Point-Counterpoint of Sphingosine 1-Phosphate Metabolism

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Abstract—Sphingosine 1-phosphate (S1P), an evolutionarily conserved bioactive lipid mediator, is now recognized as a potent modulator of cell regulation. In vertebrates, S1P interacts with cell surface G protein–coupled receptors of the EDG family and induces profound effects in a variety of organ systems. Indeed, an S1P receptor agonist is undergoing clinical trials to combat immune-mediated transplant rejection. Recent information on S1P receptor biology suggests potential utility in the control of cardiovascular processes, including angiogenesis, vascular permeability, arteriogenesis, and vasospasm. However, studies from diverse invertebrates, such as yeast, Dictyostelium, Drosophila, and Caenorhabditis elegans have shown that S1P is involved in important regulatory functions in the apparent absence of EDG S1P receptor homologues. Metabolic pathways of S1P synthesis, degradation, and release have recently been described at the molecular level. Genetic and biochemical studies of these enzymes have illuminated the importance of S1P signaling systems both inside and outside of cells. The revelation of receptor-dependent pathways, as well as novel metabolic/intracellular pathways has provided new biological insights and may ultimately pave the way for the development of novel therapeutic approaches for cardiovascular diseases. (Circ Res. 2004;94:724-734.)

Key Words: sphingolipid mediators ■ sphingosine kinase ■ sphingosine 1-phosphate phosphatase ■ sphingosine 1-phosphate lyase ■ sphingosine 1-phosphate receptors

Sphingolipid mediators, such as sphingosine 1-phosphate (S1P), ceramide, and sphingosine are derived by the enzymatic breakdown of sphingomyelin, an abundant membrane phospholipid.1 S1P is an evolutionarily conserved lipid signaling molecule with potent effects on multiple organ systems. The enzymes that metabolize S1P-like molecules are highly conserved in all eukaryotes.2 In contrast, effectors of S1P such as the G protein–coupled receptors (GPCRs) on the plasma membrane appear to be present only in higher eukaryotes.3 In contrast, effectors of S1P such as the G protein–coupled receptors (GPCRs) on the plasma membrane appear to be present only in higher organisms,2 because genomic sequencing efforts showed that the S1P receptor subfamily coevolved with the chordates. Historically, S1P has been considered as an intracellular second messenger.4 Implicit in such a notion is the assumption that S1P binds to and regulates the activity of intracellular regulatory molecules, such as enzymes, channels, and transcription factors, which would sense changes in the concentration of S1P. Despite a wealth of evidence implicating intracellular second messenger action, the validity of this model is a matter of debate,4,5 until direct targets have been molecularly characterized. Secondly, S1P has been shown to act as a ligand for G protein–coupled receptors on the plasma membrane.6 In this “extracellular” mode of action, S1P...
released from cells binds to the receptor on the plasma membrane, which then transmits intracellular signals into the interior of the cell. The third possibility can be termed as “metabolic” mode of action. Because metabolic pathways of S1P synthesis and degradation interact with essential cellular pathways, such as phospholipid synthesis, sterol metabolism, and transcriptional responses [e.g., the sterol receptor enhancer binding protein (SREBP) transcription pathway], modulation of S1P levels may greatly influence critical cellular decisions, such as cell survival, death, and differentiation. Recent development of S1P-based therapeutic tools, exemplified by the immunomodulatory agent FTY720, warrant a better mechanistic understanding of how S1P functions and is metabolized. This review will summarize the emerging knowledge of S1P function in the cardiovascular system. Next, our recent knowledge of how S1P is formed and degraded will be described, and its impact on S1P function will be discussed.

**S1P as a Bioactive Lipid Mediator in the Cardiovascular System**

High levels of S1P are found in blood, placing it in an effective position to regulate the cells of the vascular wall. Platelets, which lack the S1P lyase and therefore a rich source of S1P, release it upon activation by prothrombotic stimuli, such as thrombin, ADP, and collagen. In addition, various cells of the hemangioblastic lineage, such as monocytes, mast cells, endothelial cells, and red blood cells secrete S1P. Thus, S1P levels in serum are considerably higher than in plasma, which were estimated at ≈0.4 and 0.1 μmol/L, respectively. However, bioactive concentration of S1P may be lower because S1P bound to low-density lipoprotein (LDL) fraction may be biologically inactive. In contrast, S1P bound to the high-density lipoprotein (HDL) fraction may be biologically active. On the other hand, the concentration of S1P in blood and plasma constituents are significantly higher than the estimated kDa of S1P to its G protein–coupled receptors, which is in the range of 8 to 60 nm.

