the overall fold, topology, and residues crucial for activity. Unlike their eukaryotic counterparts, bacterial SPT and SPL lack a transmembrane helix, making them targets of choice for biochemical characterization because the use of detergents can be avoided. Both human enzymes are linked to severe diseases or disorders and might therefore serve as targets for the development of therapeutics aiming at the modulation of their activity. This review gives an overview of the sphingolipid metabolism and of the available biochemical studies of prokaryotic SPT and SPL, and discusses the major similarities and differences to the corresponding eukaryotic enzymes.**

**Keywords: sphingolipids; SPL; SPT; PLP; structure; function**

## Introduction

Sphingolipids are ubiquitous constituents of cell membranes, and their metabolites play the role of signaling molecules in eukaryotic cells.[1–4] They are strictly found in eukaryotes, with the exception of some bacterial genera and viruses.[5] Sphingolipids are composed of three modules: a sphingosine backbone called long-chain base (LCB), a polar head group, and an amine-linked fatty acid chain (Fig. 1). The common structure of the sphingosine backbone, 2-amino-1,3-diol-alkane, is unique in nature and possesses two chiral centers at carbon atoms 2 and 3. The naturally occurring diastereoisomers of sphingolipids have the (2D,3D) [or D(+)-*erythro*-] configuration.[6] The sphingosine backbone and the fatty acid chain are variable in length, degree of unsaturation, and substitution. Sphingolipids can either be produced via the degradation of existing sphingolipids or *de novo* in a synthesis pathway initiated by serine palmitoyltransferase (SPT), an enzyme located in

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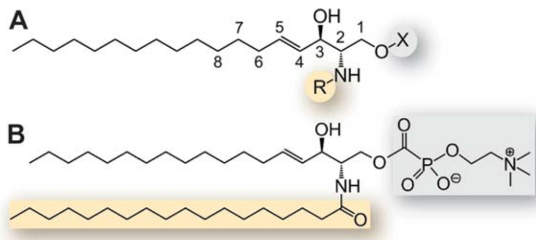


**Figure 1.** Chemical structure and diversity of sphingolipids. A: Sphingolipids d18:1$^{\Delta 4trans}$. The module R is an amine-linked fatty acid in sphingomyelin (SM) and ceramide (CER) and an H in sphingosine (SPH) and sphingosine-1-phosphate (S1P), whereas X is either a phosphocholine in SM (see panel B), an H in CER and SPH, or a phosphate group in S1P. Phytosphingolipids carry a hydroxyl group at position 4, whereas 1-deoxy-sphingoid bases (DSBs) lack the hydroxyl at position 1. B: Sphingomyelin d18:1$^{\Delta 4trans}$/18:0. For details about nomenclature, see Pata *et al*.[4] Although the singular is used in the text, each class of sphingolipid is composed of a variety of molecules differing in length, degree of unsaturation, and substitution of the fatty acid and sphingolipid chains.

the endoplasmic reticulum (ER)[2] (Figs. 2 and 3). SPT catalyzes the condensation of serine and palmitoyl-CoA to the first sphingolipid of the synthesis pathway, 3-keto-dihydrosphingosine (3-keto-dihydro-SPH)[9] (Fig. 2). This reaction is the entry point of the sphingolipid metabolism and its rate-limiting step. The enzyme 3-keto-dihydro-SPH reductase (KDS) produces dihydrosphingosine (dihydro-SPH). N-Acylation of dihydro-SPH generates dihydroceramide (dihydro-CER),[10] which is subsequently desaturated to form ceramide (CER),[11] a sphingolipid metabolic hub. A second route for the generation of CER is provided by the breakdown of complex sphingolipids by specific hydrolases[12] and of sphingomyelin (SM) by specific sphingomyelinases (SM'ases).[13] CER, bound to CER transfer protein (CERT) or integrated to vesicles, is transported to the Golgi and serves as building block for glycosphingolipids (Glyco-SPHL),[14] ceramide-1-phosphate (C1P),[15–18] and SM[19,20] (Figs. 2 and 3). CERT consists of ER- and Golgi membrane-interacting domains and a START (standing for steroidogenic acute regulatory protein-related lipid transfer) domain able to bind CER.[21,22] SM and Glyco-SPHL are relocated to the plasma membrane (PM) by vesicular transport, whereas various Glyco-CER are specifically transported from the Golgi to the inner leaflet of the PM bound to glycolipid transfer protein (GLTP).[23–25] CER deacylation by various ceramidases (CER'ase) produces the single-chain sphingolipid sphingosine (SPH).[26] SPH is the substrate of sphingosine kinase (SK),[27] which produces sphingosine-1-phosphate (S1P). S1P can be reversibly dephosphorylated by S1P phosphatase (S1PP)[28] or irreversibly degraded by S1P lyase (SPL) in one long-chain aldehyde, hexadecenal (Hex), and one polar compound, phosphoethanolamine (PE)[29] (Fig. 2). The cleavage of S1P catalyzed by SPL is the exit point of the sphingolipid metabolism. PE can serve as building block for the synthesis of certain phospholipids, for example, phosphatidylethanolamine (PtE),[30] which might in turn be involved in further signaling events.[31]

The specific inhibition or activation of the enzymes of the sphingolipid metabolism is crucial to control the concentration and the site of formation or degradation of sphingolipids, which play essential roles in living cells.[32] Together with cholesterol and SM Glyco-SPHL form detergent-resistant membrane microdomains called "lipid rafts," which are involved in the clustering of membrane proteins that are essential during signal transduction, in vesicular budding, and in the entry of pathogens.[33] In

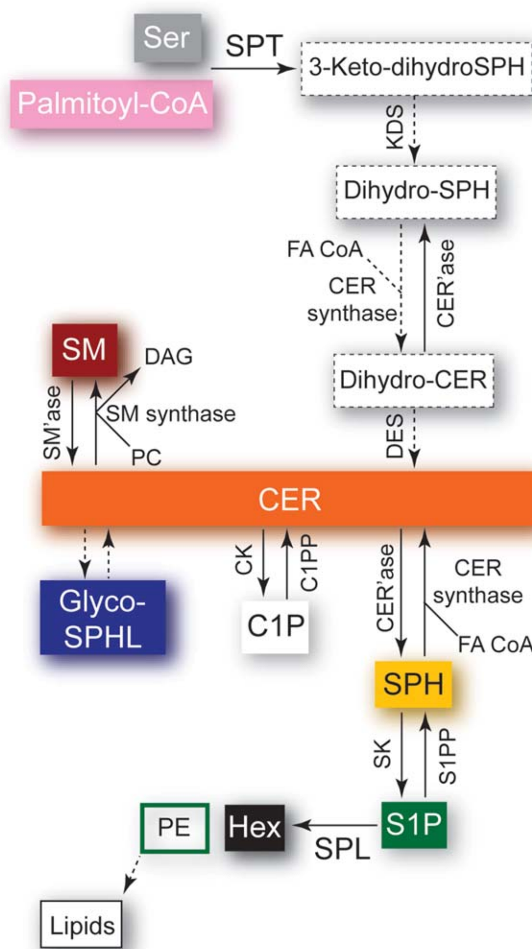


**Figure 2.** Overview of the sphingolipid metabolism. The direct pathway from entry to exit, that is, from SPT to SPL, goes from the gray and purple boxes until the black and green boxed rectangles. See text for abbreviations. FA CoA, fatty acyl-Coenzyme A; DES, dihydro-SPH desaturase; PC, phosphatidylcholine; DAG, diacylglycerol; CK, CER kinase; C1PP, C1P phosphatase. See Figure 3 for the cellular location of the reactions. Dashed boxes and arrows correspond to the respective dashed arrows in Figure 3.

