Minireview

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Sphingosine 1-Phosphate, a Key Cell Signaling Molecule*

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The bioactive sphingolipid metabolite sphingosine 1-phosphate (S1P),[1] formed by phosphorylation of sphingosine catalyzed by sphingosine kinase (Fig. 1), is an important lipid mediator that has been implicated in many biological processes. S1P has been detected in organisms as diverse as plants, yeast, worms, flies, and mammals. More than a decade has elapsed since it was first suggested that S1P can regulate cell growth (1). Because it has multiple actions and regulates many processes, only relatively recently have we begun to make major progress in unraveling its pleiotropic actions following the cloning of the enzymes that regulate its levels and identification of its specific cell surface receptors. Much still remains to be uncovered, and its name, derived from the riddle of the mysterious sphinx, remains appropriate for this enigmatic lipid.

Extracellular Functions of Sphingosine 1-Phosphate

It is now well established that S1P is the natural ligand for specific G protein-coupled receptors (GPCRs), hereafter referred to as S1PRs. To date, five members, EDG-1/$S1P_1$, EDG-5/$S1P_2$, EDG-3/$S1P_3$, EDG-6/$S1P_4$, and EDG-8/$S1P_5$ have been identified (2–5). These receptors are highly specific and only bind S1P and dihydro-S1P, which lacks the trans double bond of the sphingoid base. Although earlier studies suggested that $S1P_1$ might also bind the structurally related serum-borne phospholipid, lysophosphatidic acid (6), it is now clear that this lipid is not a ligand for any of the S1PRs and has its own closely related family of GPCRs (7). The S1PRs are ubiquitously expressed and are coupled to a variety of G proteins. Whereas $S1P_1$ and $S1P_5$ are coupled mainly to G_i, $S1P_2$ can be coupled to all G proteins, $S1P_3$ is coupled to G_i, G_q, and $G_{12/13}$, and $S1P_4$ activates G_i and G_{12} but not G_s or $G_{q/11}$ in response to S1P. As a consequence, S1P influences distinct biological processes depending on the relative expression of S1PRs as well as G proteins. Members of the S1PRs also differentially regulate the small GTPases of the Rho family, particularly Rho and Rac (8), which are downstream of the heterotrimeric G proteins and are important for cytoskeletal rearrangements and cell movement (9). Activation of $S1P_1$ stimulates Rac-coupled cortical actin formation and enhances motility (8, 10–13) whereas $S1P_2$ elicits Rho-coupled stress fiber assembly and suppresses Rac activation (14), thereby inhibiting cell migration. Interestingly, only higher eukaryotes express S1PRs, whereas lower organisms, including plants and yeast, though responsive to S1P, seem not to have them.

Understanding the biological functions of the S1PRs is still in its infancy although some major advances have emerged from recent gene disruption studies. The phenotype of *s1p*$_1$ null mice revealed the important function of $S1P_1$ in vascular maturation (11). The embryos died *in utero* between E12.5 and E14.5 because of incomplete vascular maturation (11) resulting from a failure of vascular smooth muscle cells and pericytes to migrate around arteries and capillaries and properly reinforce them. Disruption of the PDGF-BB or PDGFR-β genes in mice also resulted in defective ensheathment of nascent blood vessels (15, 16). Dysfunctional migration of $S1P_1$ null embryonic fibroblasts toward a gradient of PDGF (13) links these two phenotypes at the final steps of vascular development, underscoring the importance of $S1P_1$ and endothelial cell-pericyte communication in vascular maturation and angiogenesis. This study also revealed novel cross-talk between a receptor tyrosine kinase, PDGFR, and a GPCR, $S1P_1$. Hence, binding of PDGF to its receptor activates and recruits sphingosine kinase to the leading edge of the cell (17). This localized formation of S1P spatially and temporally stimulates $S1P_1$ (13), resulting in activation and integration of downstream signals essential for cell locomotion, such as FAK and Src, necessary for turnover of focal complexes, and the small guanosine triphosphatase Rac, important for protrusion of lamellipodia and forward movement (13, 17) (Fig. 2*A*). These results shed light on the proposed vital role of $S1P_1$ in vascular maturation (11) and angiogenesis (8, 10, 18, 19). Further support for such receptor cross-communication recently emerged from the demonstration that PDGFR is tethered to $S1P_1$ providing a platform for integrative signaling by these two types of receptors (20). In contrast, it was recently proposed that tyrosine kinase receptors, such as the insulin-like growth factor-1 receptor, transactivate $S1P_1$ through Akt-dependent phosphorylation that does not require the sphingosine kinase pathway (21). Thus, in this scheme, insulin-like growth factor-1-activated Akt binds $S1P_1$ and phosphorylates its third intracellular loop at Thr-236, which is required for Rac activation and chemotaxis (21). Further studies are necessary to validate the generality of this concept of S1P-independent activation of S1PRs.