An important question is whether S1P receptors are stimulated constitutively under basal conditions. In other words, it is not known whether S1P is a tonic factor that is constitutively present or an inducible factor that acts when needed. Although this important issue needs to be further explored, it appears that both modes of action may be relevant in different pathological contexts. Further experimentation with receptor-specific antagonists and tissue-specific knock-out animals in physiological and pathological systems are necessary to address this issue.

**S1P and the Endothelium**

The vascular endothelium is a major S1P-responsive cell type. The S1P receptor EDG-1 (S1P1) was originally isolated as an inducible gene from endothelial cells. In addition, S1P2 and S1P3 GPCRs are also expressed. Treatment of endothelial cells with S1P in vitro results in the induction of cell migration, proliferation, shape change, and survival. Unique to endothelial cells, S1P induces cellular morphogenesis into tubular, capillary-like networks. Such effects of S1P are mediated by receptor-regulated heterotrimeric G protein activation. Indeed, S1P receptor subtypes couple to different G protein α subunits, such as Gαq, Gα11, and Gα13, and small GTPases Rho and Rac. S1P is particularly critical for Rac activation in endothelial cells, whereas Rho appears to be activated by several S1P receptors, in particular by the S1P1 subtype. Indeed, S1P couples to the Gα1 subtype and inhibits the Rac GTPase in a Rho-dependent manner. Thus, S1P1 and S1P3 receptor subtypes exert opposing effects on Rac-regulated processes, such as the formation of lamelipodia (migrating front) and cell chemotaxis.

It has been proposed that S1P mediates angiogenesis, or new blood vessel formation. Vascular endothelial cell migration is strongly induced by S1P. Indeed, S1P regulates the Rho-dependent assembly of focal contacts, Rac-dependent lamellipodia formation, and integrin activation, resulting in migration on complex extracellular matrices. This mechanism may be important in wound healing, in which blood clotting would be expected to release S1P and thereby attract endothelial cells into the provisional matrix. In fact, S1P strongly induces matrix metalloprotease expression and stimulates endothelial cell invasion into 3-dimensional matrices, thereby promoting capillary sprout formation. These findings suggest that S1P may be an important angiogenic factor in different pathological settings such as tumor angiogenesis, diabetic retinopathy, and psoriasis.

Because S1P regulates the assembly of cell-cell junctions (mediated by VE-cadherin), it is likely to regulate normal vascular permeability. Indeed, it has long been appreciated that platelets secrete important factors for vascular integrity. Recent studies in cultured endothelial cells indicate that S1P stimulates barrier function in a G protein–dependent manner. Thus, under normal conditions, S1P may be important for the prevention of excessive vascular permeability. The S1P receptor agonist FTY720 phosphate is a profound inhibitor of vascular permeability in vivo, and receptor-dependent assembly of endothelial cell adherens junctions play a major role in this process. These studies illustrate a general principle of G protein–coupled receptor regulation of endothelial cell VE-cadherin assembly and permeability by S1P.

Apoptosis of endothelial cells occurs in serious biological insults, such as in sepsis, Dengue hemorrhagic fever as well as in radiation injury, thus leading to circulatory collapse. S1P signaling via the S1P1 receptor is critical for inducing endothelial cell survival. In addition, HDL–induced endothelial cell survival appears to require S1P signaling. Because vascular dysfunction leads to organ edema and compromise, a common problem associated with infectious or traumatic shock, radiation toxicity, cancer chemotherapy, immune-mediated vascular leak syndromes, and low platelet conditions (thrombocytopenias), it will be of interest to determine if S1P receptor agonists could offer new therapeutic approaches in any of these clinical scenarios. A converse strategy would be the potential to use S1P receptor antagonists in the enhancement of vascular permeability of pharmacological reagents in selected tissues, such as in the delivery of drugs into the central nervous tissues by overcoming the blood brain barrier of brain endothelial cells.
SIP treatment of endothelial cells in vitro results in the induction of inflammatory cell adhesion molecules, such as V-selectin and intercellular adhesion molecule-1. In addition, treatment of endothelial cells with tumor necrosis factor-α induced adhesion molecule expression, which was blocked by sphingosine kinase inhibitors. The physiological relevance of SIP as an inflammatory mediator is not well established in animal models and therefore requires further study.

**SIP and the Vascular Smooth Muscle**

SIP also induces potent effects on vascular smooth muscle cells (VSMCs). Differential expression of SIP receptor subtypes were seen in phenotypically distinct VSMC cell lines: adult medial VSMCs express SIP2 and SIP3 subtypes, whereas pup intimal VSMCs express SIP1, SIP2, and SIP3 subtypes. SIP induces proliferation and migration of the intimal but not the adult medial subtype of VSMCs, suggesting a role in vascular pathologies such as restenosis after angioplasty or transplant-associated atherosclerosis. The ability of the SIP1 receptor to couple to the Gq-linked p70 S6 kinase pathway is important in the induction of cell proliferation. Similarly, the induction of VSMC migration is attributed to the SIP1 receptor subtype, which is overexpressed in human atherosclerotic lesions. In contrast, SIP2, which is expressed in medial VSMCs, inhibits the small GTPase Rac and cell migration. Opposing actions of SIP1 and SIP2 receptors may be important in vascular maintenance and remodeling.