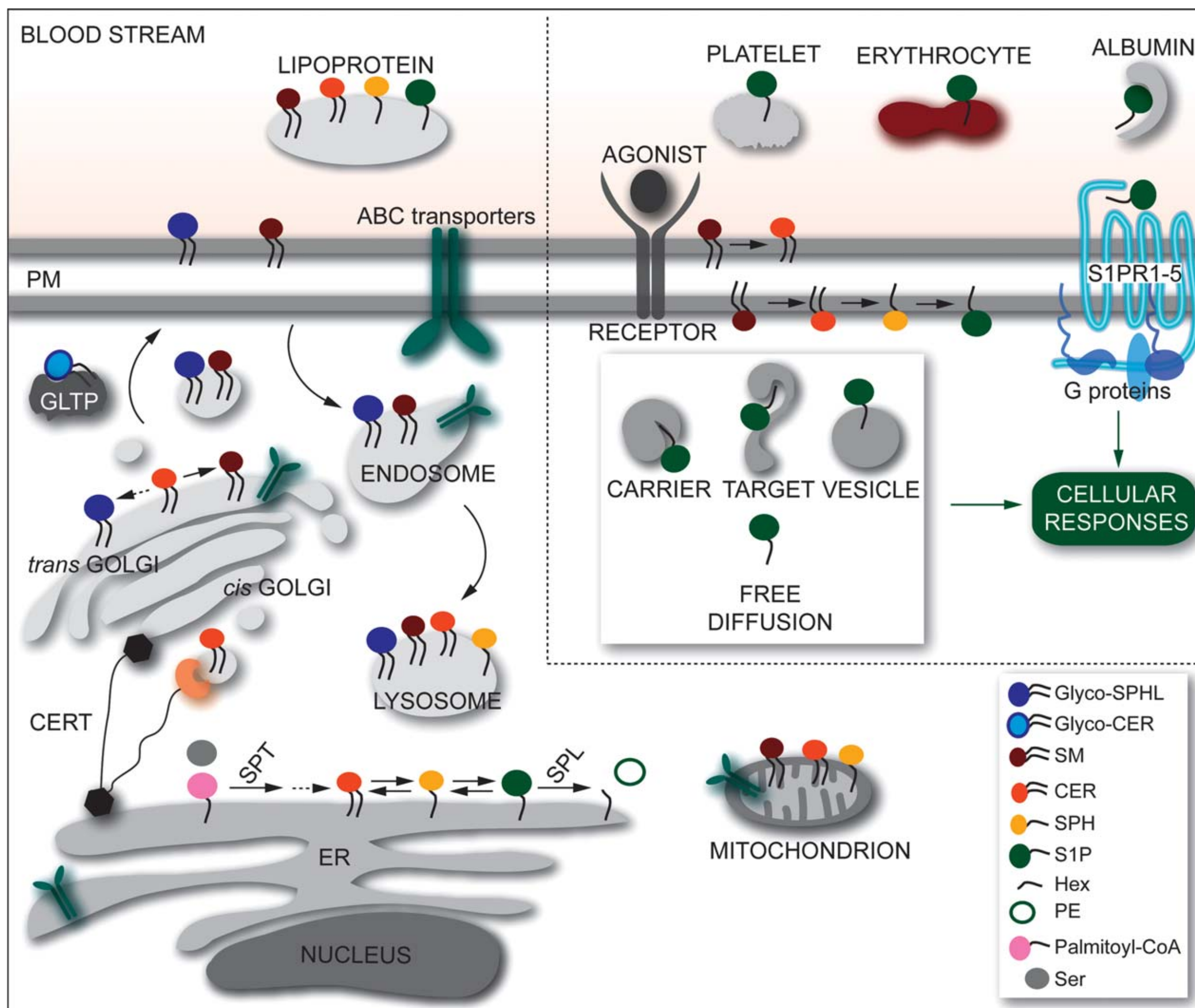


**Figure 3.** Cellular locations of sphingolipids and S1P signaling modes. The color code for the various sphingolipids is the same as in Figure 2. The dashed arrows encompass reactions that are not displayed for clarity (see Fig. 2). The left part of the scheme illustrates the formation and degradation of sphingolipids in the ER, the subsequent transport of CER to the Golgi via CERT, the formation of Glyco-SPHL and SM therein, their transport to the PM via GLTP and vesicles, and their transport into the blood and their turnover via endosome. The ER- and Golgi membrane-interacting domains of CERT are represented as black hexagons, the CER-binding START is depicted in light orange, whereas the linkers between each domains are represented as black lines. In the cell, some sphingolipids also reside in the mitochondrion membrane. ABC transporters responsible for the export, import, or flipping of various sphingolipids were identified in different cellular compartments.[7,8] The upper right part of the scheme depicts the extracellular transport of S1P in the blood, the stimulus-driven production of S1P (for example via binding of an antagonist to its cognate receptor), the putative intracellular transport modes of S1P, and the S1P extracellular signaling mode via the specific GPCRs $S1PR_{1–5}$.

addition, sphingolipids can directly interact with domains of membrane proteins, thereby modifying their activity.[34] Moreover, some sphingolipids are "bioactive," that is, a change in their concentration brings about functional consequences.[1,3] The enzymes of the sphingolipid metabolism are biochemically interconnected, meaning that an increase in synthesis of one compound is related to a decrease in the other. Such interconnectivity increases the complexity of the signaling pathway and allows the cell to modulate the levels of a signaling molecule and its antagonist (Fig. 2). In addition, the location and transport of metabolites and enzymes, allowing pathways to operate in parallel, add another level of complexity (Fig. 3). Relative amounts of sphingolipids and imbalances in their metabolism have been univocally linked to hepatitis C,[35] atopic dermatitis,[36] rheumatoid arthritis,[37] allergic response, anaphylaxis and asthma,[38] multiple sclerosis, diabetes mellitus type I, psoriasis, Alzheimer's disease and atherosclerosis,[39] cancer,[3,36,40–42] and hereditary sensory and autonomic neuropathy type 1 (HSAN I).[3] More specifically, Glyco-CER is involved in drug resistance.[43] SPH modulates the activity of various kinases involved in the regulation of the cytoskeleton, endocytosis, cell cycle, and apoptosis,[44] CER

regulates stress-induced senescence[45] and apoptosis,[46] whereas C1P is involved in the inhibition of apoptosis,[3] inflammation, vesicular trafficking, and phagocytosis.[16,47] S1P is involved in the regulation of proliferation, cell growth, cell survival, cell migration, inflammation, angiogenesis, and resistance to apoptosis.[40,48,49] It is a tumor-promoting agent antagonist to the cellular response primed by CER and SPH. Elevated amounts of S1P are found in cancer cells.[50] Because of its physicochemical properties, micromolar concentrations of S1P can be found in the cytosol (Fig. 3), unlike the fully insoluble CER, SPH, and C1P.[51] A recently identified intracellular target of nuclear S1P is histone deacetylases, suggesting that S1P is able to regulate gene transcription.[52] In addition to its intracellular signaling mode, S1P can act extracellularly in an autocrine or a paracrine way by binding to five cognate receptors[49,53,54] (Fig. 3). Consistently with its function in signaling, the concentration of S1P is tightly regulated by SK, S1PP, and SPL (Figs. 2 and 3).

SPL and SPT belong to the superfamily of pyridoxal 5′-phosphate (PLP)-dependent enzymes. PLP is covalently bound to the enzyme via a Schiff base between the aldehyde group of the PLP and the side-chain amine of a nearby lysine. Although they carry out two different reactions, SPL and SPT share a common fold, and their enzymatic mechanisms are in many aspects comparable. This article will review the recent advances in the structural and functional analysis of prokaryotic homologs of SPT and SPL, compare the two enzymes and relate them to their mammalian counterparts.