The importance of $S1P_2$ in cardiac development was revealed in the zebrafish mutant miles apart (*mil*), the $S1P_2$ orthologue, by the formation of a bilateral heart on the either side of the midline (22). Remarkably, the $S1P_2$ gene is not expressed in the migrating heart precursors; rather, it is expressed in the midline region of zebrafish embryos (22). In contrast to what might be expected, $S1P_2$ deletion in mice did not produce a similar cardiovascular or any other physiological defect (23).

$S1P_3$-deleted mice also developed normally suggesting that it is likewise nonessential for development (24). However, S1P-dependent activation of PLC and not Rho was defective in fibroblasts from these mice (24). These results suggest that $S1P_3$ is the predominant receptor coupling G_i to PLC activation and inositol 1,4,5-trisphosphate formation. Even less is known of $S1P_4$, which is mainly expressed in lymphoid and hematopoietic tissues and activates ERK1/2 (25) and PLC via pertussis toxin-sensitive G proteins (26). Of all of these GPCRs, $S1P_5$, which is expressed predominantly by oligodendrocytes and/or fibrous astrocytes in the rat brain (27), is the only one that mediates anti-proliferative effects, and it has the most unusual signaling properties. Surprisingly, ligand-activated $S1P_5$ inhibited serum-induced activation of ERK1/2, most probably because of activation of a tyrosine phosphatase (28).

Sphingosine 1-Phosphate: an Intracellular Mediator?

Does S1P exert its action solely through GPCRs? In analogy with some other lipid mediators, such as eicosanoids, which might bind to and activate nuclear receptors (29), it is tempting to speculate that S1P may also have intracellular targets. Indeed, there is abundant evidence that S1P can also function as a second messenger important for regulation of calcium homeostasis (30–32) and suppression of apoptosis (33–36). Although intracellular targets of

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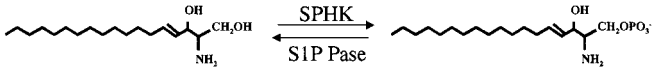
[1] The abbreviations used are: S1P, sphingosine 1-phosphate; GPCR, G protein-coupled receptor; S1PR, S1P receptor; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; PLC, phospholipase C; SPHK, sphingosine kinase.



SPHINGOSINE	S1P
Growth arrest	Stimulates growth
Enhances apoptosis	Suppresses apoptosis
Inhibits PKC	Regulates motility
Inhibits ICRAC	Regulates calcium
Decreases thermotolerance	Increases thermotolerance
Regulates endocytosis	GPCR ligand
Role in cytoskeletal rearrangements	Role in cytoskeletal rearrangements

FIG. 1. **Signaling functions of the substrate and product of the sphingosine kinase reaction.** SPHKs, using ATP as the phosphate donor, catalyze the phosphorylation of D-*erythro*-sphingosine to produce S1P. Several downstream targets and potential functions of both sphingosine and S1P are indicated. *PKC*, protein kinase C; *ICRAC*, calcium release-activated calcium current.