In some vessels, such as the middle basilar artery in the brain, SIP is a potent vasoconstrictor. In this scenario, local thrombus-induced release of SIP could trigger cerebral vasospasm. In addition, SIP was shown to induce coronary and renal artery contraction and thereby reduce blood flow to the respective organs. These in vivo findings suggest that specific SIP receptor subtypes mediate vasoconstrictive responses. Indeed, SIP1 and SIP3 are candidates because they are expressed in vascular tissue and coupled to the Gq pathway. Analysis of signaling pathways in cells derived from monogenic and bigenic S1P2- and S1P3-null mice suggests that these receptors are important for the regulation of Rho GTPase and phospholipases C/Ca2+ pathways, respectively. Both pathways have been implicated in vasoconstrictor responses and are thought to be involved in pathological conditions such as hypertension.

Recent studies also emphasize that SIP1 is an important regulator of nitric oxide (NO) synthesis in endothelial cells. SIP1 activation of its receptors results in Gq-dependent activation of phosphatidylinositol 3-kinase, subsequent stimulation of the protein kinase B/Akt, and phosphorylation of the endothelial nitric oxide synthase enzyme, resulting in the increased formation of NO. In preparations of isolated mesenteric arterioles, SIP induced a dose-dependent vasorelaxation that was dependent on the endothelium-dependent NO generation. These data suggest that in some vascular beds, SIP is an endogenous vasorelaxant factor. This is somewhat paradoxical, because SIP1 can act as a vasoconstrictor in some contexts, as described above. It is likely that differential expression and function of SIP1 receptor subtypes in normal vessels (with an intact endothelial layer) versus pathological vessels (with damaged or inflamed intima) would cause differential responses of the vascular tissue to SIP. In addition, specific vascular beds may express different receptor subtypes in endothelial cells and VSMCs and thereby determine the physiological response. More detailed information regarding SIP receptor expression patterns in normal versus pathological tissues is needed to understand the acute effects of SIP on vascular physiology so that rational pharmacological approaches can be developed.

**SIP and the Heart**

SIP potently affects the development and function of the heart. Mutation in a zebrafish SIP1 receptor called Miles Apart (which is most similar to the S1P2 receptor of mammals) results in a myocardial precursor cell migration defect and formation of the cardiac bifida condition, wherein the two primitive heart tube structures fail to coalesce and form a single mature heart structure. Gene deletion of the S1P1 receptor in the mouse did not result in obvious cardiac anomalies, suggesting that mammalian S1P1 may not be strictly orthologous to the bony fish counterpart. Alternatively, redundancy in the function of other SIP receptor subtypes in the mammals may have resulted in the different phenotypes.

Studies in adult cardiac cells showed that SIP regulates the calcium metabolism and ionic currents in cells of the sinoatrial node, which controls heart rate. In addition, SIP was shown to prevent death of cardiac myocytes on ischemia/reperfusion injury. Cardiomyocytes express S1P1, 3 receptors, suggesting that signaling pathways stimulated by these receptors may contribute to intrinsic myocardial function. Intravenous administration of SIP in vivo results in decreased heart rate, ventricular contraction and blood pressure. In an animal model of ischemia/reperfusion injury of the heart, SIP was found to be cardioprotective. These preclinical studies, together with emerging data suggesting that serum SIP levels may be predictive of recent or ongoing occlusive coronary vascular events, provide a strong impetus to further define the role of SIP signaling in cardiovascular physiology and pathophysiology.