## Structure and Function of SPL

The SPL gene has been first identified in budding yeast (*Saccharomyces cerevisiæ*),[55] and the human gene was cloned in 2000.[36,56] Yeast and mammalian SPLs are predicted single-pass ER membrane proteins with the transmembrane helix residing within the first fifth of the protein,[57] but may localize to other organelles.[36] Their N-terminus is situated in the ER lumen, where it undergoes glycosylation in yeast[57] and their active site is exposed to the cytosol.[58] Yeast SPL, called Dpl1p (dihydrosphingosine-1-phosphate lyase 1), is involved in resistance to heat stress[59,60] and nutrient deprivation,[61] in calcium homeostasis,[62,63] as well as in endocytosis.[64] In mammals, SPL plays a crucial role in the migration of T-cells from lymphoid tissues by maintaining the S1P gradient necessary for T-cells to egress from the lymph nodes into the blood stream.[36,65] Similarly, SPL plays a central role in neutrophil egress from blood to tissues.[66] Further, SPL has recently been confirmed as a central and crucial regulator of lipid homeostasis in mice liver.[67] Disruption of SPL is linked to resistance to the chemotherapeutic agents etoposide and doxorubicin.[68] SPL activity is also linked to an increase in CER levels in response to stress factors and is involved in apoptosis.[69] The SPL gene is a transcriptional target of platelet-derived growth factor (PDGF),[70] which regulates critical cellular events like cell growth, differentiation, and migration and is involved in cancer, inflammation, wound healing, and production of the extracellular matrix.[71] The human *SGPL1* gene is ubiquitously expressed with the exception of erythrocytes[72] and platelets,[73] and its expression is linked to tissues undergoing rapid cell turnover, like the intestine, colon, thymus, and liver.[36,74] *SGPL1* gene expression is downregulated in colon cancer,[68,75] whereas it is high in enterocytes under normal conditions, where the protein is involved in metabolizing dietary sphingolipids.[36,76] Notably, SPL is upregulated in certain malignant tissues, for example, in ovarian cancers[77] as well as in the skin of patients suffering from atopic dermatitis,[36,78] complicating the interpretation of its precise role in diseases. SPL has been recently directly linked to acute lung injury and might therefore be a target for the development of therapeutics.[79] Additionally, SPL is involved in developmental events, especially in vascular maturation.[36,74] Recently, the role of SPL in DNA repair and radioprotection was demonstrated.[80]

SPL is a member of the carbon–carbon lyase subclass of aldehyde-lyases (SPL, EC 4.1.2.27).[36] SPL cleaves the amphiphilic substrate S1P between carbon atoms 2 and 3, yielding one hydrophobic and one hydrophilic product. Interestingly, Saba and coworkers have recently shown that the hydrophobic product of the cleavage of S1P, *trans*-2-hexadecenal, has a signaling function in mammalian cells.[81] This indicates that the biological function of SPL is not restricted to the degradation of S1P but extends to the direct production of another bioactive lipid. This very important finding highlights the importance and the complexity of SPL in cell signaling events.

The full mechanism of accommodation and cleavage of the substrate and of release of the hydrophilic and hydrophobic products is not yet elucidated. The protein may contain a hydrophobic recognition site that interacts with the hydrophobic moiety of the substrate, whereas the hydrophilic head might be accommodated within the active site and can then be cleaved.[6] Recent advances through biochemical and structural studies, however, have improved our understanding of the function of SPL,[82] although biochemical studies are hindered by the poor solubility of phosphorylated sphingolipids, albeit zwitterionic in nature.[6] Electrostatic and hydrophobic intermolecular interactions are presumably responsible for this property.[83] Triton X-100 is so far considered the best detergent and is widely used to solubilize phosphorylated sphingolipids, because a number of other neutral and zwitterionic

detergents have been reported to inhibit the enzyme,[6,84] even though this needs to be confirmed *in vitro*. The pH optimum of SPL is in the range of 7.2–7.4. SPL shows a high level of specificity toward the stereochemistry of the substrate and cleaves exclusively the naturally occurring D-*erythro* (2D,3D configuration) sphingolipids.[36] On the other hand, the enzyme exhibits very little specificity with respect to the chain length, degree of unsaturation, and substitution, as it cleaves S1P, dihydro-S1P, phyto-S1P (PS1P) [Fig. 1(A)], as well as methyl- or dimethyl-S1P.[74,84,85] When the amino group of the substrate is modified, for example, by acetylation (*N*-acetyldihydro-S1P) or by methylation (*N*,*N*-dimethyl-S1P), the substrate is not cleaved.[84] The classical PLP-dependent enzyme inhibitors semicarbazide, iodoacetamide, and *N*-methylmaleimide inhibit SPL to various extent, presumably by binding to either the cofactor, as shown for semicarbazide,[82] or to a sulfhydryl group from a cysteine.[74] Deoxypyridoxine also inhibits SPL, possibly by competing with PLP.[6,36] There are known substrate analogs that inhibit SPL, for example, 1-deoxydihydrosphingosine-1-phosphonate,[36,86] the *threo* isomer of dihydro-S1P,[87] and 2-vinyldihydro-S1P.[36,88] FTY720 (also called fingolimod) is an immunomodulatory drug phosphorylated by sphingosine kinase 2[89] that targets the S1P receptor 1.[90,91] FTY720 inhibits SPL *in vivo*, when orally administered to mice.[36] THI (2-acetyl-4-tetrahydroxybutyl-imidazole), found in the food colorant caramel colorant III, also inhibits SPL *in vivo*,[36,65] although the mechanism of inhibition is not understood,[74] as that of FTY720. A novel specific inhibitor of SPL, LX2931, is undergoing clinical trials.[37,92] The divalent cations $Ca^{2+}$ and $Zn^{2+}$ have also been reported to inhibit the activity of SPL.[93] It should be noted that biochemical results in the literature have been so far nearly invariably obtained using crude extracts or cell fractionation samples. Given the tight interconnectivity of S1P-related enzymes, this approach might introduce non-negligible bias.

Some bacteria, for example, *Myxococcus xanthus* and *Symbiobacterium thermophilum*, also carry a gene coding for a SPL. The structures of SPL from the thermophile *Symbiobacterium thermophilum* (StSPL) [Fig. 4(A)] and from an N-terminal truncation of *Saccharomyces cerevisiae* (Dpl1p) lacking the transmembrane helix reveal that the protein is a homodimer or a multiple thereof.[82] Interestingly, the naturally occurring form of StSPL lacks a transmembrane domain and the recombinant protein is *in vitro* active, whereas the truncated Dpl1p fails to cleave S1P, dihydro-S1P, and PS1P *in vitro*.[82] The cofactor occupancy is variable in several crystal forms of both SPLs. This indicates that the cofactor is labile in at least one subunit and that the two active sites might therefore carry out different functions. The very N-terminal parts of both proteins are flexible, suggesting that they might play a role in the activity or in the binding of other proteins, as is often the case for flexible regions.

The presence of PLP in the active site allows for straightforward spectrophotometric characterization of wild type (WT) and mutants. Electron transfers as well as the microenvironment of PLP are reflected by enzyme-specific changes in the visible spectrum.[95–101] WT StSPL features a broad peak at 420–460 nm and a shoulder at 360 nm.[82] Upon addition of S1P d18:1$^{\Delta 4trans}$, two transient new peaks at 403 and 420 nm appear. These peaks correlate with the enzymatic activity and were used to perform an analysis of StSPL mutants.[82]

Already in 1974[88] and 1993,[6] a reaction mechanism was proposed based on general knowledge of the biochemistry of PLP-dependent enzymes. Our recently published structural and functional investigations of purified SPLs[82] shed a new light into the mechanism (Fig. 5). In the resting state of the enzyme, the PLP is covalently linked to the Lys residue 311, a complex called internal aldimine (step I). The amino group of the substrate replaces the lysine as PLP-binding partner (step III), and the covalent PLP-S1P adduct, named external aldimine [Fig. 4(B)], undergoes a retro-aldol cleavage by a yet unidentified base. The base was proposed to be C276 (in StSPL, corresponding to C317 in human SPL[56]), but our mutagenesis analysis did not confirm this finding.[82] The formed adduct decomposes into a long-chain aldehyde that is released from the active site (step IV). A quinonoid intermediate (step V) is formed and a proton is stereospecifically incorporated (step VI). PE is then released (step VII) from the second external aldimine (step VI). Our mutagenesis analysis revealed that the highly conserved internal aldimine K311 as well as the neighboring K317 are strictly required for activity. Interestingly, Y482 located at the very C-terminus of the protein and covering the active site entrance is involved in enzymatic activity, suggesting an important role for the C-terminal part of SPL.[82] Likewise, H129, belonging to the neighboring subunit, seems to be important for activity, because mutating it into Ala drastically impairs activity both in StSPL and Dpl1p.[82] Mutating A103 into proline suppresses activity.[82] Surprisingly, this residue is not conserved in human, because it is replaced by T148, suggesting that human SPL might feature a slightly different reaction mechanism or kinetic properties than StSPL and Dpl1p, at least concerning the role of this active-site loop. The same conclusion might be drawn for the inactive Y105F StSPL mutant compared to the corresponding Y174F Dpl1p mutant, which is partly active.[82] Altogether, the recently published results highlight the dual role of lysines in catalysis and in the stabilization of reaction

