

Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signaling

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Summary

The phosphorylated sphingolipid metabolites sphingosine 1-phosphate (S1P) and ceramide 1-phosphate (C1P) have emerged as potent bioactive agents. Recent studies have begun to define new biological functions for these lipids. Generated by sphingosine kinases and ceramide kinase, they control numerous aspects of cell physiology, including cell survival and mammalian inflammatory responses. Interestingly, S1P is involved in cyclooxygenase-2 induction and C1P is required for the activation and translocation of cPLA₂. This suggests that these two sphingolipid

metabolites may act in concert to regulate production of eicosanoids, important inflammatory mediators. Whereas S1P functions mainly via G-protein-coupled receptors, C1P appears to bind directly to targets such as cPLA₂ and protein phosphatase 1/2A. S1P probably also has intracellular targets, and in plants it appears to directly regulate the G protein α subunit GPA1.

Key words: Ceramide 1-phosphate, Ceramide kinase, Sphingosine 1-phosphate, Sphingosine kinase, Inflammation, Cancer

Introduction

Ceramide is the central core lipid in the metabolism of sphingolipids. It is produced via a de novo biosynthetic pathway beginning with the condensation of serine and palmitoyl-CoA by the enzyme serine palmitoyl-CoA transferase. Ceramide is also produced by hydrolysis of sphingomyelin by sphingomyelinases. It can be phosphorylated by ceramide kinase (CERK) to ceramide 1-phosphate (C1P), or utilized for the synthesis of sphingomyelin or glycosphingolipids. Ceramide can also be broken down by ceramidases to sphingosine, which in turn is phosphorylated by sphingosine kinases (SphKs) to generate sphingosine 1-phosphate (S1P). S1P is degraded by specific phosphatases that regenerate sphingosine or by a lyase that cleaves it irreversibly into ethanolamine 1-phosphate and palmitaldehyde (Fig. 1). Recently, there has been great progress in cloning and characterizing some of the enzymes involved in sphingolipid metabolism. However, we still have only limited insight into the regulation of most of these. For further information, the reader is referred to excellent recent reviews (Futerman and Hannun, 2004; Ogretmen and Hannun, 2004).

Ceramide itself is an important second messenger in various stress responses and growth mechanisms, and formation of ceramide by the hydrolysis of sphingomyelin or de novo synthesis occurs in response to many inducers of stress, such as TNF α , γ -interferon, 1 α ,25-dihydroxyvitamin D₃, interleukin 1 (IL-1), UV light, heat, chemotherapeutic agents, FAS antigen and nerve growth factor (NGF) (Hannun and Obeid, 2002; Pettus et al., 2002). The emerging role of ceramide in the transduction of stress and apoptotic responses has necessitated a mechanistic understanding of its action. This has led to the identification of several candidates for ceramide-regulated enzymes, including ceramide-activated protein

kinase (CAPK), protein kinase C ζ , cathepsin D and ceramide-activated protein phosphatase (CAPP) (Hannun and Obeid, 2002).

The interconvertible ceramide-derived metabolites S1P and C1P, also represent an important class of bioactive lipid mediators. There are two mammalian SphK isoenzymes, SphK1 and SphK2, which catalyze the phosphorylation of sphingosine to S1P (Spiegel and Milstien, 2003). Although sphingosine may also be an important physiological regulator, because it can inhibit protein kinase C as well as induce cell-cycle arrest and apoptosis, S1P has distinct roles in cell growth and survival, angiogenesis, vasculogenesis, neuritogenesis, and immune function, and the number of reports on S1P-mediated cell signaling has exploded in recent years (Spiegel and Milstien, 2003). Extracellular actions of S1P are mediated by its interaction with a family of five specific G-protein-coupled receptors (GPCRs), S1P₁-S1P₅ (Goetzl and Rosen, 2004; Spiegel and Milstien, 2003). In addition, similarly to other potent lipid mediators, S1P also has intracellular actions independent of these receptors (Spiegel and Milstien, 2003).

C1P is another phosphorylated bioactive sphingolipid whose importance has only recently begun to be appreciated. Although C1P was identified more than a decade ago, tantalizing hints of its potential biological functions have only appeared in the last few years (Liang et al., 2003; Pettus et al., 2003a). C1P was originally detected in HL-60 human leukemia cells, where it was shown to be formed by phosphorylation of sphingomyelin-derived ceramide by a kinase that was functionally and physically separable from diacylglycerol kinase (Kolesnick and Hemer, 1990). CERK is now known to be a distinct but close relative of SphKs that catalyzes the phosphorylation of ceramide to give C1P (Sugiura et al., 2002), and its cloning has helped to uncover new physiological