S1P have not yet been identified (making this a controversial area) several lines of evidence strongly support a role for intracellular actions of S1P. (i) Sphinganine 1-phosphate (dihydro-S1P), which is identical to S1P and only lacks the 4,5-*trans* double bond, binds to all of the S1PRs and activates them, yet does not mimic all of the effects of S1P, especially those related to cell survival (17, 34, 37, 38). (ii) Microinjection of S1P, as well as caged S1P, which elevate intracellular S1P, have been shown to mobilize calcium (32) and enhance proliferation and survival (34, 37). (iii) Yeast do not possess GPCRs, yet levels of phosphorylated long chain sphingoid bases regulate environmental stress responses and survival (39–42) in a manner reminiscent of the function of S1P in mammalian cells. (iv) Finally, recent evidence implicates S1P in calcium signaling and mobilization in yeast (43) and in higher plants (44).

The Sphingolipid Rheostat: a Conserved Stress Regulator

Ceramide (*N*-acylsphingosine) and sphingosine, the precursor of S1P (Fig. 1), are associated with cell growth arrest and are important regulatory components of stress responses and apoptosis (see accompanying minireview by Hannun and Obeid (72)). In contrast, S1P has been implicated in cellular proliferation and survival (33, 45). Whereas stresses increase *de novo* ceramide synthesis or activate sphingomyelinases and ceramidase and elevate levels of ceramide and sphingosine leading to apoptosis, many other stimuli, particularly growth and survival factors, activate SPHK, resulting in accumulation of S1P and consequent suppression of ceramide-mediated apoptosis (33). Thus, it has been suggested that the dynamic balance between intracellular S1P *versus* sphingosine and ceramide and the consequent regulation of opposing signaling pathways are important factors that determine whether cells survive or die (33).

This sphingolipid rheostat concept has important clinical implications. For example, increased S1P or decreased ceramide can prevent radiation-induced oocyte loss in adult wild-type female mice, the event that drives premature ovarian failure and infertility in female cancer patients (34, 46). This effect was not mimicked by dihydro-S1P nor was it blocked by pertussis toxin, indicating (in agreement with previous studies (33, 35, 38, 47–50)) that the cytoprotective effects of S1P are likely S1PR-independent. The balance between sphingosine and S1P also has been suggested to determine the allergic responsiveness of mast cells (51). Moreover, the protective action of high density lipoprotein against the development of atherosclerosis and associated coronary heart disease has also been correlated with resetting of the sphingolipid rheostat (52).

The sphingolipid rheostat is evolutionarily conserved, as it also plays a role in regulation of stress responses of yeast cells (40–42). In these lower eukaryotic cells, the sphingolipid metabolites ceramide and sphingosine have been implicated in heat stress responses as decreased phosphorylated long chain sphingoid bases dramatically enhanced survival upon severe heat shock (40, 41). Recently, it was reported that sphingosine is required for endocytosis in *Saccharomyces cerevisiae* and for proper actin organization (53, 54). Whether sphingosine plays such a role in mammalian cells is an open question.

Metabolism of Sphingosine 1-Phosphate

A prerequisite to understanding how cells regulate intracellular levels of an important signaling molecule such as S1P is a complete description and characterization of enzymes responsible for its production and degradation. Recently, two different isotypes of sphingosine kinase, the most important enzyme regulating S1P levels in eukaryotic cells, have been cloned and characterized (55, 56). Although highly similar in amino acid composition and sequence and possessing five conserved domains, sphingosine kinase type 1 is much smaller than type 2 and expressed mainly in the cytosol (Fig. 3). In contrast, SPHK2 additionally has several predicted transmembrane regions and a proline-rich SH3-binding domain, suggesting a different subcellular location. Importantly, these two ubiquitously expressed isoenzymes have different kinetic properties and also differ in the temporal patterns of their appearance during development (55, 56), implying that they perform distinct cellular functions and may be regulated differently. To date, sphingosine kinases have also been characterized in yeast *S. cerevisiae* (57) and plant *Arabidopsis thaliana* (58), and homologues have been identified in *Drosophila melanogaster* and *Caenorhabditis elegans* by data base searches, suggesting that sphingosine kinases are a unique family of lipid kinases and further supporting the notion of evolutionarily conserved roles for S1P.