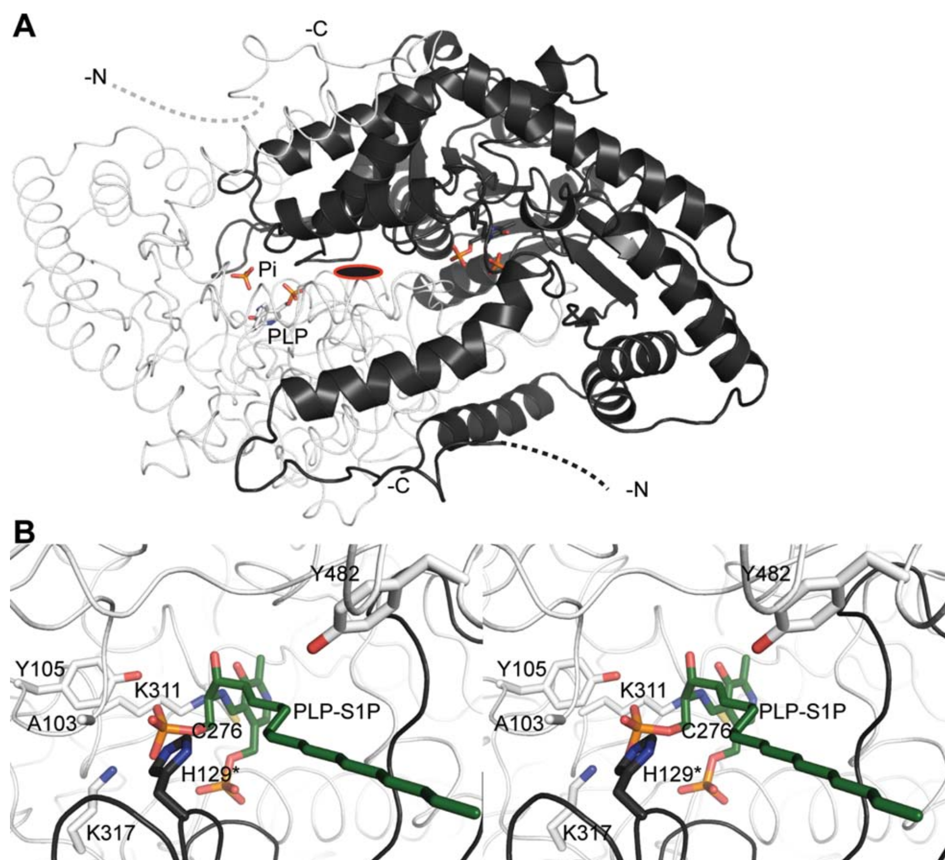


**Figure 4.** X-ray structure of StSPL. A: Dimeric structure of WT StSPL (PDB code 3MAD[82]). Subunit A is colored light gray, the subunit B dark gray, and represented in cartoon and ribbon, respectively. PLP and the covalently bound K311 as well as a phosphate ion appear in ball-and-stick in the color of the corresponding subunit and atom colors and are labeled in subunit A. Residues 1–57 are not visible and are represented by dashed lines in the color of the corresponding subunit. The N- and C-termini are indicated. The subunits are related by a twofold axis perpendicular to the plane of the sheet, indicated as a red-framed black ellipse. B: Stereo representation of the active site of subunit A showing the modeled external aldimine PLP-S1P in green (corresponding to step III in Figure 5). Residues important for activity appear in ball-and-stick in the color of the subunit. The asterisk denotes a residue belonging to the subunit B. Details about the modeling are given in Ref. [82]. This and the following structural figures were prepared with PyMOL.[94]

intermediates,[82] as recently demonstrated for K88 of the PLP-dependent enzyme cystathionine β-synthase from *Drosophila melanogaster*.[96]

## Structure and Function of SPT

As for SPL, the effect of deleting the SPT gene was first studied in yeast.[102] It clearly turned out that SPT is strictly required to produce sphingolipids and that it is crucial for sphingolipid homeostasis.[103] Recently, yeast and human SPT has been shown to be negatively regulated by the yeast integral ER membrane proteins ORM1/2—ORMDL1/2/3 in human—by forming a complex with SPT and with the phosphoinositide phosphatase Sac1, termed SPOTS, and its formation is initiated by a yet unidentified sphingolipid intermediate. This provides a hint on the interconnectivity of the sphingolipid and the phosphoinositide metabolisms[104,105] as well as on the regulation of the rate-limiting step of the sphingolipid metabolism.

In yeast and mammals, SPT is an ER membrane-associated complex.[106] Human SPT is a heteromer composed of the SPTLC1 subunit and either SPTLC2 or 3.[107] SPTLC1 and SPTLC2 share about 21% sequence identity and 45% similarity, whereas SPTLC2 and 3 share 68% identity and 84% similarity.[107] SPTLC2 and 3 contain the PLP-binding residues and are therefore considered as the catalytic subunits.[107,108] They have different acyl-CoA recognition affinities, increasing the variety of the sphingolipids produced. Importantly, despite its lack of cofactor, SPTLC1 is strictly required for activity.[109] A so-called small subunit of the human SPT, named ssSPT, is responsible for maximal activity of the protein.[110] The known isoforms, ssSPTa and ssSPTb, both activate a SPTLC dimer (either 1-2 or 1-3), with different preferences of acyl-CoA chain lengths.[110]

SPT (EC 2.3.1.50) is a member of the α-oxoamine synthase (AOS) subfamily.[102,111,112] The enzyme catalyzes the formation of 3-keto-dihydro-

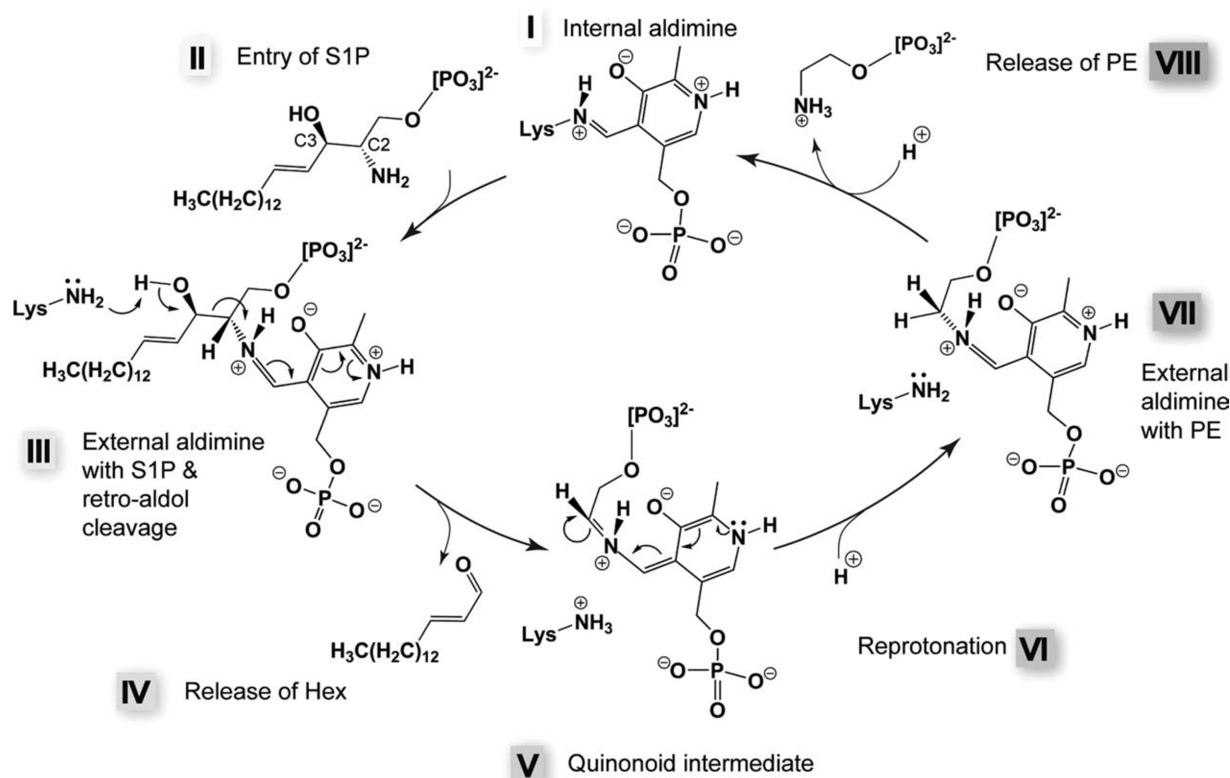


**Figure 5.** Proposed SPL reaction mechanism.[82] In the resting state, a lysine (311 in StSPL) forms a Schiff base with the aldehyde group of PLP (step I). The incoming substrate (S1P in the scheme, step II) triggers transaldimination and replaces the lysine as Schiff base partner of PLP. The resulting S1P-PLP complex is called external aldimine [step III, model presented in Figure 4(B), colored green]. After retro-aldol cleavage (step III), the long-chain aldehyde product (Hex in the scheme) leaves the active site (step IV) and a quinonoid intermediate is formed (step V). A stereospecific reprotonation (step VI) of this intermediate yields the second external aldimine (step VII). We propose that one of the two conserved lysines strictly required for activity carries out the retro-aldol cleavage in step III as well as the reprotonation (step VI). PE is then released (step VIII) and the internal aldimine is re-formed (step I).