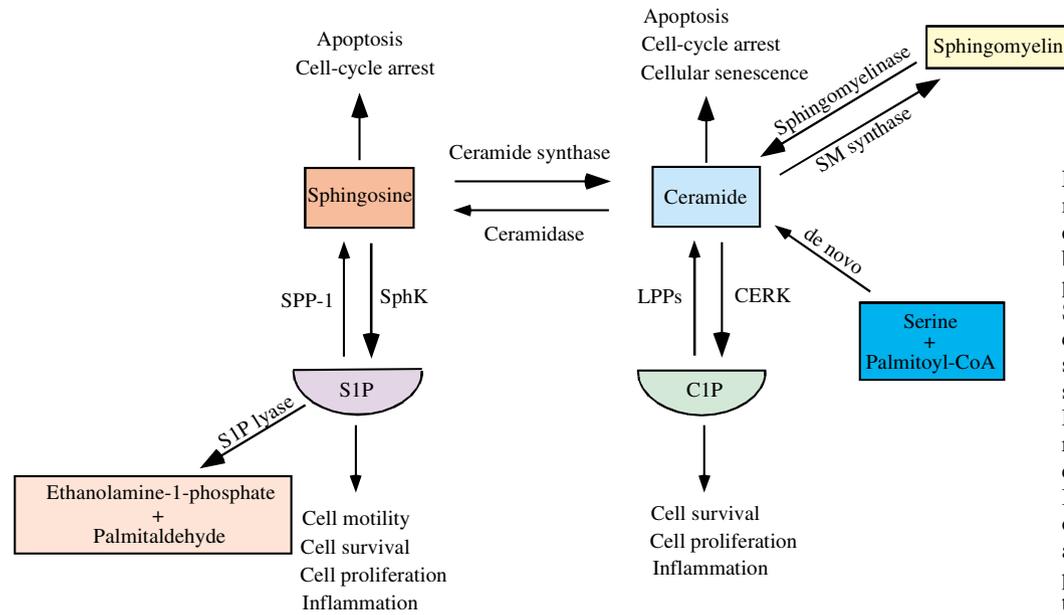


Fig. 1. Bioactive sphingolipid metabolites. The schematic depicts the sphingolipid biosynthetic pathway: LPP, lipid phosphate phosphatase; SPP-1, S1P phosphatase-1; CERK, ceramide kinase; SphK, sphingosine kinase; SM, sphingomyelin. Differential/opposing signaling roles of sphingosine, S1P, ceramide and C1P are indicated. Because of the interconvertibility of these molecules, the activity of a single enzyme in the depicted pathways may affect the fate of the cell.

functions for C1P. Here, we focus on the emerging roles of S1P and C1P in inflammatory responses and touch on recent insights into their mechanisms of action, and their functions in plants.

Functions of S1P in cell migration and lymphocyte trafficking

Diverse external stimuli, including PDGF, VEGF and TNF- α (reviewed in Spiegel and Milstien, 2003), stimulate SphK1 to generate intracellular S1P, which can function in an autocrine or paracrine fashion to activate S1P receptors present on the surface of the same or a nearby cell. This type of signaling is important for migration of cells towards PDGF and has important implications for vascular maturation. Activation of the S1P₁ receptor stimulates downstream signals important for cell locomotion (Hobson et al., 2001; Rosenfeldt et al., 2001), whereas S1P₂ acts to dampen this. Thus the net responses to S1P depend on the relative expression levels of these two receptors and their activation in response to PDGF (Goparaju et al., 2005).

Interest in the functions of S1P in the immune system has increased recently owing to the discovery that the potent immunosuppressive drug FTY720, which is now in clinical trials for kidney transplantation and multiple sclerosis, is a sphingosine analogue that is phosphorylated by SphK2 and functions as a S1P mimetic to induce sequestration of T lymphocytes in thymus and lymph nodes (Brinkmann et al., 2002; Cyster, 2005; Goetzl and Graier, 2004; Mandala et al., 2002). Adaptive immunity depends on circulation of T and B cells between secondary lymphoid organs to monitor antigens. Studies with mice, whose hematopoietic cells lack S1P₁, have established that this S1P receptor is essential for lymphocyte recirculation and tissue homing (Matloubian et al., 2004). Although it is not completely understood how S1P gradients regulate lymphocyte trafficking, it has been suggested that low concentrations of S1P (between 10 and 100 nM) are optimal for enhancing chemotaxis of

lymphocytes to chemokines and some cytokines (Graier and Goetzl, 2002; Rosen and Goetzl, 2005). By contrast, the higher concentrations of S1P (100 to 1000 nM) in the blood inhibit chemokine-induced movement of T cells from high endothelial venules into secondary lymphoid organs (Rosen and Goetzl, 2005). Several studies suggest that the chemotactic responsiveness of T cells to S1P increases between the time of entry into and exit from secondary lymphoid organs (Lo et al., 2005). However, the mechanisms that regulate S1P₁ expression and signaling are unknown. Although much remains to be learned, it is clear that S1P, acting through S1P₁, is a major regulator of T-cell development, B- and T-cell recirculation, lymphocyte homing and chemotactic responses to chemokines (Goetzl and Rosen, 2004). This pattern of lymphocyte responses suggests that, new forms of immunotherapy that specifically target this S1P receptor on immune cells might be uniquely valuable for suppression of organ-graft rejection without impairment of host defences against infections.