Sphingosine kinase is activated by numerous external stimuli including PDGF (45), nerve growth factor (59), muscarinic acetylcholine agonists (31), cytokines such as tumor necrosis factor-α (38) and interleukin-1β (60), vitamin D3 (61), and cross-linking of the immunoglobulin receptors FcϵRI (62) and FcγRI (63) and GPCRs, including S1PRs themselves (64). Overexpression of SPHK1 in NIH 3T3 fibroblasts resulted in enhanced proliferation (48), growth in soft agar, and tumor formation in NOD/SCID mice (65). An elegant study used a sphingosine kinase inhibitor and a dominant negative mutant of this enzyme to demonstrate that sphingosine kinase contributes to cell transformation mediated by oncogenic H-Ras (65). Overexpression of SPHK1 also protected against apoptosis, particularly death induced by ceramide elevation (35, 48). The cytoprotective effect was attributed to inhibition of activation of caspase-2, -3, and -7 and of the stress-activated protein kinase, JNK (c-Jun NH_2-terminal kinase) (35).

Specific sphingoid base phosphate phosphohydrolases were first identified in yeast and shown to be important regulators of heat stress response (40, 66). Deletions of these S1P phosphatases led to increased thermotolerance, whereas overexpression reduced it (40, 41), substantiating a role for phosphorylated sphingoid bases in heat stress responses. Based on homology with the yeast gene, a mammalian S1P phosphatase has been cloned that only degrades phosphorylated sphingoid bases (67). Overexpression of this unique S1P phosphatase altered the dynamic balance between S1P and sphingosine/ceramide in mammalian cells and, consequently, markedly enhanced apoptosis (67). Although several other mammalian lipid phosphate phosphohydrolases that can degrade S1P have been identified (68) it seems unlikely that they would play an important role in S1P metabolism (due to their lack of specificity), although further studies are necessary to confirm this.

S1P can also be degraded by S1P lyase, a pyridoxal-dependent enzyme, to ethanolamine phosphate and hexadecanal. S1P lyase, like S1P phosphatase, appears to be localized to the endoplasmic reticulum. Yeast lyase deletion mutants exhibited cell cycle arrest (39). Interestingly, disruption of the S1P lyase gene in the slime mold *Dictyostelium discoideum* resulted in aberrant morphogenesis as well as enhanced viability during stationary phase and provided resistance to the anti-cancer drug cisplatin, thus suggesting a role for S1P in survival and development of even this primitive multicellular organism (69).

An important question, to which there are only fragmentary answers, is how is S1P transported inside and outside cells? Recent studies in *S. cerevisiae* implicated the yeast oligomycin resistance gene (*YOR1*), a member of the ABC family of proteins, in the transport of S1P. Interestingly, the cystic fibrosis transmembrane regulator (CFTR), a unique member of this family with high homology to YOR1, was recently shown to regulate uptake of S1P (70). It will be important in the future to determine whether other members of the ABC family that translocate lipids across the plasma membrane are also S1P translocators (see accompanying minireview by van Meer and Lisman (73)).