SPH from serine and palmitoyl-CoA (Fig. 2), which is the rate-limiting step in sphingolipid synthesis. The bacteria *Sphingomonas paucimobilis* EY2395$^T$, *Sphingomonas wittichii* RW1, *Sphingobacterium multivorum*, and the bacteria-predating bacterium *Bdellovibrio stolpii* carry SPT homologs that have recently been identified and characterized.[113–117] Unlike *Sphingomonas paucimobilis* and *Sphingomonas wittichii* SPTs, which are cytosolic enzymes, the latter two SPTs are peripheral inner membrane proteins. Notably, the physiological function of the sphingolipids in bacteria is unknown.

The structure of *Sphingomonas paucimobilis* EY2395$^T$ SPT (SpSPT) was solved in 2007 at 1.3 Å resolution[117] [Fig. 6(A)]. The first 20 and the last nine residues of the SpSPT sequence are not visible in the electron density map. Unlike eukaryotic SPTs, the enzyme is a homodimer with one active site per monomer. Each active site is composed of residues from both subunits. The role of the presumed $Mg^{2+}$ ions sitting near the PLP [brown spheres in Figure 6(A)] remains to be clarified. Like StSPL, SpSPT produces an absorption spectrum characteristic of PLP-dependent enzymes, featuring a 416- to 426-nm and a 332- to 338-nm peak. The spectrum allows one to follow the equilibrium between the ketoenamine and enolimine tautomers of the Schiff base formed by PLP and K265 [Fig. 6(B)]. The ratio of those tautomers is modified upon addition of serine. A transient 505- to 515-nm peak, characteristic for a quinonoid intermediate, appears upon addition of the fatty acyl-CoA substrate.[116,118,119] In 2009, a structural and functional study of *Sphingobacterium multivorum* SPT (SmSPT) was published (PDB code 3A2B).[113] The structure, solved at 2.3 Å resolution, is similar to that of SpSPT, with minor differences in the N-terminal region.[117] The structure of a third SPT from *Sphingomonas wittichii* RW1 (SwSPT) was solved in 2010 at 2.1 Å resolution by Campopiano and coworkers.[114] SwSPT shares a similar fold with SpSPT and SmSPT, and its active site is nearly identical to that of SpSPT. Interestingly, the expression of the SwSPT gene seems to be coupled with a specific acyl

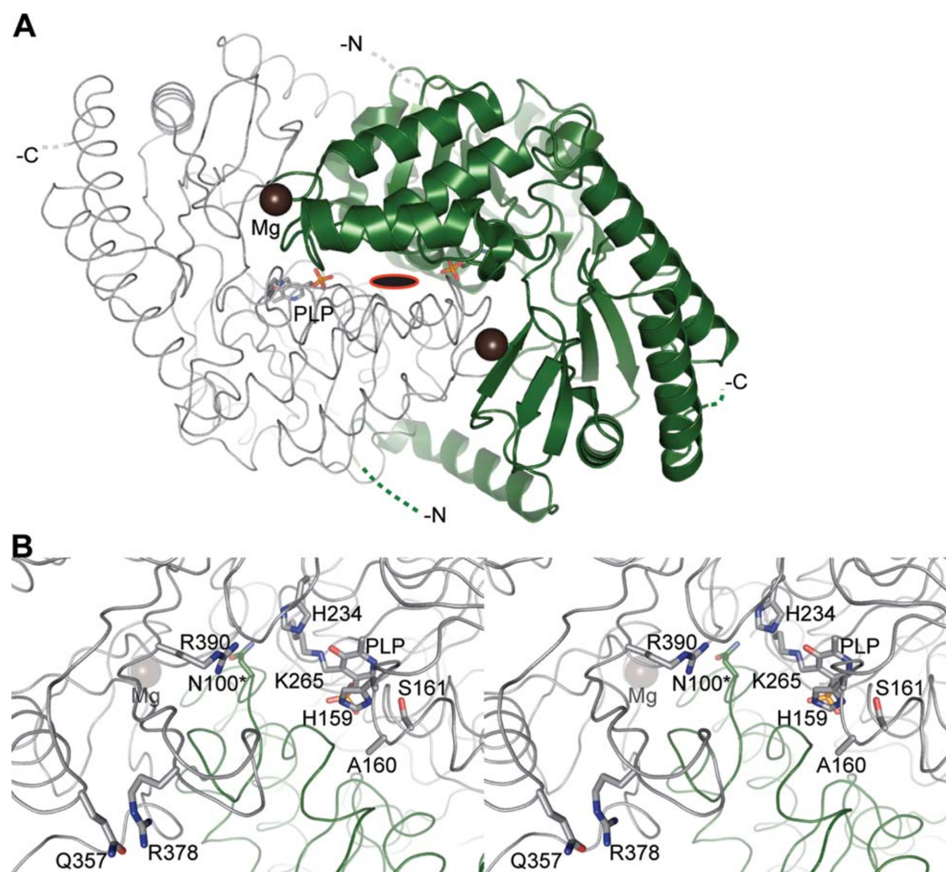


**Figure 6.** X-ray structure of SpSPT (PDB code 2JG2)[117]. A: Same as Figure 4(A) for holo-SpSPT. Subunit A is colored pale gray, whereas subunit B is dark green. The first 20 and the last nine residues of each subunit are represented by dashed lines. The bound Mg ions appear as brown spheres. B: Stereoview of the active site. The internal aldimine of subunit A as well as residues important for activity are shown as in Figure 4(B).

carrier protein.[114] This finding might provide new insight into the substrate accommodation into the active site of SPT.

Several compounds are known as potent inhibitors of SPT, for instance, sphingofungin B, myriocin, lipoxamycin, and viridofungin A,[102] and are thought to mimic a reaction intermediate. In addition, L-cycloserine has recently been found to inhibit SpSPT.[111]

The reaction mechanism of SPT has been extensively studied, using, along with biochemical knowledge on the AOS family,[112] the characteristic UV–visible spectra of the protein at various reaction stages of the WT and mutant forms.[106,118–121] Briefly, the internal aldimine between PLP and K265 (in SpSPT) (Fig. 7, step I) undergoes transaldimination with L-serine to yield PLP-L-serine external aldimine (step III). Binding of the second substrate, a fatty acid conjugated to either coenzyme A (CoA) or to acyl-carrier protein (ACP), as recently suggested in Ref. [114] (step IV), triggers a conformational change and ends up in α-deprotonation of serine. A first quinonoid intermediate is formed (step V). The resulting carbanion attacks the conjugated fatty acid to generate a condensation product (step VI), which is then decarboxylated to yield a second quinonoid intermediate (step VII). Protonation at $C_\alpha$ gives the external aldimine with the product, 3-keto-dihydro-SPH (step VIII), which is released from the active site (step IX)). The conserved residue H159, part of the HAS motif conserved throughout the AOS family,[117] plays crucial roles in catalysis [Fig. 6(B)].[118] It stacks against the pyridine ring of PLP, recognizes and anchors the carboxylate group of serine, regulates the conformation of the serine-PLP external aldimine to prevent unwanted reactions, and acts as acid catalyst in the condensation reaction.[111] Another histidine, H234, plays a role in the reaction intermediate stabilization, as its position might suggest.[111] In 2009, the structure of the external aldimine PLP-L-serine of SpSPT was reported,[119] highlighting the crucial role of N100 from the neighboring subunit in binding the carboxylate of the external aldimine.[119] Residue R378, the conformation of which is stabilized by a hydrogen bond with Q357,[119] is involved in the stabilization of the quinonoid intermediate although it is not conserved in all SPTs.[119] This residue, found in a mobile region of the protein, has two known conformations and is shown to play a crucial role in the binding of serine