**SIP and Vascular Development**

Knock-out of the SIP1 receptor in the mouse elucidated an essential role for SIP in vascular maturation during development. The maturation/stabilization of blood vessels was defective in S1P1−/− mice: it was found that pericyte coverage of the dorsal aorta and the cranial artery were incomplete. This led to endothelial cell morphological changes and vessel instability, ultimately resulting in hemorrhage and death at embryonic 12.5 to 14.5 days of development. The authors suggested two potential mechanisms: first, regulation of pericyte migration by SIP1, and second, the regulation of adhesion of endothelial cells and pericytes (heterotypic cell-cell adhesion) by SIP, expressed in either or both cell compartments. Tissue-specific knock-out studies indicate that endothelial-specific deletion of SIP1 phenocopies the total knock-out phenotype, suggesting that signaling within the endothelial compartment is critical for proper vascular stabi-
lization/development. In contrast, deletion of S1P1 in VSMCs did not lead to embryonic lethality, suggesting that S1P1 in the mural cells is not essential for vascular maturation or development. Second, it suggests the existence of an endothelium-dependent signaling in trans to the pericyte in the regulation of vascular maturation. S1P1 is known to regulate the activity and localization of adhesion molecules, such as cadherins and integrins, and thus it is likely that defective endothelial cell/pericyte heterotypic cell-cell adhesion is involved in this process. Further mechanistic studies to define this pathway may lead to new insights in the understanding of vascular maturation, especially as it pertains to disease processes such as diabetic retinopathy, tumor angiogenesis, among others. Because S1P is a potent regulator of vascular maturation, strategies to increase S1P production and signaling may lead to new ways of stimulating therapeutic arteriogenesis and venogenesis in situations in which new collateral vessels are desired, such as in the ischemic myocardium and in diabetic limbs.

A recent article proposed that S1P1 may function downstream of the platelet-derived growth factor (PDGF) receptor-β and regulate PDGF-BB induced pericyte migration. However, this mechanism is unlikely to be generally applicable because different clones of S1P1−/− cells exhibit wide variability in their migratory response to PDGF-BB. In addition, the phenotype of PDGF-β receptor-null mice is distinct from the S1P1−/− mice; in the former, pericytes are lacking in brain microvessels, whereas in the latter case, they are mislocalized in the arteries, suggesting that different mechanisms are operative in vascular maturation pathways regulated by these two important growth factors.

These recent findings emphasize the emerging role of S1P as an important multifunctional mediator in the cardiovascular system. Although known cardiovascular actions of S1P are primarily receptor-dependent, recent studies implicating the potent regulatory role of S1P metabolic enzymes raise the possibility that intracellular S1P metabolism may exert significant influence over cardiovascular development, physiology and pathology. Further knowledge on the enzymes of S1P metabolism is needed not only for better understanding of the sphingolipid signaling system in the cardiovascular system, but also for the rationale design and utility of novel therapeutics in this area.

**Metabolism of S1P**

The structure of all complex sphingolipid molecules consists of three components: a polar head group (such as phosphocholine for sphingomyelin), a long-chain sphingoid base backbone, and a fatty acid in amide linkage with the base. The predominant long-chain base in mammalian cells is C18 sphingosine, although varying amounts of C16 dihydrosphingosine and phytosphingosine (collectively called long-chain bases, LCBs) are also present and are enriched in certain cell types. The latter two LCBs differ from sphingosine in lacking the C4,8 double bond (considered to impart biological activity to sphingosine, S1P, and ceramide), and by the presence of additional hydroxyl groups on the carbon chain. S1P-like molecules are present in all eukaryotes, although substantial diversity exists in the chain length and structure of the LCB and phosphorylated long-chain base (LCBP) molecules of different species.

A common product of sphingolipid breakdown is ceramide, which is generated primarily by hydrolysis of membrane sphingomyelin (Figure). Ceramide can be further catalyzed by ceramidases to generate sphingosine, which can be either reacylated to ceramide or phosphorylated through the actions of sphingosine kinase, generating S1P. Recent studies in numerous cell types indicate that ceramide formed through the de novo synthesis pathway (initiated by the condensation of serine and palmitoyl-CoA in a step catalyzed by serine palmitoyltransferase) can also contribute to the regulation of apoptosis, phagocytosis, insulin gene expression, and cholesterol esterification. Thus, changes in intracellular S1P levels may potentially be elicited by the availability of ceramide and sphingosine derived from degradation of higher order sphingolipids (the sphingomyelinase pathway) or from stimulation of sphingolipid biosynthesis (the de novo pathway) (Figure). In addition, hydrolysis of sphingosylphosphorylcholine by autotaxin (lyosphospholipase D) in the plasma may also be capable of generating S1P, although the physiological relevance of this reaction remains to be determined.

Once formed, S1P can be degraded by dephosphorylation, catalyzed by either S1P phosphatase (SPP) or type 2 phosphatidate phosphohydro-
lases, or through its irreversible cleavage at the C$_{2-3}$ bond to a long-chain aldehyde and ethanolamine phosphate, catalyzed by S1P lyase.$^1$