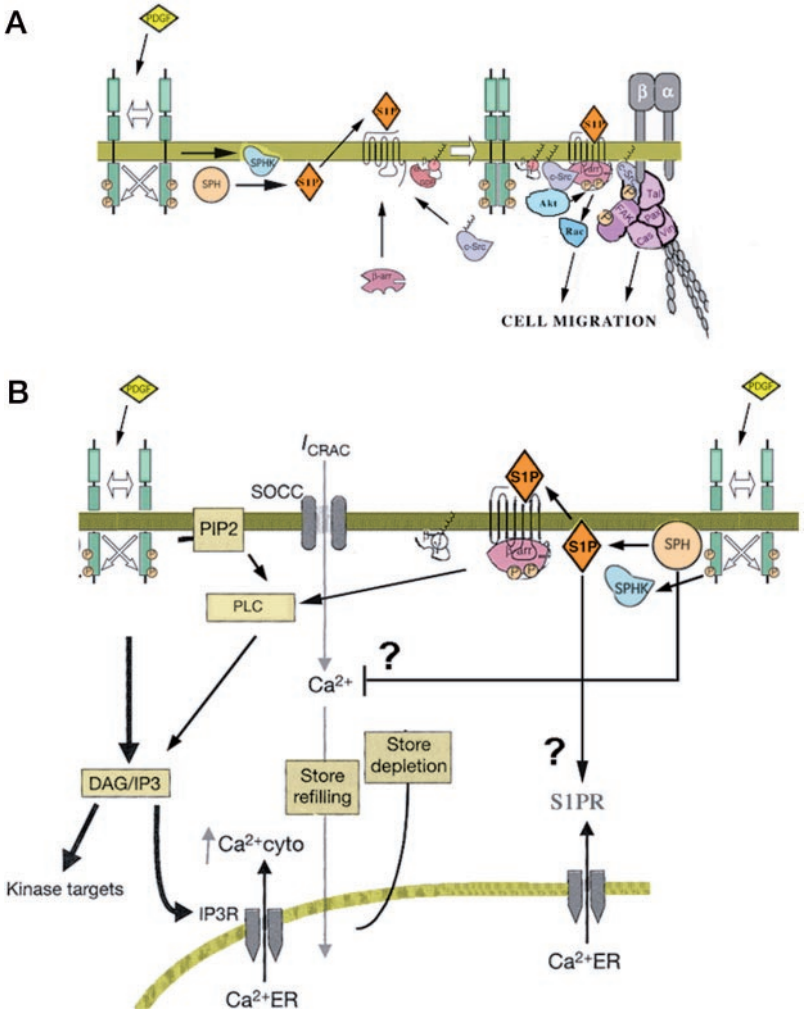


FIG. 2. **Receptor tyrosine kinase transactivates S1P receptors.** This scheme depicts cross-communication between a tyrosine kinase growth factor receptor, PDGFR, and S1P receptors. Binding of PDGF to PDGFR results in activation and translocation of SPHK to the plasma membrane and restricted generation of S1P. S1P in turn activates its receptors leading to recruitment and/or activation of downstream signaling molecules, including Src, FAK, and Rac, important for cell migration (*A*) or other downstream signaling, such as phospholipase C that regulates calcium levels (*B*). S1P can mobilize calcium from internal sources either via an unidentified inositol 1,4,5-trisphosphate (IP_3)-independent receptor on the endoplasmic reticulum (*ER*) or by activation of S1PRs that stimulate phospholipase C. Stimulation of SPHK also results in decreased sphingosine levels that normally block the store-operated calcium release-activated calcium current leading to refilling of the stores (modified from Ref. 13). *DAG*, diacylglycerol.