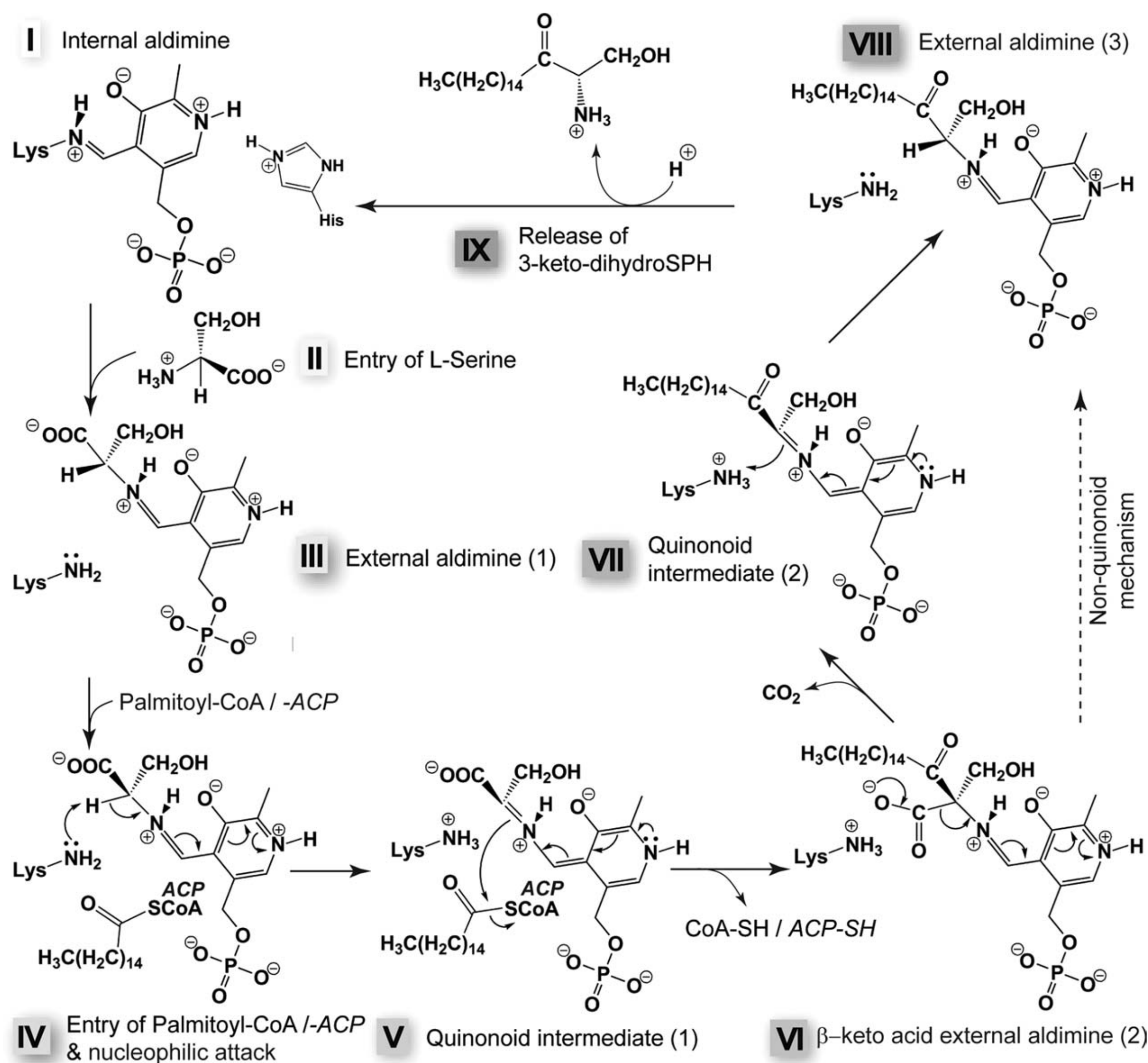


**Figure 7.** Proposed SPT reaction mechanism.[119,121] In the resting state, the PLP is linked to the enzyme via K265 (in SpSPT). Please note that the imidazole ring of H159 pi-stacks the pyridine ring of the PLP [see Fig. 6(B)]. For clarity, this residue is not shown in the subsequent steps (see comment below). The incoming amino acid (in this case serine) enters the active site (step II) and forms an external aldimine with PLP (step III). The external aldimine undergoes a nucleophilic attack, most likely by the internal aldimine-forming K265, after the second substrate, the acyl-CoA or the acyl-ACP (in this case palmitoyl), has entered the active site (step IV). The active site is expected to undergo conformational changes upon binding of the conjugated acyl. After the nucleophilic attack, a first quinonoid intermediate is formed (step V), the acyl carrier (either reduced CoA or ACP) leaves the active site, and a second external aldimine (with a β-keto acid) is formed (step VI). Decarboxylation of this intermediate produces the second quinonoid intermediate (step VII), which gets reprotonated to form the third external aldimine of the reaction cycle, between PLP and the reaction product (step VIII). The product 3-keto-dihydroSPH is then released (step IX). The proposed mechanism is based on the work of the Campopiano group.[111,119,121] Importantly, H159 stabilizes by H-bond either the carboxyl group of the PLP-bound Ser (step III) or the carbonyl of the conjugated acyl (steps IV and V) and of the intermediates depicted in steps VI to VIII. For further explanations on the mechanism and especially the role of H159, please refer to the review by Ikushiro and Hayashi.[106]

as well as of the inhibitor cycloserine.[111] Interestingly, R390 of SpSPT located nearby is conserved in all SPTs and is strictly required for activity in *Sphingomomas wittichii* (R370).[121] This recent finding shed a new light into the role of arginine in the reaction mechanism. As in Ref. [96], the ε-amino group of K265 may play a critical role by abstracting the Cα proton in step IV and by stabilizing the carbanionic intermediate (step VI).

SPT can metabolize other amino acids than serine.[122] In the case where alanine is used in the condensation reaction, 1-deoxy-sphingoid bases (DSBs) are synthesized. DSBs lack the hydroxyl group at position 1 [Fig. 1(A)] and cannot be degraded by

**Table I.** *Mutations of SPT Causing Hereditary Sensory and Autonomic Neuropathy Type 1 (HSAN I)*

| Subunit | Human SPT | Yeast SPT | SpSPT corresponding residue[a] | Location in SpSPT[a] | References |
|---|---|---|---|---|---|
| SPTLC1 | C133W/Y | C180W | N100 | Active site | 110,111 |
| SPTLC1 | V144D | V191D | D121 | Surface exposed | 106,107,111 |
| SPTLC2 | V359M | N.R.[b] | V246 | Surface exposed | 112 |
| SPTLC2 | G382V | N.R.[b] | G268 | Active site | 112 |
| SPTLC2 | I504F | N.R.[b] | G385 | Surface exposed | 112 |

[a] See Figure 8.
[b] Not reported.