C1P and S1P in inflammatory responses

Eicosanoids (e.g. prostaglandins and leukotrienes) are well-established mediators of inflammatory responses with roles in the pathogenesis of cancer and inflammatory disorders such as atherosclerosis, asthma, osteoarthritis and Alzheimer's disease. The formation of arachidonic acid (AA) via the activation of phospholipase A₂ is the initial rate-limiting step in eicosanoid biosynthesis (Clark et al., 1995). In many cases, inflammatory agonists induce activation and translocation of group IVA cytosolic phospholipase A₂ (cPLA₂ α) in a Ca²⁺-dependent or -independent manner, a process whose mechanism and mediators have not been completely elucidated (Scott et al., 1999). Depending on the basal levels of expression of downstream enzymes in the eicosanoid pathway – for example, cyclooxygenase 2 (COX-2) – their transcription (if necessary and this varies by cell type) can be induced in a secondary rate-limiting step (Scott et al., 1999). Increased COX-2 synthesis in

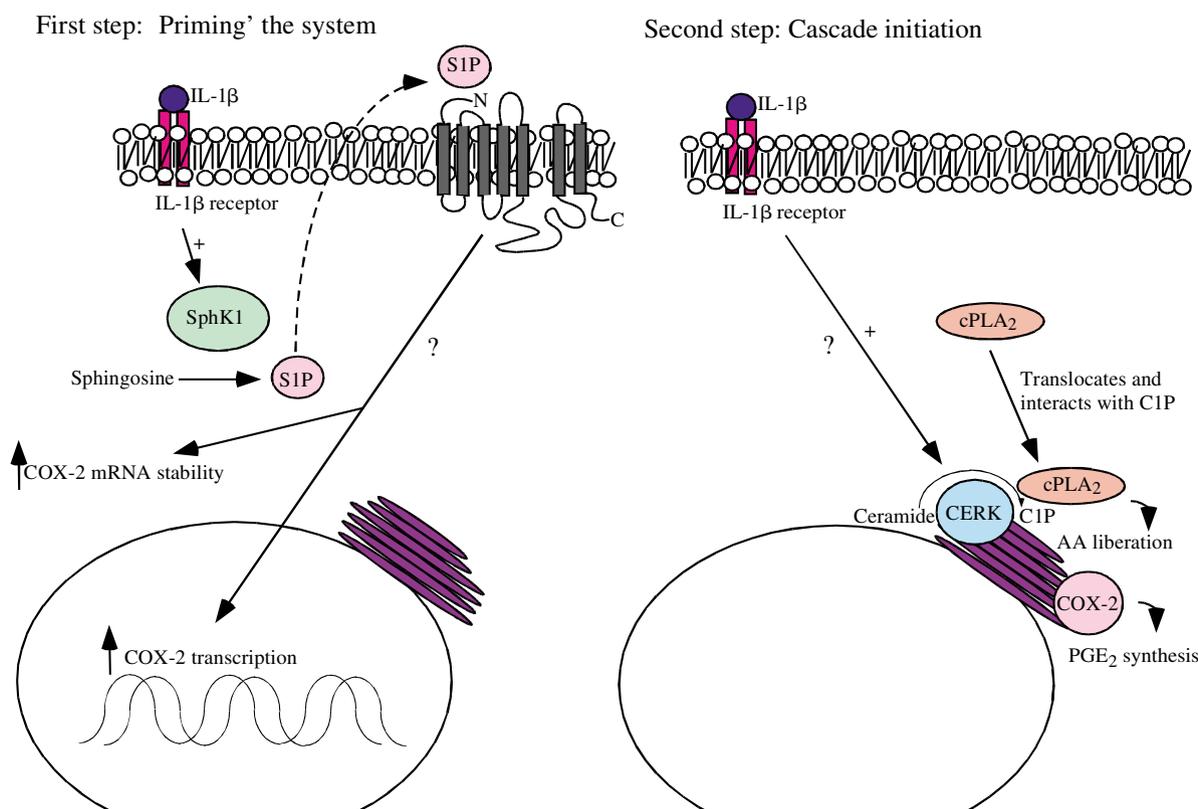


Fig. 2. Roles of SphK1 and CERK in eicosanoid synthesis. The roles of S1P and C1P in eicosanoid synthesis in response to inflammatory cytokines are indicated, beginning with the first step, induction of cyclooxygenase-2 (COX-2). This 'primes' the cell for PGE₂ synthesis and requires the generation of S1P by SphK1, followed by a receptor-mediated signaling event initiated by interaction of S1P with its receptor. The second step of prostanoid biosynthesis in response to IL-1 β , involves the activation of cPLA₂ and generation of arachidonic acid (AA), which requires CERK and the generation of C1P.

most cases occurs prior to cPLA₂ α activation and is termed 'priming' the system for optimal response. COX-2 then utilizes the AA released by cPLA₂ to initiate the prostaglandin-synthesis pathways (Scott et al., 1999). In the leukotriene pathway, the initial enzyme, lipoxygenase (LO), also utilizes AA as a substrate.