The metabolic relationships between ceramide, sphingosine, and S1P are of great importance in determining cell behavior and are, thus, of significant therapeutic interest. In many cases, ceramide and sphingosine oppose the actions of S1P. Similarly, modulation of intracellular levels of ceramide and/or sphingosine versus S1P results in opposing effects on cell function and survival. Thus, a conceptual framework coined the “sphingolipid rheostat” has been proposed, in which the ratio between intracellular ceramide/sphingosine and S1P is important for biological outcome.$^3$ Although conceptually useful, this model does not take into account the possibility that large fluctuations in the levels of sphingolipid mediators can occur in specific subcellular locales and, thus, may mediate specific biological effects even though total changes in levels of the mediators may be modest. These issues warrant the development of new tools and technologies to image dynamically and at high resolution the concentration and localization of sphingolipid mediators and their enzymes within specific cellular and membrane subdomains. Further, the molecular targeting of sphingolipid metabolizing enzymes to specific subcellular organelles, determination of the composition and content of sphingolipid species within organelles under physiological conditions such as angiogenesis, hypoxia, ischemia/reperfusion, and other stresses, and evaluation of the activity and expression of endogenous sphingolipid metabolizing enzymes in organelles under these same conditions should help to address this issue.

**Sphingosine Kinases**

Sphingosine kinase (SK) requires ATP and a divalent cation for the phosphorylation of the hydroxyl group on the first carbon of sphingosine into S1P.$^7$ d-erythro-sphingosine, the most abundant long-chain base in most mammalian cells, serves as a substrate for SK from all known sources. SK activity has been demonstrated in mammalian tissues and in a variety of plant, yeast, and animal species.$^{59,60}$

Like other lipid kinases, including diacylglycerol kinase and phosphatidylinositol 3-kinase, SK acts as a signaling hub for a variety of upstream effectors in many cell types. SK activation has been demonstrated in response to a wide range of agonists including PDGF, nerve growth factor, epidermal growth factor, tumor necrosis factor (TNF-α), basic fibroblast growth factor, vascular endothelial growth factor (VEGF), muscarinic acetylcholine agonists, phorbol ester and protein kinase C activation, cross-linking of the FcγRI and FcεRI receptors, estrogen, and absicic acid (in plants).$^4$ Further, *Saccharomyces cerevisiae* SK is optimally active and induced by stress conditions, including heat shock and nutrient deprivation.$^{59,61}$ The agonists mentioned above have been shown to stimulate SK activation and S1P generation; conversely SK inhibitors block at least some downstream signaling effects and biological endpoints of these agonists. Thus, SK activation has been implicated in diverse biological endpoints.$^{62}$ However, many studies utilize N,N′-dimethyl sphingosine (DMS) to inhibit the activity of SK, and it is known that this compound influences other signaling molecules such as protein kinase C and ion channels.$^{63-65}$ Thus, in many situations, rigorous demonstration of obligatory requirement for SK, for example, disruption of SK genes, is still lacking. Nevertheless, the SK enzyme appears to be involved in a host of physiological and clinical settings.

Yeast genetic approaches led to the identification and characterization of all three major genes of S1P metabolism. S1P lyase was the first to be identified (see later), and by using forward genetic strategies and suppressor screens, SK and S1P phosphatase (SPP) were subsequently cloned.$^{52,66-69}$ The predicted protein sequences of yeast SK enzymes contain a diacylglycerol kinase homologous domain, suggesting the existence of a conserved lipid kinase structural domain wherein nucleotide binding and lipid substrate binding motifs reside. Comparison of yeast, murine, human, and *Drosophila* SK proteins and a putative *Caenorhabditis elegans* SK-predicted protein sequence reveal the presence of five highly conserved domains.$^{70}$ An invariant positively charged GGTKG motif was identified in the lipid kinase domain. In addition, SK1 contains a second region bearing strong resemblance to the proposed ATP-binding site of diacylglycerol kinase. Mutation of (human SK1) glycine 82 to aspartate in this region demonstrated that this glycine residue is required for nucleotide binding and resulted in a catalytically inactive, “dominant-negative” SK mutant.$^{71}$ This mutant blocked exogenous SK activation by TNF-α without altering baseline SK activity or S1P levels. This finding suggests that SK may provide dual roles in mammalian cells, first a housekeeping function required for sphingolipid turnover and ceramide clearance and a second, regulatable signaling function. A lysine in close proximity to glycine 82 was also found to be necessary for maximal catalysis. In consideration of these findings, an SK nucleotide-binding motif of SGDXXG was proposed, structurally relating the SK catalytic site to the glycine-rich loop found in protein kinases.$^{72}$ Recently, a novel activation mechanism of SK1 by extracellular signal-activated (ERK)-2 protein kinase was proposed, in which the serine$^{225}$ of human SK1 was phosphorylated, resulting in increased catalytic activity and membrane translocation.$^{73}$ Thus, growth factor or hormonal stimulation of cells which often result in ERK activation, may induce S1P release into the extracellular milieu (see later).