SPL. Accumulation of DSBs is toxic and was observed in cells expressing mutants of SPTLC1 and 2 linked to hereditary sensory and autonomic neuropathy type 1 (HSAN I) (Table I).[108,123–125] These mutations most likely affect the residues involved in interaction with partner proteins (as hypothesized in Ref. [108]) or with the ssSPT subunit [Fig. 8(A)] or residues involved in PLP binding and substrate accommodation into the active site [Fig. 8(B), residues N100 and G268]. They result in a complete to partial loss of enzymatic activity. It turned out that some of these mutations perturbed the active site to facilitate the formation of the external aldimine with Ala.[123,125,126] Interestingly, the accumulation of DSBs, rather than the reduced catalytic activity of SPT, seems to be the major factor responsible for HSAN I.[122,126] In addition, elevated levels of DSBs were recently observed in patients suffering from diabetes mellitus.[122] As alanine and serine derive from the glycolytic chain, this may suggest a link between the two pathologies. The corresponding HSAN I-causing mutations on the yeast SPT also result in reduced activity. However, the mutated subunit (2 or 3) is still able to form a heterodimer with subunit 1, indicating that the reaction mechanism is conserved from yeast to human.[109] Moreover,

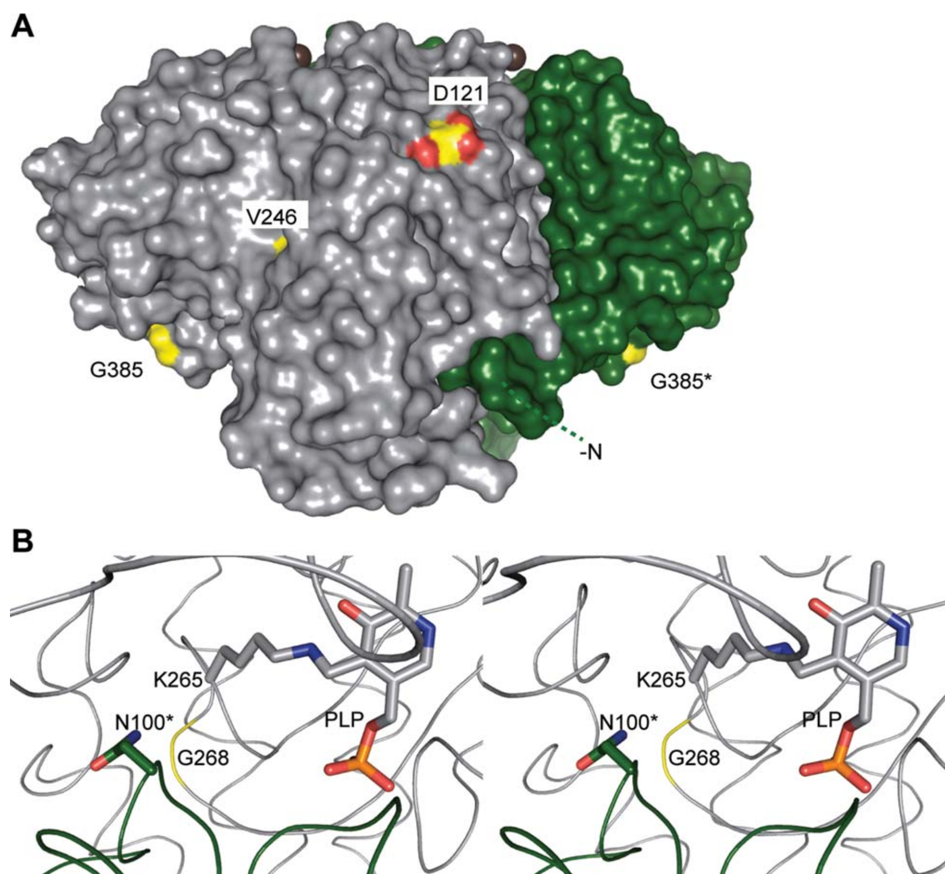


**Figure 8.** Hereditary sensory and autonomic neuropathy type 1 (HSAN I)–linked mutations on SpSPT. A: Surface representation of holo-SpSPT (PDB code 2JG2[117]). Subunit A is colored pale gray, whereas subunit B is dark green. The first 20 residues of subunit B are represented by a green dashed line. The residues of SpSPT corresponding to mutations linked to HSAN I are colored yellow and atom color. The bound Mg ions appear as brown spheres. Compared to Figure 6(A), the dimer was rotated 90° anticlockwise along an axis parallel to the plane of the sheet. This figure is linked to Table I. B: Stereoview of the active site. The residue G268 is colored yellow.

SPTLC1 was shown to physically and functionally interact with ABCA1 and to inhibit the transport of cholesterol,[127] providing evidence for coupling of the cholesterol and the sphingolipid metabolisms and a hint on a possible onset of atherosclerosis.

### StSPL and SpSPT: Comparison

In addition to covalently linking PLP to the enzyme, K311 in StSPL and K265 in SpSPT most likely also play a catalytic role,[82,106] mainly in the nucleophilic attack and protonation events. Likewise, H129 in StSPL and N100 in SpSPT, both located on an active site loop belonging to the neighboring subunit, are also key players in activity, demonstrating that the active form of both enzymes is a dimer or a multiple thereof.

The biological function of the encoding sphingolipid-related enzymes in bacteria and the origin of the corresponding genes are intriguing. The thermophile *Symbiobacterium thermophilum* has a significantly high number of genes acquired by horizontal transfer from different organisms.[128] Notably, horizontal transfer of genes predicted to be involved in the biosynthesis of sphingolipids, including a putative SPT, has been reported between an algae and its DNA virus.[129] The precise function of the proteins in the virus is unknown although they are thought to activate host cell death, thereby promoting the virus dissemination, to temporarily inhibit cell death to extend the length of infection, and to be involved in the virus recognition mechanism by the host cell by modulating the lipid profiles of the host/virus membranes.[129] Indeed, in pathogenic bacteria and viruses, sphingolipid-related enzymes can mimic antigens during infection and escape from the immune system,[5] modify the properties of the membrane to allow the entry of the pathogen,[130] act as a virulence factor to promote infection, and interfere with the sphingolipid metabolism to promote infection,[130] as found for *Listeria ivanovii* SM'ase[131] and *Pseudomonas aeroginosa* CER'ase.[132] In nonpathogenic bacteria such as *Symbiobacterium thermophilum*, it seems more likely that the main biological roles of StSPL are to degrade phospho-SPHL found in the environment and to contribute to the symbiotic relationship, similar to that between the symbiotic *Bacteroides* genus and a mammalian host in the intestine.[5]

The precise location of S1P and its mode of accommodation into the active site of SPL are still unknown, as are the exit modes of the hydrophilic product PE and of the hydrophobic product Hex. Likewise, the accommodation mode of the fatty acid into SPT and the exit path of the produced sphingolipid are still unknown. A conformational change is known to occur upon binding of the conjugated fatty acid in active site of SPT. In general, large conformational changes in SPL and SPT are expected to

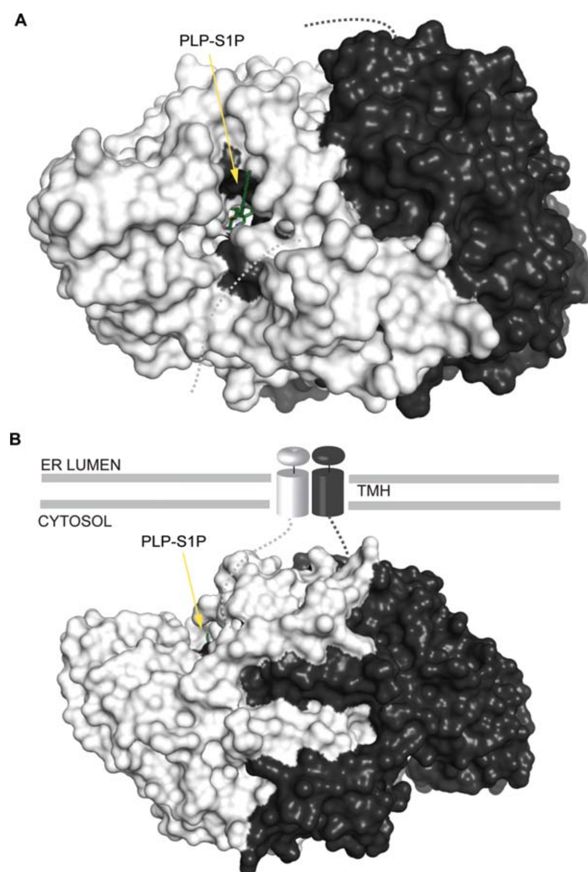


**Figure 9.** Putative membrane-bound SPL. Proposed topology of the membrane-bound mammalian and yeast SPL homodimer.[82] StSPL containing a modeled PLP-S1P external aldimine is colored as in Figure 4 and displayed as surface. A: Compared to Figure 4(A), the protein was rotated 90° anticlockwise along an axis parallel to the plane of the sheet such that the putative active site entrance faces the reader. B: The protein is oriented as in Figure 4(A). TMH stands for transmembrane helix.