Ceramide was initially implicated as a signaling molecule for inflammatory responses. A cell-permeable ceramide analog increases prostaglandin production, and, although ceramide alone has little effect, it enhances IL-1-stimulated PGE₂ production (Pettus et al., 2005). In addition, sphingomyelinase treatment dose-dependently enhances IL-1-stimulated PGE₂ production (Pettus et al., 2005). Subsequently, ceramide and A23187 were shown to synergize to induce the translocation of cPLA₂ α , but again ceramide alone had no effect. Thus, it was suggested that ceramide regulates eicosanoid synthesis by enhancing activation of cPLA₂ α . However, recent studies have shown that C1P rather than ceramide functions as the proximal mediator of AA release (Pettus et al., 2003a). Several lines of evidence support this concept. First, unlike ceramide, exogenous C1P alone can stimulate AA release and PGE₂ formation. Second, treatment of cells with spider venom sphingomyelinase D to produce C1P from membrane sphingomyelin can elicit the AA response, whereas sphingomyelinase C, which produces

ceramide, has no effect. Third, inflammatory agonists, such as IL-1 β , induce rapid increases in endogenous C1P levels within the proper time frame for AA release. Importantly, downregulating CERK by RNAi dramatically inhibits AA release and PGE₂ synthesis in response to ATP, the calcium ionophore A23187 and IL-1 β (Pettus et al., 2003a). Furthermore, C1P-induced AA release requires cPLA₂ α activity. These results thus suggest that C1P acts upstream of this step in eicosanoid synthesis (Pettus et al., 2004) and thereby regulates inflammatory responses (Fig. 2).

Since ceramide, C1P, sphingosine and S1P are interconvertible, they might function as components of a 'rheostat' that regulates immune cell functions, including mast cell responsiveness, neutrophil and macrophage priming, chemotaxis, and survival of many types of immune cells (Goetzl and Rosen, 2004; Olivera and Rivera, 2005; Spiegel and Milstien, 2003). Activation of SphK1 by a variety of cytokines and concomitant formation of S1P are important for various inflammatory responses (Goetzl and Rosen, 2004; Olivera and Rivera, 2005; Spiegel and Milstien, 2003). A recent study showed that ceramide, sphingosine, and S1P can all induce COX-2 in A549 human lung cells and macrophages (Pettus et al., 2003b) but S1P is the most potent. In response to TNF- α treatment, there is a significant increase in the levels of S1P, and downregulation of SphK1, but not SphK2, blocks