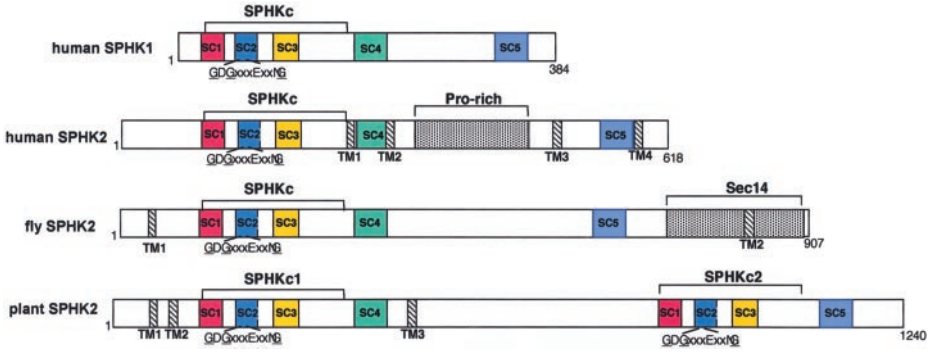


FIG. 3. **Structural organization of the sphingosine kinase family.** All SPHKs have five conserved domains, labeled *SC1* to *SC5* for convenience. All SPHK catalytic domains contain the conserved ATP binding sequence, GDG*XXXEXX*NG. Human SPHK2 contains a proline-rich region, which is known to bind to SH3 domains, and four transmembrane regions (*TM*). It is also noteworthy that *Drosophila* SPHK2 has a SEC14 domain and a phosphatidylinositol-binding domain at the COOH terminus. SEC14 is a lipid-binding domain that is present in a homologue of an *S. cerevisiae* phosphatidylinositol transfer protein and in RhoGAPs, RhoGEFs, RasGEF, and neurofibromin. Although type 1 SPHK from *A. thaliana* is a *bona fide* sphingosine kinase (58), a data base search revealed an unusual putative type 2 SPHK with a duplicated catalytic domain.

Universal Intracellular Roles of Sphingosine 1-Phosphate: from Plants to Higher Eukaryotes

Recent studies indicate that S1P, known to be important for calcium regulation in animal cells, is also involved in calcium-dependent signaling of calcineurin in yeast (43) and in the plant *A. thaliana* (44). It was initially suggested that, in mammalian cells, S1P mobilizes calcium from internal sources in an inositol 1,4,5-trisphosphate-independent manner (30). Although many studies appear to support this concept (31, 32, 62), the direct receptor on the endoplasmic reticulum has yet to be identified. In mast cells, FcεRI cross-linking leads to activation of SPHK and conversion of sphingosine to S1P. Not only can S1P mobilize calcium (62), but perhaps more importantly, sphingosine blocks the store-operated calcium release-activated calcium current (I_{CRAC}) activated by agonists. Hence, upon depletion of internal calcium stores, metabolism of sphingosine by conversion to S1P catalyzed by SPHK lowers sphingosine levels and leads to the disinhibition of I_{CRAC} (71) and a net increase of cytosolic calcium (Fig. 2*B*).

Recently, an intriguing study showed that S1P is a new calcium-mobilizing molecule in plants (44). The plant hormone abscisic acid produced in roots during desiccation stress is transported to the leaves, where it decreases stomatal opening by direct activation of plasma membrane calcium channels. When plants were grown in drought conditions, the levels of endogenous S1P increased. Exog-

enously applied S1P, but not dihydro-S1P, stimulated calcium oscillations and stomata closure, just as drought conditions do. Moreover, the effect of abscisic acid was blocked by treatment with a SPHK inhibitor. Together, these data suggest that S1P might act as a second messenger in plants and that S1P regulates plant guard cell aperture.

Perspectives and Future Directions

The results of the many studies carried out only within the last few years that are described in this review provide strong support for the notion that S1P functions as both a first messenger and a second messenger. In summary of its most well established functions to date, S1P acts extracellularly by binding to members of the S1PR family of GPCRs, thereby regulating cell movement, and it acts intracellularly to regulate survival and Ca^{2+} homeostasis. Future challenges include further characterization of the specific physiological roles of the various S1PRs, identification of the intracellular targets of S1P, the sources of S1P, and elucidation of its transport into and out of cells. The number of genes known to be involved in S1P metabolism has increased rapidly during the last years, yet it is likely that other isoforms will be identified and much more needs to be learned. Structure-function analysis of these gene products, as well as characterization of their topology, localization, and mechanisms of activation will enhance understanding of the cellular functions of S1P. The development of antagonists or agonists of S1PRs and of inhibitors or activators of enzymes that affect the intracellular concentration of S1P may provide the basis for the development of novel therapeutics.

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