Subsequently, a second mammalian SK isoform was cloned.$^{74}$ Murine and human SK2 proteins contain the same five conserved domains found in SK1. However, the two isoforms diverge widely at the N-terminus, with SK2 manifesting a unique N-terminal extension.$^{70}$ In addition, heterogeneity in the N-terminus was found in different SK2 isoforms, which is suggestive of alternative splicing or transcriptional start sites.$^{75}$

Biochemical distinctions between SK1 and SK2 include differential responses to high salt and detergents and the ability of SK2 but not SK1 to efficiently phosphorylate unusual substrates such as the SK inhibitor d, l-threo-dihydrosphingosine, the immune modulatory prodrug FTY720,$^{23,75}$ and phytosphingosine. Further, recent studies implicating translocation of SK1 to the membrane as a mechanism of activation have not been demonstrated for SK2.$^{76}$ However, the presence of a nuclear localization
sequence in SK2 was recently demonstrated.77 Interestingly, overexpression of SK2 induces cell-cycle arrest, a finding that is in sharp contrast to SK1, which generally promotes cell survival and proliferation.77 In addition, SK2 overexpression was shown to induce apoptosis by virtue of a BH3-like domain that interacts with Bcl-xL.78 The mechanism of how SK2 promotes these effects is not known. However, it is likely that SK2 acts in an intracellular locale that is different from that of SK1. SK2 is more promiscuous than SK1 in terms of substrate utilization, and SK genes of C elegans and Drosophila melanogaster bear more resemblance to SK2 than to SK1, suggesting that SK1 may have evolved later than SK2.

As an important regulatory enzyme, it is not unexpected that SK activity should itself be subject to multiple levels of control. For example, phosphorylation of SK1 by protein kinase C was shown to activate SK in response to VEGF stimulation in endothelial cells.79 Protein kinase C–dependent activation of SK1 mediated SK translocation from cytosol to plasma membrane in HEK293 cells.76 However, translocation of wild-type SK was associated with increased appearance of S1P in the media, indicating that SK translocation may facili- tate S1P export and access to extracellular receptors.

A recent study demonstrating that SK can itself be exported from cells via a nonclassical pathway and function as an ectoenzyme provides an alternative mechanism in which S1P formation may occur in the extracellular milieu.77 In addition, SK may also be shuttled between the cytosol and various intracellular organelles.80 Thus, mechanisms that regulate cellular or extracellular localization of SK may bring S1P production into the immediate vicinity of S1P receptors or potentially other intracellular targets.

TNF–α-regulated SK activation is likely to be important in nuclear factor-κB (NF-κB) activation and inhibition of apoptosis.81 TNF–α induces the protein-protein interactions between SK1 and the TNF receptor–associated factor (TRAF2).82 Thus, SK activation contributes to and dissects TNF-mediated biology, ie, inflammation and apoptosis. As earlier, the pathophysiological significance of these mechanisms needs to be pursued in whole animal models of inflammation and apoptosis.

The relationships between S1P metabolism, lipoproteins, and vascular cells may be highly relevant to the development of atherosclerosis. Specifically, mitogenic effects of oxidized LDL on vascular smooth muscle cells, which contribute to the atherogenic process appear to require the activation of SK.83 Thus, by influencing the state of SK activation and intracellular S1P generation, lipoproteins may affect vascular cell proliferation, migration, adhesion, retraction, and survival.

In summary, various mechanisms by which SK activity is regulated, including translocation, export, phosphorylation, protein-protein interactions, and potentially transcriptional regulation, and the apparently opposing roles of SK1 and SK2 on cell survival suggest that S1P synthesis involves a complex interplay of molecular events, some of which may provide novel avenues for therapeutic intervention. In addition, metabolic activation of novel therapeutics such as FTY720 by the SK2 isomer,83 warrants further studies on expression, regulation, and genomic and epigenetic mechanisms that regulate this important family of enzymes.

**S1P Phosphatases**

The dephosphorylation of S1P is catalyzed by two distinct classes of lipid phosphatases. Lipid phosphate phosphohydrolases (LPPs) encompass a family of membrane-bound phosphatases characterized by magnesium independence, N-ethylmaleimide insensitivity, and the ability to dephosphorylate a variety of lipid substrates including phosphatidic acid, lysophosphatidic acid (LPA), S1P, and ceramide 1-phosphate.84 Three conserved domains, characterized by the motifs KX,RP, PSGH, and SRX,HX,D, have been identified among all members of the LPP family and are also found in other phosphatases85 and are likely to constitute the active site of the lipid phosphatases. Whereas LPP1 localizes to the plasma membrane with its active site facing the extracellular space, LPP3 resides in the endoplasmic reticulum (ER), Golgi, and lipid raft membrane subdomains.86