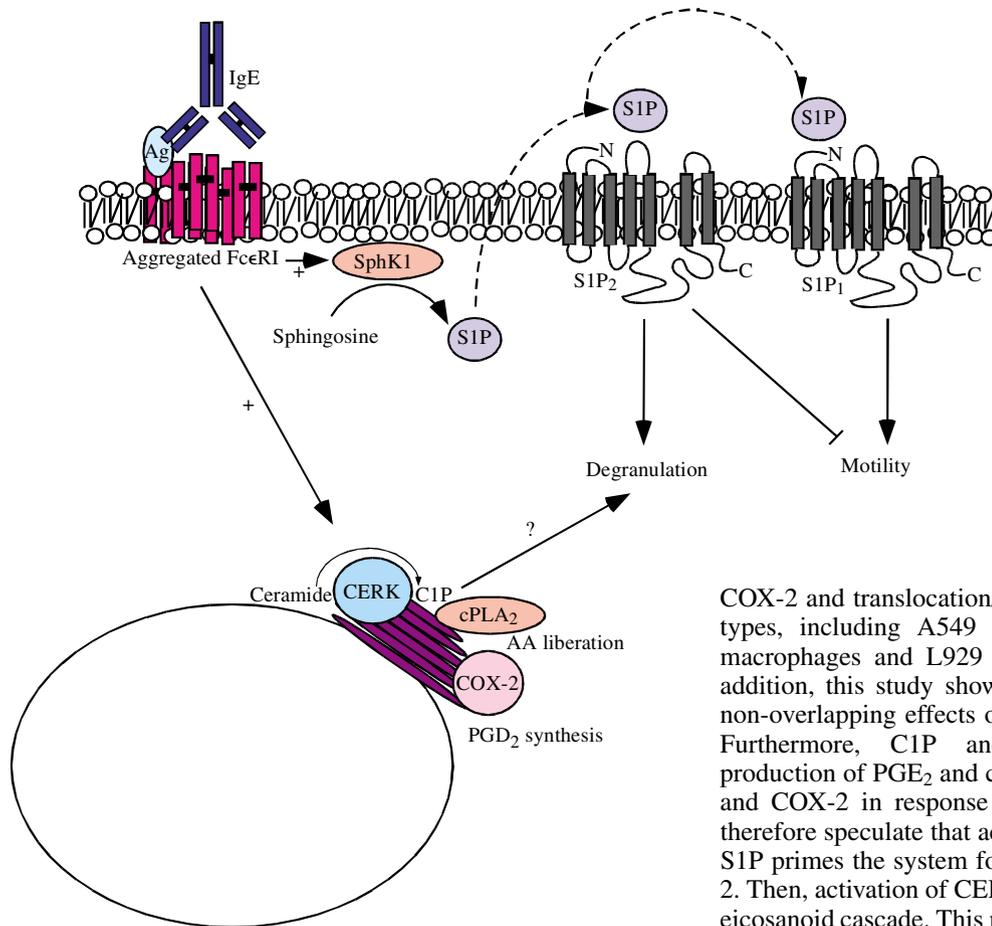


Fig. 3. SphK1 and CERK in mast cell functions. Crosslinking of the high affinity IgE receptor FcεRI on mast cells translocates and activates SphK1, increasing S1P formation. S1P in turn transactivates S1P₁ and S1P₂ receptors, leading to mast cell degranulation and cell motility. Furthermore, CERK may also regulate degranulation of mast cells.

this, as well as PGE₂ production. Conversely, RNAi directed against S1P lyase or S1P phosphatase, which should increase S1P levels, enhances TNF-induced COX-2 and PGE₂ production. Importantly, expression of SphK1 is necessary for the induction of COX-2 by ceramide and sphingosine, but not S1P, indicating that these two sphingolipid metabolites act through their conversion to S1P (Fig. 2).

These recent studies go a long way towards clarifying the complex body of information on actions attributed to sphingolipid metabolites on inflammatory processes. However, although evidence now strongly supports roles for C1P and S1P in regulation of eicosanoid synthesis, the mechanisms involved require further clarification. Important questions remain. How are SphK and CERK regulated in response to these inflammatory agonists, and are C1P and S1P components of a linear regulatory pathway or do they act independently? As regards the first question, almost nothing is known about how SphK and CERK are activated in response to these inflammatory agonists. Since both of these lipid kinases are activated by calcium, A23187 and ATP may activate them by increasing intracellular calcium levels. Both enzymes are also phospho-proteins and utilize lipid cofactors, thus these are also possibilities for activation of the enzymes. Therefore, future studies of regulation of SphK and CERK in immune cells are warranted. As for the second question, a recent study demonstrated that IL-1β activates both SphK1 and CERK, and that these two kinases independently regulate induction of

COX-2 and translocation/activation of cPLA₂α in diverse cell types, including A549 lung adenocarcinoma cells, J774.1 macrophages and L929 fibroblasts (Pettus et al., 2005). In addition, this study showed that S1P and C1P have unique, non-overlapping effects on COX-2 and cPLA₂α, respectively. Furthermore, C1P and S1P synergistically stimulate production of PGE₂ and can recapitulate activation of cPLA₂α and COX-2 in response to IL-1β (Pettus et al., 2005). We therefore speculate that activation of SphK1 and production of S1P primes the system for PGE₂ synthesis by inducing COX-2. Then, activation of CERK and formation of C1P triggers the eicosanoid cascade. This mechanism would ensure coordinated activation/translocation of cPLA₂ and induction of COX-2, the enzymes that generate and metabolize AA, respectively, in the pathway for formation of prostaglandins as well as other eicosanoids (Fig. 2).

Mast cells are also important players in inflammatory responses, and S1P and C1P are also thought to regulate many of their functions, including degranulation and chemotaxis (Jolly et al., 2004; Jolly et al., 2005). Cross-linking of the high-affinity receptor for IgE (FcεRI) on mast cells activates SphK1, leading to generation and secretion of S1P, which in turn activates its receptors S1P₁ and S1P₂ on mast cells. Although activation of S1P₁ and Gi signaling are important for cytoskeletal rearrangements and migration of mast cells towards antigen, they are dispensable for FcεRI-triggered degranulation (Jolly et al., 2004). However, S1P₂, whose expression is upregulated by FcεRI cross-linking, is required for degranulation. Furthermore, RNAi directed against SphK1 and SphK2 clearly showed that SphK1 and S1P are required for degranulation of mast cells (Jolly et al., 2004). Activation of SphK1 and, consequently, S1P receptors by FcεRI triggering thus seems to play a crucial role in mast cell functions and might be involved both in the movement of these cells to sites of inflammation and in their degranulation.

CERK and its product, C1P, could also function in degranulation of mast cells because overexpression of CERK enhances degranulation of RBL-2H3 cells induced by A23187 (Mitsutake et al., 2004). However, the interconversion of C1P with the other bioactive sphingolipid metabolites was not considered in this study, and it is possible that overexpression

of CERK or treatment of cells with C1P merely influences the levels of S1P. Establishing whether S1P and C1P have non-overlapping roles in this case therefore warrants more comprehensive studies (Fig. 3).