take place to accommodate and release molecules with different physicochemical properties. The enzymes lacking a transmembrane domain may interact with the membrane and accommodate their substrates or release their product via a transiently exposed hydrophobic stretch. Such a mechanism is known for *Bacillus cereus* and *Listeria ivanovii* SM'ases, two prokaryotic soluble homologs of membrane-bound eukaryotic SM'ases. There, a β-hairpin and a loop penetrate the lipid bilayer to accommodate the substrate in the active site.[131,133] This phenomenon is not restricted to sphingolipid-related proteins. Other examples of peripheral membrane proteins include the SPL structural homolog *E. coli* GadB[134,135] bound to the membrane at low pH, the hepatitis C virus protein N3S-4A,[136] carnitine palmitoyltransferase 2,[137] the peripheral membrane protein *E. coli* pyruvate oxidase,[138] and *A. aeolicus* sulfide:quinone oxidoreductase.[139] The N-terminal part of SPL and SPT varies across species, especially

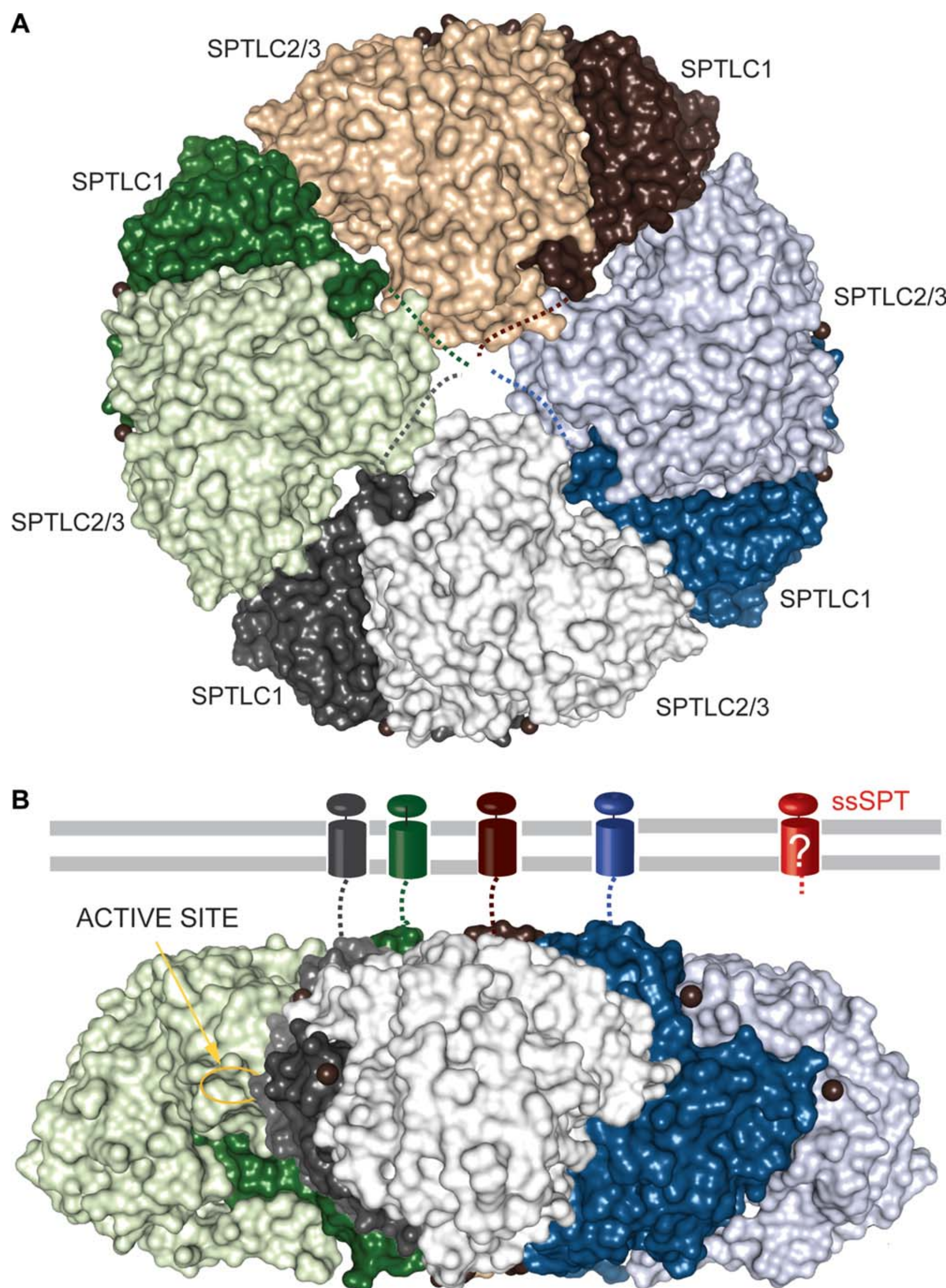


**Figure 10.** Putative membrane-bound SPT. Proposed topology of the membrane-bound mammalian and yeast SPT heteromer based on the assumption of Hornemann *et al*.[140] SpSPT dimers in surface representation are colored green, brown, blue, and gray where SPTLC1 is colored with the darker shades and SPTLC2/3 with the darker shades. A: The orientation of the brown dimer corresponds to that of Figure 8(A), whereas the blue, gray, and green dimers are rotated by, respectively, 90, 180, and 270° in the plane of the sheet and assembled to form a disk. B: Lateral view of the octamer. Compared to the orientation in (A), the disk was rotated 90° anticlockwise along an axis parallel to the plane of the sheet. The four transmembrane helices of the SPTLC1 subunit are represented as in Figure 9. The active site of the green subunit SPTLC2/3 is indicated. Note that the topology of ssSPT, colored red, is unknown.

between prokaryotic and eukaryotic proteins,[106,115] and may carry out species-specific functions. In addition, it would not be surprising if the flexible N-termini were found to initiate conformational changes before substrate accommodation and product release.

On the other hand, it should not be excluded that SPL and SPT may interact with a specific hydrophobic molecule carrier protein. Indeed, it was proposed that the expression of SPT is coupled with an acyl carrier protein in *Sphingomonas witchii* RW1.[114]

## Mammalian Counterparts: Similarities and Differences

Although the respective active sites of SPL and SPT are most likely conserved from bacteria to human, a distinctive feature of eukaryotic SPLs and SPTs is the presence of transmembrane domains. In the absence of biophysical or structural data, the topology, oligomeric state, and relative orientation of the active site to the lipid bilayer remain elusive. Mammalian SPL is thought to be a homooligomer (Fig. 9), whereas SPT may be a heterooligomer. Hornemann *et al*. proposed that SPT forms a high-molecular-weight complex, most likely an octamer.[140] Following this view, a possible octameric architecture is proposed in Figure 10. Given the well-described quaternary structure of PLP-dependent enzymes and the participation of both subunits to one active site, the oligomers are most likely formed by association of dimers. The third subunit of eukaryotic SPT (Fig. 10(B), red) adds another level of complexity to the challenge of defining the topology of the protein.

Another characteristic of eukaryotic SPT is the presence of PLP in only one subunit. This suggests that there is a single active site per dimer. Interestingly, it has been reported that the cofactor is labile in one subunit of StSPL and of a truncated form of Dpl1p.[82] This may reflect regulation mechanisms mostly specific to eukaryotic enzymes. Further experimental data are needed to understand the differences in the two active sites and its consequences for the function of the enzymes.

## Conclusion

The two PLP-dependent enzymes SPT and SPL play crucial roles in sphingolipid metabolism. Recent structural and functional analyses of prokaryotic SPL and SPT allowed describing the basic structure–function relationship of these enzymes. Nevertheless, important questions remain unanswered, such as the precise topology, the oligomeric state, the regulation mechanism of both enzymes, as well as the mode of substrate accommodation and product release in the prokaryotic as well as in the eukaryotic representatives. Finding answers to these questions is important for basic and applied research because entry and exit of the sphingolipid metabolism are targets of choice for drug design aimed at regulating the effects of given sphingolipid metabolites.[39]

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