What role do the LPPs play in cell and animal physiology? It has been proposed that the primary function of LPPs is to remove lysophospholipids within the vicinity of the interface between the plasma membrane and extracellular space, thereby attenuating signals propagated from lysophospholipid (LPA and S1P) receptors.87 The recent demonstration that LPP enzymes interfere with intracellular lysophospholipid metabolism, independent of the bioavailability of receptor ligands suggest their potential to regulate intracellular signaling events.88,89

The *Drosophila wunen* genes, which encode phosphatidic acid phosphatases, provide evidence that LPPs influence metazoan biology through their effects on cell migration and survival.90 It is likely that by dephosphorylating their endogenous substrate(s), the *wunen* enzymes create a repellant molecule, forming a gradient that directs the migrating germ cells away from tissues expressing *wunen* and toward the mesoderm, which lacks *wunen*.

Recently, the phenotype of the mouse *LPP3* (which is capable of dephosphorylating S1P) deletion was reported.91 Severe embryonic developmental defects, such as defective formation of the choioallantois, placenta, yolk sac vasculature, and shortened anterior-posterior axis and duplication of the axial structures were observed, suggesting an essential function of this molecule in early mouse development. The authors noticed similarities between *LPP3*-null embryos and *Wnt* signaling pathway phenotypes and showed that LPP3 downregulates the canonical *Wnt* signaling pathway, for example, β-catenin–induced transcriptional responses. These findings reinforce the concept that enzymes of S1P metabolism may regulate postembryonic angiogenesis.

The existence of a phosphatase specific for S1P was established definitively with identification of two yeast proteins.92 Based on sequence homology to yeast S1P phosphatases, *hSPP1* and *mSPP1* were identified.93 Both these mammalian S1P phosphatases localize to the ER (based on overexpression studies of GFP-tagged proteins), reduce S1P and dihydroS1P levels by catalyzing their dephosphorylation, and induce apoptosis on overexpression by enhancing the formation of ceramide. The ER localization of S1P phospha-
S1P Lyase
S1P lyase catalyzes the irreversible cleavage of S1P at the C2,3 carbon bond to yield a long-chain aldehyde and ethanolamine phosphate. This enzyme is a pyridoxal 5'-phosphate–dependent member of the carbon-carbon lyase subclass of aldehyde-lyases. Whereas the enzyme recognizes only the natural d-erythro isomer, it is more promiscuous with regard to the type and chain length of sphingoid base. S1P lyase activity is found in all mammalian tissues examined, with the exception of platelets.

Analysis of the yeast S1P lyase sequence revealed an N-terminal hydrophobic domain that probably serves to anchor the protein to the membrane and significant homology to glutamate decarboxylase and other pyridoxal 5'-phosphate–dependent enzymes, especially in the region of the predicted cofactor-binding site (see later). Isolation of the yeast enzyme enabled the identification of functional S1P lyase homologs within the genomes of man, mouse, fly, and nematode. Comparison of the S1P lyase protein sequences of these diverse species reveals conservation of the N-terminal hydrophobic domain and a 20 amino acid stretch (amino acids 344 to 364) surrounding and including the cofactor binding lysine, as predicted by homology to the cofactor attachment site of class V aminotransferases. Positively charged amino acids within this stretch may interact with the phosphate groups of the substrate, cofactor, or both. Altered S1P lyase expression in a variety of cell lines and mutant model systems has yielded pronounced effects and severe phenotypes. For example, S. cerevisiae S1P lyase–null mutants are highly resistant to heat stress and nutrient deprivation, whereas overexpression of the enzyme can correct specific defects of endocytosis. In mouse embryonal carcinoma cells, disruption of the S1P lyase gene enhances cellular differentiation in response to retinoic acid. In Drosophila, C. elegans, and Dictyostelium, S1P lyase expression is required for embryogenesis, reproduction, survival, and movement, and loss-of-function mutations confer resistance to chemotherapy. These observations indicate that S1P lyase has important and, in some cases, essential functions in cells and organisms and can also influence the response to pharmacological agents.

A human S1P lyase-GFP fusion protein localizes to the ER. Whereas the S1P phosphatases are poised to provide sphingosine or dihydrosphingosine substrates to ceramide synthase for conversion to ceramide, S1P lyase may provide a local influence over serine palmitoyltransferase which, like these other enzymes, also resides in the ER and appears to be inhibited by LCBPs. One structural model postulates the enzyme is positioned in the ER membrane with its active site facing the cytosol and its N-terminal domain within the lumen. Mutation of human S1P lyase K353 to leucine is associated with complete loss of S1P lyase activity without affecting localization to the ER, whereas N-terminal truncation of the first 58 amino acids encompassing the predicted membrane-spanning domain (amino acids 39 to 59) leads to loss of membrane association without loss of enzyme activity. Mutation of either of two conserved cysteines (Cys317Ser, Cys218Gly) nearly or totally inactivates the enzyme.