cPLA₂ as a target of C1P

Unlike S1P, C1P is not thought to act through a cell surface receptor. It probably functions instead at the intracellular level. cPLA₂α is an obvious potential target and recent work supports the idea that C1P regulates this enzyme by direct interactions (Pettus et al., 2003a). cPLA₂α has a calcium-dependent lipid-binding (C2/CaLB) domain similar to the C2 domain of protein kinase C (PKC) (Clark et al., 1991). C1P, an anionic lipid, might therefore interact directly with this domain in a fashion analogous to that by which phosphatidylserine interacts with the C2 domain of PKC. Alternatively, C1P could also indirectly activate cPLA₂α since exogenous C1P has been reported to induce calcium mobilization (Tornquist et al., 2004). We have recently discovered that C1P is indeed a direct activator of cPLA₂α that interacts with its C2/CaLB domain (Subramanian et al., 2005). This was a somewhat unexpected finding because the current dogma is that zwitterionic lipids, such as phosphatidylcholine, rather than anionic lipids such as C1P, bind to the C2/CaLB domain (Hixon et al., 1998). Nevertheless, there is significant interaction between C1P and cPLA₂α or its C2/CaLB domain at 300 nM calcium (Subramanian et al., 2005), which is in agreement with early findings suggesting that association of cPLA₂ with membranes requires 300 nM calcium (Clark et al., 1991).

Importantly, C1P specifically activates cPLA₂α, both by an allosteric mechanism and by lowering the dissociation constant of the enzyme for phosphatidylcholine-rich vesicles (Subramanian et al., 2005). Given the latter effect, C1P could be involved in the recruitment of cPLA₂α to the Golgi complex. CERK localizes to the Golgi complex in HUVECs, COS-1 and A549 cells (Carre et al., 2004) (our unpublished findings), and C1P can thus be generated in the appropriate cellular compartment for recruitment of cPLA₂α in response to inflammatory agonists. Recent work indicates that C1P interacts with a novel site within the C2/CaLB domain [near calcium binding region II (CBR II) of cPLA₂α] and does not compete with the interaction sites for phosphatidylcholine (CBRI and CBRIII) or PtdIns(4,5,)₂ (the catalytic domain) (Subramanian et al., 2005).

Regulation of cell survival by C1P and S1P

Other possible direct targets of C1P are protein phosphatase 1 and protein phosphatase 2A (PP1 and PP2A). Ceramide is an activator of these serine/threonine protein phosphatases (also known as CAPPs), which have been linked to ceramide-induced apoptosis (Chalfant et al., 2002). Activation of PP1 by ceramide leads to dephosphorylation of SR proteins, a family of serine/arginine-domain proteins that modulate mRNA splicing, reducing the levels of the anti-apoptotic protein Bcl-x(L) and increasing the levels of apoptotic Bcl-x(S). Interestingly, C1P is a potent inhibitor (IC₅₀ 50 nM) of both PP1 and PP2A in vitro (Chalfant, 2004). This observation ties in well with the mitogenic/survival effects of C1P, because inhibition of these phosphatases has been linked to activation

of the ERK1/2 pathway and increased DNA synthesis (Dougherty et al., 2005; Hancock et al., 2005).

This potential of C1P to function as an inhibitory signaling molecule regulating PP1 activity is also directly relevant to the role of alternative splicing in cancer. Chemotherapeutic agents induce a pro-apoptotic change in the alternative splicing of caspase 9 and Bcl-x pre-mRNA (Massiello et al., 2004). This effect is mediated by ceramide-dependent activation of PP1 (Massiello et al., 2004). Since C1P is a potent inhibitor of PP1, one can hypothesize that C1P generated by CERK antagonises ceramide action (e.g. activation of PP1 and subsequent effects on Bcl-x and caspase-9 alternative splicing) and is thus pro-survival and cytoprotective. This idea has also been recently alluded to by Gomez-Munoz and co-workers, who demonstrated that C1P induces the expression of Bcl-x(L) and cell survival (Gomez-Munoz et al., 2005). Unfortunately, they did not examine the expression of Bcl-x(S). If indeed Bcl-x(L) levels do increase at the expense of Bcl-x(S), then CERK may be a crucial switch that regulates the fate of a cell in response to apoptotic agonists by having opposite effects on the alternative splicing of Bcl-x and caspase 9 pre-mRNA. By analogy with SphK1, which converts pro-apoptotic sphingosine to anti-apoptotic S1P, CERK may also play a homeostatic role regulating the balance between ceramide and C1P. Therefore, SphK1 and CERK may both be key determinants of the balance between cell death and cell survival (Fig. 1).

Intracellular targets for S1P

As previously mentioned, S1P acts through cell surface receptors but might also have direct intracellular targets. Several crucial studies have clearly shown that, S1P has specific intracellular actions that are independent of its cell surface receptors. For example, activation of Ras and ERK signaling pathways by VEGF requires SphK1 and is independent of S1P receptors (Shu et al., 2002; Wu et al., 2003), in contrast to cytoskeletal rearrangements and cell locomotion (Olivera et al., 2003). Similarly, TNF-α stimulates SphK1, leading to the activation of the transcription factor nuclear factor NF-κB, which is essential for the prevention of apoptosis (Xia et al., 2002). In addition, S1P inhibits male germ cell apoptosis independently of its receptors, possibly by inhibiting NF-κB and Akt phosphorylation (Suomalainen et al., 2005). SphK1 also enhances endothelial cell survival through PECAM-1-dependent activation of PI3K/Akt and regulation of Bcl-2 family members, without activating S1P receptors or ERK signaling (Limaye et al., 2005). Moreover, S1P can induce calcium mobilization independently of GPCRs (Meyer zu Heringdorf et al., 2003). Lastly, numerous studies in plants (see below), yeast, *Dictyostelium*, *Drosophila* and *Caenorhabditis elegans* revealed that S1P has important regulatory functions in these diverse lower organisms, which are devoid of S1P receptors (Herr et al., 2004; Herr et al., 2003; Min et al., 2005). Degradation of S1P by S1P lyase in *Drosophila*, for example, regulates the flux of sphingolipid metabolites into phospholipid synthesis and controls the release of sterol-regulatory-element-binding protein (SREBP) from cell membranes, as a feedback-control mechanism regulating synthesis of fatty acids and phospholipids (Dobrosotskaya et al., 2002). Clearly, an important missing

piece of the intracellular S1P signaling puzzle is the identification of direct intracellular targets.

C1P and S1P signaling in plants

The accelerated cell death 5 (*ACD5*) gene product in *Arabidopsis thaliana* was recently identified as a CERK (Liang et al., 2003). Plants that have an *ACD5* mutation initially develop properly but eventually undergo spontaneous cell death. Furthermore, *ACD5* mutants are more susceptible to infection by *Pseudomonas syringae*. They exhibit increased accumulation of ceramide before they shrivel and die. An important role of plant CERK might therefore be to remove excess ceramide, keeping the plant healthy and resistant to environmental stress. These are intriguing findings because, in mammalian cells, CERK activity and C1P levels are kept low compared with ceramide levels (10% of ceramide levels), and, even when CERK is activated, only a small decrease in the total ceramide levels results (our unpublished findings). Nevertheless, they indicate that CERK might be cytoprotective in plants, converting pro-apoptotic ceramide to pro-survival C1P, and playing a role analogous that of SphK1 in mammalian cells.

Recent studies suggest that SphK and S1P also have important functions linking the perception of abscisic acid (ABA) to reductions in guard-cell turgor (Ng et al., 2001). The drought hormone ABA activates SphK in *A. thaliana* and the S1P formed is involved in both inhibition of stomatal opening and promotion of stomatal closure (Coursol et al., 2003). Inhibition of SphK attenuates ABA-mediated regulation of guard-cell inwardly rectifying K^+ channels and slow anion-channels, which are involved in the regulation of stomatal pore size (Coursol et al., 2003). The action of S1P is impaired in *A. thaliana* knockout lines that lack the sole prototypical G protein α subunit GPA1, which indicates that heterotrimeric G proteins are downstream targets for S1P.

Notice that, whereas in mammals the prevalent long-chain base is sphingosine, which has a chain length of 18 carbon atoms and an E-double bond between C4 and C5, in plants the predominant long-chain bases are dihydrosphingosine and phytosphingosine, a saturated and 4-hydroxylated form of dihydrosphingosine (Fig. 4B). In common with S1P, phyto-S1P inhibits stomatal opening and promotes stomatal closure in *A. thaliana*, and these actions are also impaired in guard cells of GPA1-knockout plants.

Importantly, these effects of S1P are unlikely to be mediated by a GPCR because guard cells lacking the only *A. thaliana* GPCR-like protein, GCR1, have responses to ABA and S1P opposite to those of GPA1 mutants (Pandey and Assmann, 2004). Moreover, GCR1 has no significant sequence similarity to any of the conserved S1P receptors and does not bind either S1P or phyto-S1P. Therefore, the S1P signal in guard cells may be transduced by a direct interaction between S1P and GPA1