Overexpression of human S1P lyase in mammalian cells induces apoptosis in response to serum deprivation and other apoptotic stimuli, diminishes intracellular S1P levels, and increases stress-induced ceramide generation. S1P lyase–induced apoptosis requires its enzyme activity and can be blocked by either addition of exogenous S1P or by treatment with the ceramide synthase inhibitor fumonisin B1. In contrast, products of the lyase reaction had no effect on apoptosis. Further, apoptosis induced by S1P lyase is associated with cytochrome c release from mitochondria, suggesting involvement of the intrinsic apoptotic pathway. These studies demonstrate that S1P lyase is capable of regulating mammalian cell survival through the modulation of intracellular levels of both S1P and ceramide. Interestingly, the Drosophila S1P lyase knockout model demonstrates abnormalities of embryonic and postembryonic programmed cell death, indicating that lyase expression is involved in regulating cell survival in vivo. Future studies aimed at determining whether changes in lyase activation or expression affect cell survival under physiological conditions in mammals will be
important in determining the role of this enzyme and the potential for its modulation as a therapeutic intervention for conditions caused at least in part by dysregulated apoptosis.

In light of recent studies implicating S1P lyase in the regulation of sterol and fatty acid metabolism through an influence on SREBP cleavage in Drosophila,7 and the role of yeast S1P lyase in regulating the flow of sphingolipid metabolites into phospholipid synthesis,110 it is tempting to postulate that S1P lyase may provide an important and central role in coordinating lipid metabolism in response to changing intrinsic (developmental, genetic) and extrinsic (environmental, nutritional) conditions in metazoans.

The human S1P lyase gene maps to chromosomal region 10q21.111 Notably, chromosomal deletions have been found within this region in many human tumor types. Although no direct contribution of S1P lyase to tumor biology has been established, it is clear that loss of S1P lyase leads to accumulation of intracellular S1P in all existing S1P lyase loss-of-function models, and this would be expected to potentiate cancer cell proliferation and survival. Interestingly, a study investigating mechanisms of resistance to the cancer chemotherapeutic agent cisplatin using the Dictyostelium model system identified S1P lyase mutations as protective against the drug, providing another mechanism by which loss of S1P lyase function may protect cancer cells, possibly through inhibition of apoptosis induced by DNA damage or cellular stress.112

In summary, S1P lyase is an important regulator of intracellular S1P levels. Lack of S1P lyase expression in some cells, for example the platelet, is clearly important in the S1P biosynthetic capacity of a given cell type. The regulation of S1P lyase gene expression and activity may provide the cell with an additional barometer of changing environmental or developmental stimuli and a mechanism by which the cell coordinates and adjusts intracellular S1P levels to meet the needs of the organism.

**Conclusions and Future Perspectives**

It is now clear that S1P metabolism in cells provides an evolutionarily conserved metabolic/signaling network that is critical for important cellular decisions. This is exemplified by early studies in S cerevisiae heat stress responses, sterol metabolism, endosomal trafficking, and phospholipid synthesis and by subsequent findings in simple metazoans. Additional evidence supporting an intracellular, receptor-independent role for sphingolipids in critical signaling events was provided by the recent demonstration that SREBP-dependent transcriptional responses that regulate phospholipid synthesis require sphingolipid metabolic enzymes such as SK, SPP, and S1P lyase.7,113 This ancient paradigm should be vigorously explored in vertebrate systems, in particular in the cardiovascular system, from the study of which the SREBP pathway was originally elucidated. In addition, emerging evidences suggest that modulation of S1P metabolic enzymes exert profound effects on cell survival, death, and differentiation, potentially by modulation of membrane physiology, structure, and trafficking. Moreover, feed-back and feed-forward autoregulation of the S1P metabolic pathway constitute additional level of fine control. In addition, a potential second messenger function of S1P or sphingosine in specific subcellular compartments is possible.

The counterpoint to this metabolic/intracellular signaling system is the GPCR-dependent signaling by S1P in vertebrates and chordates.5 We have begun to realize that S1P receptors play important roles in vascular tone control, vascular permeability, heart physiology, cardiovascular development, inflammation, immunity, and angiogenesis. As GPCRs constitute the most “druggable” class of receptors, it is perhaps timely that the first S1P receptor agonist, FTY720, is currently undergoing phase III clinical trials for transplant rejection indications.8 As FTY720 administration was recently shown to regulate vascular permeability and endothelial cell integrity,23 it is likely that S1P receptor agonists/antagonists may find utility in cardiovascular therapeutics. As our knowledge of S1P metabolism and function increases, it should become possible to fully harness the potential of this multifunctional lipid mediator in the control of human maladies.

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**References**


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