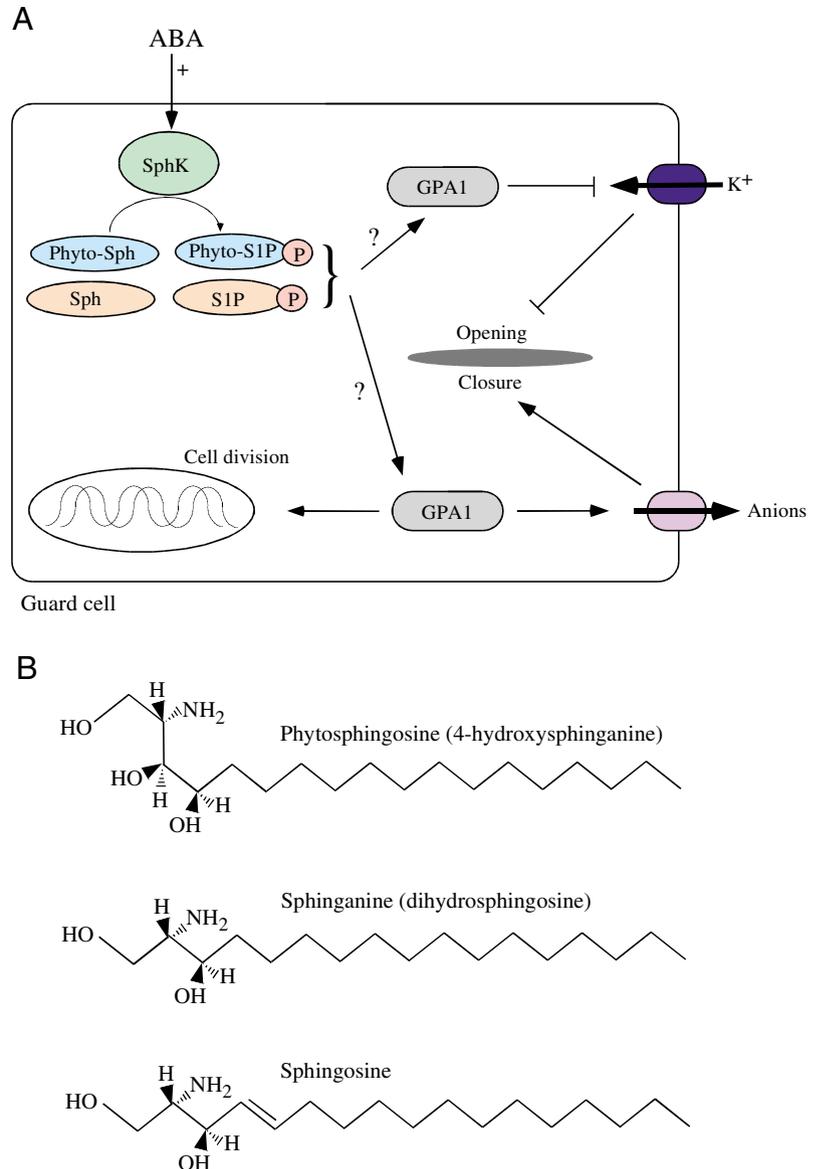


Fig. 4. (A) SphK and S1P signaling in responses of *A. thaliana* guard cells to abscisic acid (ABA). ABA stimulates SphK and formation of S1P and/or phyto-S1P. Phosphorylated sphingoid bases, acting through the heterotrimeric G protein GPA1 by a mechanism that is still not known, inhibit inwardly rectifying K^+ channels and activate slow anion channels, thereby inhibiting stomatal opening and promoting stomatal closure. S1P and/or phyto-S1P can also stimulate cell proliferation in a GPA1-dependent manner. (B) Structures of phytosphingosine, dihydrosphingosine, and sphingosine.

(Fig. 4A). Alternatively, S1P might act through an unidentified intermediate similar to AGS (activator of G protein signaling) – a protein that can activate heterotrimeric G proteins independently of GPCRs (Cismowski et al., 2001; Vaidyanathan et al., 2004).

Interestingly, GPA1 regulates not only guard-cell function but also plant cell division (Ullah et al., 2001). The discovery that it is a downstream component of S1P and phyto-S1P signaling in guard cells raises the exciting possibility that phosphorylated sphingoid bases play a role in other G-protein-mediated processes in plants, particularly those related to cell

proliferation. Moreover, this might help to reveal the enigmatic intracellular actions of S1P in mammals. Future work, therefore, will probably reveal novel mechanisms of S1P signaling through G proteins in diverse eukaryotes.

Perspectives

Because of the strong association between chronic inflammation and cancer (Lawrence et al., 2005; Nakanishi and Toi, 2005), coordinate regulation of inflammatory responses to cytokines by C1P and S1P suggests they may be ideal pharmacological targets for development of novel anti-inflammatory and anti-cancer therapies. The development of such drugs could be of key importance in light of the recent reports on the side effects of COX-2 inhibitors, such as VIOXX. The cause of these side-effects is unknown, but several possibilities exist. First, a COX-2 inhibitor might have non-specific cellular targets, and thus long-term administration could dysregulate important signaling pathways required for proper cell maintenance. Second, some basal level of prostaglandins synthesized by COX-2 may be required for normal homeostasis. Third, abnormal eicosanoids could be produced from excess AA, resulting from its lack of use by inhibited COX-2. Specific SphK1 inhibitors would clearly not have the same non-specific effects as COX-2 inhibitors. Moreover, CERK inhibition in conjunction with SphK1 inhibition or possibly even with VIOXX or Bextra might overcome the third possibility through their negative effects on cPLA₂, decreasing availability of AA. If specific eicosanoids produced by basal level COX-2 activity are important for normal cell functions, this would clearly be the most difficult problem to overcome. Inhibition of CERK to block inflammatory responses could potentially be as useful as COX-2 inhibitors were hoped to be. Since other eicosanoids, such as leukotrienes and thromboxanes, also regulate inflammatory responses, CERK inhibitors could have the added benefit of blocking all eicosanoid synthesis by inhibiting AA release in response to inflammatory mediators.

C1P and S1P have been shown to regulate important cellular processes in plants as well as mammals, demonstrating the broad and important role for these lipids in cell signaling cascades conserved across evolution. We hope that recent exciting and provocative results discussed here will stimulate the scientific community to undertake the daunting tasks of identifying intracellular targets for C1P and S1P and developing clinically useful therapies based on this knowledge.

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